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INVITED REVIEW

Current and Future Therapeutic Strategies for Lentiviral Eradication from Macrophage Reservoirs

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Abstract

Macrophages, one of the most abundant populations of leukocytes in the body, function as the first line of defense against pathogen invaders. Human Immunodeficiency virus 1 (HIV-1) remains to date one of the most extensively studied viral infections. Naturally occurring lentiviruses in domestic and primate species serve as valuable models to investigate lentiviral pathogenesis and novel therapeutics. Better understanding of the role macrophages play in HIV pathogenesis will aid in the advancement towards a cure. Even with current efficacy of first- and second-line Antiretroviral Therapy (ART) guidelines and future efficacy of Long Acting Slow Effective Release-ART (LASER-ART); ART alone does not lead to a cure. The major challenge of HIV eradication is viral latency. Latency Reversal Agents (LRAs) show promise as a possible means to eradicate HIV-1 from the body. It has become evident that complete eradication will need to include combinations of various effective therapeutic strategies such as LASER-ART, LRAs, and gene editing. Review of the current literature indicates the most promising HIV eradication strategy appears to be LASER-ART in conjunction with viral and receptor gene modifications via the CRISPR/Cas9 system.

Keywords Macrophages · Lentiviruses · HIV · Antiretroviral therapy · Latency reversal agents · CRISPR/Cas9 · Eradication

Introduction

Macrophages, one of the most abundant populations of leukocytes in the body, are mesoderm-derived hematopoietic cells. The majority of tissue-resident macrophages are derived from the primitive yolk sac, developing without monocyte intermediates (Mowat et al. 2017; Varol et al. 2015). Macrophages migrate from the yolk sac prior to birth and throughout life self-renew their respective tissue-specific pools. Additionally, adult bone-marrow-derived monocytes aid in replenishing tissue-resident macrophages within the gastrointestinal tract and dermis (Ginhoux and Jung 2014). Macrophages are characterized by avid phagocytosis to clear debris associated with both tissue development and inflammatory processes (Varol et al. 2015).

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Resident tissue-specific macrophages are incredibly diverse with the ability to adapt to environmental cues allowing for shifts in their functional state (Wynn et al. 2013). Categorical systems have been applied to functional phenotypes of macrophages; however, all fall short by oversimplifying the heterogeneous nature of this cell type (Mowat et al. 2017). A recent model explains resident tissue macrophages are acted upon by niche specific transcription factors that program the macrophage to the specific needs of each tissue environment (T'Jonck et al. 2018). Transcription factors elicit an ongoing process allowing for various phenotypic states within tissue compartments (Collin and Bigley 2018). Additionally, rather than the previously suggested M1 (pro-inflammatory) and M2 (anti-inflammatory) model, there is likely a spectrum of phenotypes allowing for intermediate cells with intermediate potentials (Collin and Bigley 2018). This idea is further supported by the Immunological Genome Project that discovered minimal overlap of high transcriptional diversity amongst macrophages (Wynn et al. 2013).

Macrophage Polarization

Within tissue compartments, macrophages function as the first line of defense against pathogen invaders. As mentioned

above, tissue-specific macrophages are directed by transcription factors within their individual tissue environments/niches to polarize towards different phenotypic states (T'Jonck et al. 2018). Phenotypic states have been categorized based on cell surface markers, cytokine production, and phagocytic activity (Moghaddam et al. 2018). The classic division of macrophage phenotype, while acknowledged to be an oversimplification, divides macrophage responses into pro-inflammatory and anti-inflammatory. Classically activated or proinflammatory macrophages are the M1 phenotype. M1 polarization occurs following acute tissue injury or infection. Interferon-γ (IFN-γ) and CD40 ligands, LPS, and/or GM-CSF stimulate M1 polarization. The process of M1 polarization regulates gene expression of cytokine receptors (IL2RA, IL6R, and IL15R), cell marker activation (CD38, CD69, and CD97), and cell adhesion molecules (ICAM1, Integrin α L, and mucin 1). Once activated M1 macrophages secrete nitric oxide and numerous proinflammatory cytokines including TNF-α, and IL-β (Wynn et al. 2013). M1 polarization increases the destruction of engulfed pathogens (Essandoh et al. 2016).

In contrast to the classical activation pathway, alternative M2 polarization is more complex. M2 polarization is subdivided into four additional subgroups (M2a, M2b, M2c, and M2d) based on gene expression profiles. Specific sets of cytokines, immune complexes, and Toll-like receptor ligands stimulate the polarization of each subgroup. The M2 phenotype is for the most part anti-inflammatory, playing an integral role in phagocytosis of debris and tissue repair. Molecular pathways of M1/M2 are covered extensively in the following review articles (Moghaddam et al. 2018; Sang et al. 2015).

Macrophages and Viruses

Inevitably, when antigens enter the body, tissue-resident macrophages serve as a vital barrier to tissue infection. Macrophages become M1 polarized in response to surface antigens, phagocytose pathogens and elicit an appropriate immune response via cytokine signaling of other leukocyte subsets. Similarly, with a proportion of viruses, macrophages phagocytose infected cells, remove cellular debris, present viral antigens to stimulate the adaptive immune response and promote tissue healing and repair through anti-inflammatory signaling. A subset of viruses has evolved to regulate macrophage polarization in a manner that promotes progression of viral infection (Sang et al. 2015). By regulating the macrophage phenotype, viruses can "hijack" macrophage signaling to optimize cell permissiveness, promote continued tissue inflammation, and downregulate tissue repair (Sang et al. 2015). Upregulation of the M1 proinflammatory phenotype promotes the spread of a virus and increased tissue damage. When the normal polarization cascade is disrupted, an "over-inflamed" M1 state can ensue leading to the destruction of macrophages, rendering them unable to clear viral infections (Sang et al. 2015).

Within tissue compartments, macrophages perform surveillance functions to attenuate damage by foreign invaders. Surveillance consists of numerous endocytic processes that engulf and remove invading pathogens (Mercer and Greber 2013). In contrast to macrophage-mediated endocytosis, some viruses target macrophages to evade the immune system and downregulate an effective immune response. Some viruses have evolved to evade the mononuclear phagocytic system (MPS) by undergoing adaptations to remain functional in the presence of activated macrophages as well as modulate macrophage activation via macrophage depletion, virus-mediated inflammatory and cytokine responses, modulation of type I IFN signaling, IL-10 production, and use of miRNA signaling pathways (Sang et al. 2015).

Macrophages and Lentiviruses

Lentiviruses, a type of retrovirus, are species-specific, single-stranded RNA viruses that infect domestic animals, non-human primates, and humans. Rare exceptions to species-specificity occur, as with the feline immunodeficiency virus which infects predominantly felines; however is also able to infect non-human primates as well as cultured human leukocytes (Power 2018). Lentiviruses can be macrophage-tropic and form latent infections using macrophages as viral reservoirs (Narayan and Clements 1989). The lentiviruses infect and replicate within macrophages leading to primary clinical disease affecting the brain, lungs, and lymph nodes. The ability of lentiviruses to infiltrate the CNS is a key feature of the viral pathogenesis. Lentiviral infection of the CNS compartment not only serves as an important reservoir for viral rebound but also can lead to a wide spectrum of neurocognitive dysfunctions. In contrast to lentiviral T cell interactions, it is accepted that macrophages are resistant to lentiviral cytopathic effects (Reynoso et al. 2012). This lack of cytopathic effect leads to long-lived, lentiviral-infected macrophages which release abundant secretory products shown to increase tissue damage not limited to but including neurotoxicity (Meltzer et al. 1990). A better understanding of the role the macrophage plays in lentiviral pathogenesis regarding cell adhesion and entry, macrophage modulation, and ultimately virus eradication will aid in the advancement towards a cure.

Lentiviruses can be divided into non-immunosuppressive, such as bovine immunodeficiency virus (BIV), caprine arthritis-encephalitis virus (CAEV), maedi-visna virus (MVV), and equine infectious anemia virus (EIAV), and immunosuppressive, including feline immunodeficiency virus (FIV), simian immunodeficiency virus (SIV), and human immunodeficiency virus (HIV) (Mamik et al. 2016; Power 2018). Naturally occurring lentivirus infections in domestic

species can serve as important models to better understand pathogenesis as well as effective treatment regimens in regards to HIV. HIV-1 remains to date one of the most extensively studied viral infections. Even with the extensive efforts employed to better understand disease prevention, treatment, and finding a cure, HIV remains a global health crisis.

In the following sections, review of the various naturally occurring animal models of lentiviruses including contributions to pathogenesis as well as the efficiency of therapeutics pertaining to macrophages will be discussed. An in-depth discussion of current and future HIV therapeutics will follow. Table 1 lists lentiviruses and their corresponding species-specificity, cell tropism, accessory proteins, and references.

Bovine immunodeficiency virus

Bovine immunodeficiency virus was identified in Louisiana in 1969 at Louisiana State University in a 9-year-old, Holstein cow. Today, BIV has been identified in cattle worldwide. BIV causes persistent viral infection; however, has not been directly linked to a specific disease. Clinical manifestations described in association with BIV include lymphadenopathy, CNS lesions, decreased milk yield, and bovine paraplegic syndrome. Unlike HIV, BIV is considered to have a non-pathogenic nature which allows for lentiviral research to better understand the pathogenesis and effective treatment with very little biosafety risk. BIV antiserum and capsid antigen have been shown to cross-react with EIAV, SIV, and HIV-1. Cross-reactivity of these four viruses is due to a highly conserved p10 capsid protein. Interestingly, BIV has the most complex genome organization of all the lentiviruses (Bhatia et al. 2013). Like some strains of HIV, BIV has been shown to have strong neurotropism. One study using PCR and PCR-*in situ* hybridization showed BIV proviral DNA within a wide range of cells including not only microglia and macrophages within the brain but also neurons and brain microvascular endothelial cells (Zhang et al. 1997). Of note, BIV, HIV-1, HIV-2, SIV, and EIAV share similar *tat* protein function which includes binding of TAR at the 5' end of viral RNA which then interacts with positive transcription elongation factor beta (P-TEFβ). Hexamethylene bisacetamide (HMBA)-inducible protein 1 (HEXIM1), a component of inactive P-TEFβ, plays an integral role in Tat-mediated viral transcription activation. Bovine HEXIM1 (BHEXIM1) and human HEXIM1 (HHEXIM1) are 80.5% homologous in their amino acid sequences, with highly conserved central and C-terminal regions (Guo et al. 2013). Overall, the BIV model has been somewhat underutilized and could provide a valuable means to explore lentiviral specific genes and how they affect viral replication (Bhatia et al. 2013). As far as treatment of BIV, the literature is markedly sparse and appears BIV-related disease manifestations are treated on a symptomatic basis.

Small ruminant lentiviruses (CAEV and MVV)

CAEV and MVV strains represent a spectrum of lentiviral variants that can infect both caprine and ovine species and therefore have been termed small ruminant lentiviruses

Table 1 Lentiviruses of domestic and primate species

Virus	Host	Target cells	Receptor	Accessory proteins	References
HIV	H	LYM, MO, Mϕ, DC, MG	1° Receptor: CD4 Co-receptors: CCR5 or CXCR4	*tat, rev, vif, vpr, vpu, nef*	(Cavrois et al. 2017; Jakobsdottir et al. 2017; Joseph et al. 2015; Zheng et al. 2017)
SIV	NHP	LYM, Mo, Mϕ, DC, MG	1° Receptor: CD4 Co-receptors: CCR5 or CXCR4	*tat, rev, vif, vpr, vpu, vpx, nef*	(Merino et al. 2017; Micci et al. 2014; Sugimoto et al. 2017)
FIV	F, (N-HP)	LYM, Mo, MΦ, MG, Astro	1° Receptor: CD134 Co-receptor: CXCR4	*rev, vif*	(Eckstrand et al. 2017; Hu et al. 2012; Meeker and Hudson 2017; Meeker et al. 2012; Meltzer et al. 1990; Power 2018)
BIV	B	LYM, Mo, MΦ, FBLC, N, MG, BMEC	CXCR5 suspected	*tat, rev, vif, vpw, vpy, tmx*	(Bhatia et al. 2013; Guo et al. 2013; Zhang et al. 1997, 2014)
SRLV (CAEV and MVV)	O, C	Mo, MΦ, DC, MG, BMEC, LYM, EC of MaGd	Not yet identified; mannose receptor suspected	*rev, vif, vpr*	(Blacklaws 2012; Crespo et al. 2013; Narayan and Clements 1989)
EIAV	E	Mo, MΦ, EndoC, FBLC	Equine lentivirus receptor 1 (ELR1)	*S1(tat)* *S2* *S3(rev)*	(Du et al. 2015; Leroux et al. 2004; Lin et al. 2013; Liu et al. 2017a, b; Ma et al. 2014; Tang et al. 2014)

Host: *H* Human, *NHP* Non-human primate, *E* Equine, *B* Bovine, *C* Caprine, *O* Ovine, *P* Porcine, *F* Feline

Target Cells: *Astro* Astrocyte, *DC* Dendritic cells, *BMEC* Brain microvascular endothelial cells, *EC of MaGd* Epithelial cells of mammary gland, *EndoC* Endothelial cells, *FBLC* Fibroblast-like cells, *LYM* Lymphocyte, *Mϕ* Macrophage, *MG* Microglia, *Mo* Monocyte, *N* Neuron

(SRLV). MVV was discovered in Iceland in the 1950s by Bjorn Sigurdsson in sheep. Dr. Sigurdsson noted a slowly progressive, pneumo-encephalitic disease complex caused by a virus that sustained chronic replication, but at a very slow rate; hence the name lentivirus was given (*lentis* meaning slow in Latin). MVV was the first lentivirus discovered in domestic and primate species (Narayan and Clements 1989). The first cases of CAEV were discovered in 1970, in a herd of young pure-bred dairy goats. SRLVs are macrotropic with cytokines playing a key role in cell tropism. *In vitro* studies of SRLV macrophage tropism show that IFN-γ restricts viral replication by delaying macrophage maturation; however, IL-8, GM-CSF, IL-16, IL-1β, IL-4, and IL-10 appear to promote viral replication (Crespo et al. 2013). Macrophages play an integral role in SRLV pathogenesis by disseminating virus, secreting antigen, and secreting numerous cytokines that regulate the body's immune response to the virus. Interestingly, Crespo et al. showed that macrophages categorized as M2 phenotype are most susceptible to viral infection. SRLV strains induced upregulation of M2 phenotype markers with increased expression of mannose receptor and DC-SIGN. M2 macrophages are long-lived and highly motile; therefore, viral affinity for M2 macrophages is important in the context of both dissemination of virus as well as the formation of a persistent reservoir. Additionally, M2 macrophages have poor B7 expression leading to decreased T cell response. The underlying mechanism of viral affinity for M2 macrophages may be due to use of different entry receptors on the M2 macrophage. Once the M2 tropism is fully understood, therapeutics could potentially target macrophage phenotypic switching towards the M1 phenotype. Such a therapeutic could lead to decreased viral replication and dissemination as well as reduction of reservoir cells (Crespo et al. 2013).

The entry receptors for CAEV and MVV have yet to be identified; however, it appears that the two viruses may use different receptors for entry. Like HIV, mannose receptors and DC-SIGN are thought to be possible entry receptors for MVV. Additionally, Fc receptors on macrophages and dendritic cells have been suggested as a means of viral entry via virion-antibody complex attachment to Fc receptors with cellular internalization and viral infection (Blacklaws 2012). Without knowing the route of cell entry, it is difficult to guide antiviral therapy. Numerous vaccine studies have been attempted to no avail; protective antigens for SRLV have yet to be identified. In fact, the presence of antibodies in goats with CAEV appeared to increase viral internalization (Narayan and Clements 1989). This phenomenon was also noted early in HIV in which the presence of antibodies enhanced macrophage infection (Takeda et al. 1988). As a better understanding of the SRLV viral pathogenesis is developed, this lentiviral model could be beneficial to study monocyte/macrophage infection as SRLV disease progression lacks lymphocyte depletion and subsequent immune dysfunction noted in other lentiviruses (Blacklaws 2012).

Equine Infectious Anemia virus

Unlike other lentiviruses that cause chronic, progressive disease, EIAV has a defined, rapidly progressive disease course (Leroux et al. 2004). EIAV has a narrow cell tropism including tissue macrophages, peripheral blood monocyte-derived macrophages and endothelial cells (Du et al. 2015; Liu et al. 2017a, b). EIAV is unique to other lentiviruses with a single entry receptor, equine lentivirus receptor-1 (ELR1), that utilizes pH-dependent endocytosis for viral entry into macrophages (Jin et al. 2005). In many cases of EIAV, the immune system can control the virus and leads to asymptomatic carriers. This effective immune response makes EIAV a value model to explore key immune factors relating to lentiviruses (Ma et al. 2014).

EIAV is one of only two lentiviruses that has an effective vaccine available for commercial use. Clinical studies reported that the $EIAV_{FDDV13}$ attenuated vaccine is approximately 80% effective at inducing immune protection against EIAV. The vaccine confers protection by blocking the ELR1 receptors with an EIAV SU protein, upregulating secretion of an ELR1-transcript variant identified as soluble ELR1 (sELR1) and increasing expression of IFN-β by toll-like receptor 3 (TLR3) (Lin et al. 2013; Ma et al. 2014). Upregulation of secretion of sELR1 is shown to exert antiviral effects and contribute to viral resistance induced by the vaccine (Lin et al. 2013). Unfortunately, soluble viral receptors were constructed and tested for the HIV-1 receptor (CD4) in the mid to late '90s; sCD4+ treatment Phase I and II clinical trials were unable to show effective antiviral properties *in vivo* (Norkin 1995). Like HIV, TLR3 activation on macrophages has been suggested to block subsequent macrophage infection. In the EIAV model, the upregulation of TLR3 stimulates poly I:C expression which stimulates the release of cytokines such as IFN-γ, known to have strong antiviral effects (Ma et al. 2014).

The macrophage-tropic nature of EIAV allows for extensive analysis of lentiviral infection of macrophages and its effect on viral pathogenesis. Macrophages can express numerous proteins that have important effects on tissue environments throughout the body, therefore understanding how lentiviruses overtake cellular machinery and manipulate protein expression will allow for better therapeutics to block viral pathogenesis and reestablish normal biological processes within tissue compartments. Du et al. completed an extensive proteomic analysis of EIAV using innovative iTRAQ, parallel reaction monitoring (PRM), and Orbitrap technology to elucidate important viral manipulations of protein expression in the EIAV model (Du et al. 2015). Major findings included identification of 210 proteins of interest relating to various biological processes including oxidative phosphorylation, protein folding, RNA splicing, and ubiquitylation. Relating to the oxidative phosphorylation pathway, there was upregulation of two proteins, NADH dehydrogenase 1 alpha

subcomplex 9 (NDUFA9) and NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV2), which are believed to play an important role in mitochondrial ATP production. Viral manipulation of these two proteins to increase ATP provides the increased energy required to support viral replication in macrophages. Mitochondria can also play a role in host defense via mitochondrial antiviral signaling proteins (MAVS) which induce production of reactive oxygen species (ROS). One of the many functions of the COX5B protein is to suppress production of ROS. In EIAV infection, COX5B is upregulated by the virus, strategically suppressing ROS production and antiviral signaling by mitochondria. Intracellular transport is integral to viral replication; therefore, viruses will manipulate cytoskeletal membrane proteins to enhance trafficking of viral components. One such cytoskeletal protein, ROCK1, is associated with intracellular viral transport and was shown to be enhanced by EIAV infection. ROCK1 inhibition in the equine herpesvirus 1 (EHV-1) model has shown a reduction of viral capsid accumulation. Ubiquitylation of proteins is utilized by the body to signal degradation as well as signal transduction, DNA repair, and endocytosis. EIAV significantly altered expression levels of proteins associated with ubiquitylation likely to aid in entry, replication, and egress of the virus. While this study provides extremely valuable data regarding lentiviral macrophage manipulation, functional and mechanistic studies are still required to better understand how protein regulation can be used to eradicate rather than enhance lentiviruses (Leroux et al. 2004).

While the EIAV model is not identical to HIV; it offers unique opportunities to study aspects of lentiviruses in general that are not possible in other models. By comparing the viral pathogenesis, and host response that leads to a more rapidly progressive disease course and host-viral immune control, specific EIAV and HIV variances can be identified and used to enhance viral eradication efforts.

Feline immunodeficiency virus

Like HIV, FIV is characterized by long-lasting viral persistence with progressive immune depletion and neurocognitive dysfunction. Typical transmission of FIV occurs during fighting (biting) via the blood. Transmission can also occur via oral, rectal, vaginal, intramuscular, intravenous, intraperitoneal, subcutaneous, and transplacental exposure. Unlike HIV, FIV uses the transmembrane glycoprotein, CD134 (OX40), as its primary cell surface receptor, in conjunction with the co-stimulatory chemokine receptor CXCR4. Two, cysteine-rich domains, CRD1 and CRD2, on the CD134 receptor are the FIV *env* binding sites. Long-term tracking of FIV viral isolates identified two FIV subsets; CRD2-dependent and CRD2-independent isolates. Similar to HIV-1 CCR5-dependent isolates, CRD2-dependent isolates appear to have increased transmission efficiency (Eckstrand et al. 2017). As shown with HIV, FIV strains can also use heparan sulfate proteoglycans (HSPG) and DC-SIGN as binding receptors to facilitate viral entry (Geijtenbeek et al. 2000; Hu et al. 2012; Jones et al. 2005). Cells permissive to FIV include CD4+ and CD8+ T cells, CD21+ B cells, monocytes, macrophages, microglia, and astrocytes. Astrocytes have CD134 receptors, making them permissible to FIV receptor-mediated viral entry. Astrocytes lack the CD4 receptor making HIV unable to enter via receptor-mediated, CD4/CCR5 viral entry. In regards to HIV infection, the lack of a CD4/CCR5 receptor on astrocytes does not rule out viral infection; viral entry could occur via an alternative route such as non-receptor mediated viral entry. Productive HIV infection of astrocytes is hotly debated; however, astrocyte viral infection has been shown in the FIV model (Power 2018). Like HIV, FIV is detected within the CNS compartment as early as 7 days post-inoculation. While perivascular macrophages and astrocytes within the brain are FIV permissive, FIV appears to preferentially infect ramified microglia. Typical histological changes include widespread gliosis, glial nodules, white matter pallor, and neuronal injury and loss. Associated neuroinflammatory marker manifestations include increases in Iba-1, IL-6, and decreases in MAP-2 and PPAR-γ.

Neurological disease is a major feature of HIV and FIV, and the FIV model provides a useful means to explore viral entry into the brain as well as design novel therapies to inhibit CNS infection. The mode of lentiviral entry into the brain remains to be fully elucidated. Possible viral entry sites include transmigration of free virus or virus within infected leukocytes directly through the blood-brain barrier (BBB) or indirectly through the blood CSF barrier (BCB) via the choroid plexus (CP). Using an *in vitro* cell culture insert system, Hudson et al. showed leukocyte trafficking through the BBB is controlled in varying capacities by brain microvascular endothelial cells (BMEC), astrocytes and microglia. Specifically, astrocytes increase trafficking of peripheral blood mononuclear cells (PBMCs), while microglia appear to block or decrease the transmigration of PBMCs, and presence of FIV increases transmigration of CD8+ T lymphocytes and monocytes into brain (Hudson et al. 2005). An *in vitro* BBB model of FIV showed that migration of cell-free virus through the BBB is minor; however, activated and FIV-infected MYA-1 cells (MYA-1 is an IL-2 dependent cell line established from feline T-lymphoblastoid cells permissible to FIV infection) are able to readily migrate through the BBB. Additionally, migration of the activated and FIV-infected MYA-1 cells is increased when TNF-α was present on either side of the BBB model (Fletcher et al. 2009).

The CP provides a barrier between the peripheral blood and cerebrospinal fluid (CSF) and could represent a site of not only viral replication but trafficking of virus-infected cells into the CSF and peripheral nervous system. Infected T-lymphocytes express CD40-ligand and are known to infiltrate

the CP during lentiviral infection. CD40-ligand present on the lymphocytes possibly stimulates proliferation of macrophages within the CP. An *in vitro* model of feline CP showed a marked proliferation of CP macrophages when exposed to FIV and rhuCD40-ligand. Additionally, supernatants from the CP cultures exposed to FIV led to significant neurotoxicity when added to feline cortical brain cultures, predominantly containing neurons, astrocytes, and small numbers of microglia (Bragg et al. 2002b). Additional studies completed by Meeker et. al, showed CP epithelium supports differentially regulated macrophage, T cell, and monocyte trafficking through the CP and the presence of FIV increases macrophage trafficking from the CP into the cerebral ventricles. CP macrophages have been shown to have relatively low to an undetectable production of FIV RNA; however, when feline CD4+ T-cell are exposed to the infected CP macrophages, a robust T-cell infection is noted (Bragg et al. 2002a). Much is to be gained from continued investigation of the FIV model in regards to trafficking of the virus and virus-infected cells through the BBB and BCB.

A topic of utmost importance in the current era of effective viral suppression via antiretroviral therapy is the presence of viral reservoirs leading to viral rebound in the absence of antiretroviral drugs. For this review, a cellular reservoir will be defined as a cell that contains integrated, replication competent provirus and persists over a long period of time (Eckstrand et al. 2017). The semantics of viral reservoirs remain highly debated in regards to latency. True viral latency refers to a stage of the viral life cycle in which pre- or post-integrated virus is present in a reversibly, nonproductive state. Numerous studies have reported various outcomes regarding the nonproductive versus very low productive nature of infection in microglia and perivascular macrophages within the brain; therefore, the term reservoir versus latent reservoir will be used to avoid this debate.

Viral reservoirs can be categorized in two ways, one by anatomical location and the other by cell type. Anatomical locations that contain viral reservoirs include brain, gut-associated lymphoid tissue (GALT), lymph nodes and the reproductive tract. Specific cell types identified as viral reservoirs include CD4+ T cells, CD8+ T cells, CD21+ T cells, monocytes, macrophages, dendritic cells, microglia, and astrocytes (Eckstrand et al. 2017).

Microglia serve as an important viral reservoir. Microglia self-sustain within the CNS compartment (Ginhoux and Prinz 2015; Gomez Perdiguero et al. 2015; Meeker and Hudson 2017) and are able to sustain viral replication in the absence of other leukocytes (Honeycutt et al. 2016; Meeker and Hudson 2017). Formation of microglial reservoirs is associated with chronic, long-term activation of microglia and perivascular macrophages. A recent review by Meeker et. al, highlighted major neuroinflammatory and neurodegeneration insights concluded from naturally occurring and experimentally-induced FIV models which include i) lentiviral infection has an early and progressive course requiring early therapeutic intervention to protect against lentiviral induced neurodegeneration, ii) low-grade inflammation occurs within the CNS even when patients are on ART, iii) low grade ongoing CNS inflammation has the potential to be reversed, iv) tau pathology is noted in conjunction with long-term FIV-associated neuroinflammation within aged cats, v) lentivirus within the CNS may prime neuronal glutamate receptors making them more susceptible to toxicity, vi) neuronal damage is likely due to gradual calcium accumulation rather than an acute increase, and vii) activation of brain macrophages is the most likely cause of neurotoxicity (Meeker and Hudson 2017). This information allows for the creation of innovative drug therapies that can then be tested within the FIV model to prevent or improve the neurodegenerative effects of the virus.

One such drug therapy is a protease inhibitor, TL-3. Oral administration of TL-3 in FIV-infected cats reduced the viral load during early infection which limited the effect that virus had on the CNS. Even once the drug was removed, FIV-infected cats treated with TL-3 in the early stages of infection did not develop CNS lesions. These findings are indicative that a certain viral threshold is necessary for the virus to cross the BBB. Additionally, FIV-infected cats treated with TL-3 after CNS disease had been initiated showed a reversal of CNS deficits indicating a neuroprotective effect (Huitron-Resendiz et al. 2004).

To address lentiviral induced neuronal damage secondary to calcium dysregulation, a p75 neurotrophin receptor ligand, LM11A-31, was tested on an *in vitro* FIV model. Both HIV and FIV lead to similar changes of neurons including beading of neuronal dendrites, shrinkage of the neuronal cytosol, and an overall reduction in MAP-2 staining. Astrocytes also show signs of degeneration including retraction of astrocytic processes and an increase in a total number of processes. Addition of LM11A-31 to neural cultures at the time of FIV inoculation and continued treatment over a seven-day time course markedly reduced neuronal and astrocytic degeneration. To assess LM11-31's ability to stabilize neuronal calcium levels, an in vitro model was constructed consisting of primary feline neuronal cultures with the addition of medium from infected choroid plexus macrophages. This model accurately recapitulates the rapid increase of intracellular calcium within neurons during lentiviral CNS pathogenesis. The addition of LM11A-31 to this *in vitro* model did not block the acute rise in calcium; however, it did block the gradual calcium increase that is responsible for calcium destabilization. Of note, LM11A-31 did not effectively decrease viral production (Meeker et al. 2012).

Intranasal insulin treatment has been tested *in vivo* in the FIV model to assess if insulin can be neuroprotective and decrease viral expression in the brain. Insulin receptors are

present on neurons and glia. While insulin receptor expression appears unaffected in the presence of both HIV or FIV, insulin supplementation was shown to decrease inflammatory gene expression, reduce HIV-1 p24 levels and increase PPAR-γ expression in an *in vitro* HIV-infected primary human microglia culture model. FIV-infected cats were given daily intranasal insulin treatments for 6 weeks. Insulin treatment reduced inflammatory gene expression (CXCL10 and IL-6), reduced FIV RNA detection in the brain, and increased PPAR-γ expression. Postmortem immunohistochemical (IHC) evaluation of the brain of FIV-infected cats post-intranasal insulin therapy showed a reduction of Iba-1, IL-6 and restoration of PPAR-γ and MAP-2 staining compared to untreated FIV-infected cats. Additionally, these postmortem IHC anti-inflammatory changes are corroborated with antemortem behavioral testing that showed a decrease in neurological deficits of FIV-infected treated versus untreated cats (Mamik et al. 2016).

While numerous antiretroviral drugs available to humans have been tested on and shown efficacy in the FIV model; financial and logistical constraints restrict the life-long use of ART as a realistic treatment for FIV infected cats. A recent review outlined the therapeutic recommendations for FIV-infected cats with the recommendation of nucleoside analog reverse transcription inhibitor, Zidovudine (AZT), to be used only when stomatitis, neurological signs, or recurring infections were present in associated with FIV infection (Hartmann 2015).

Rather than long-term treatment, prevention is a more realistic goal in cats. Vaccines have been extensively studied as a possible means for complete prevention of lentiviruses. Five isolates of FIV (A-E) have been identified based on viral isolation throughout the world; slightly less than the prevailing seven HIV isolates that have been identified. Ideal vaccine effectiveness would confer protection against all viral strains of a given host; however, this proves to be a very daunting task. To confer viral protection, an effective vaccine needs to produce high levels of multiple types of anti-FIV T cell responses; however, much of the current research has been focused on B-cell or antibody-based vaccines. In 2002, a dual-subtype FIV vaccine, Fel-O-Vax, was released in the United States. Fel-O-Vax was constructed using infected whole cells (IWC) of two inactivated subunits of FIV-A and FIV-D with an FD-1 adjuvant. The initial vaccine studies show a protection rate of 56%. While 56% seems like an unacceptable prevention efficiency for a vaccine, it is still well above even the most effective HIV vaccine that has been tested which was 31.2% effective. One major hurdle of producing a productive FIV or HIV vaccine is optimizing the selection of viral Gag and Pol epitopes that are highly conserved and protective while avoiding selection of non-protective, replication enhancing epitopes. Optimal viral epitopes for a vaccine will need to balance induction of broadly neutralizing antibodies to the virus, but not to the specific vaccine epitopes rendering the vaccine inactive. Additionally, the vaccine will need to induce both a mild T cell activation as well as broadly neutralizing antibodies without stimulating excessive T cell activation that enhances viral replication (Sahay and Yamamoto 2018).

HIV/SIV

Unlike the previously discussed macrophage-tropic lentiviruses, HIV and SIV, have pronounced macrophage and T cell tropism. Discovered in the early 1980s, HIV remains to date a worldwide health crisis. Introduction of antiretroviral therapy in the 1990s drastically increased the life expectancy of individuals infected with HIV within the Western world; however, many underdeveloped areas of the world still do not have an adequate supply of antiretroviral drugs to treat and control HIV. The remaining text will discuss HIV pathogenesis and the current and future therapeutic strategies to control and eradicate HIV with a focus on the macrophage as a critical reservoir.

HIV Life Cycle (Reference Fig. 1)

HIV Entry

The CD4 entry receptor along with co-receptors CCR5 and CXCR4 can bind to HIV-1 viral envelope protein, gp120 (Rodrigues et al. 2017) (Fig. 1a). In comparison to T lymphocytes, macrophages have a significantly lower expression of CD4 receptors. Specific strains of HIV-1 have evolved with increased affinity for CD4, allowing effective receptor binding of the virus to macrophages with low CD4 expression, termed M-tropic strains. Additional strains have also evolved that have a predilection for CCR5, classified as R5-tropic viruses, as well as CXCR4, classified as X4-tropic viruses. An abundance of surface receptors and proteins have been identified that aid in the attachment and absorption of HIV-1 including lectin-like receptors, integrins, syndecans, proteoglycans, and cytokine/chemokine receptors (CCR1, CCR2, CCR2b, CCR8, CXCR6, CX3CR1, formyl peptide receptor 1, G protein-coupled receptor 1, GPR15, apelin receptor, and CCBP2) (Rodrigues et al. 2017; Woodham et al. 2016). The promiscuous and intricate nature of HIV attachment and entry make therapeutics to block viral entry a formidable task.

HIV Fusion

Once the virus is bound to the CD4 receptor, the envelope glycoprotein 120 (gp120) undergoes a conformational change, into a six-helix bundle structure, leading to destabilization of the envelope spikes generating gp-41-mediated

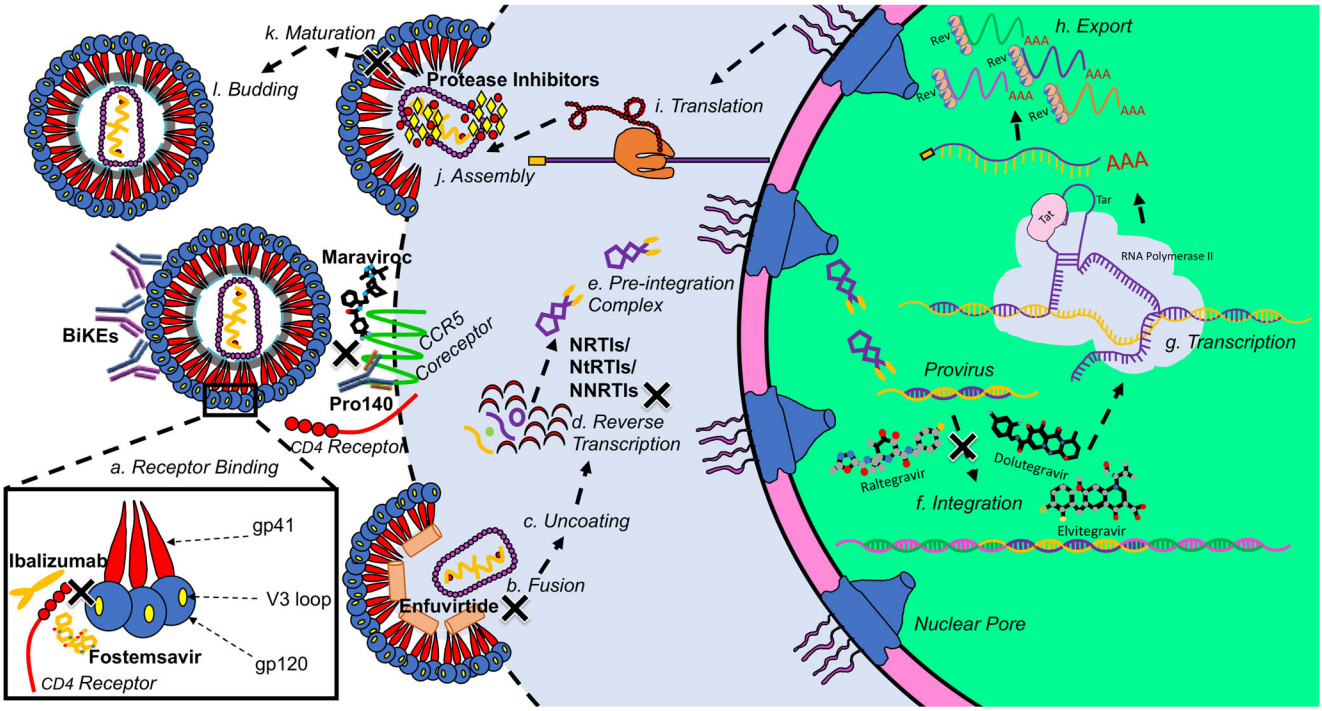

Fig. 1 HIV Life Cycle Within CD4+/CCR5+ Cell and Antiretroviral Drugs (Site and Mode of Action). The CD4 entry receptor along with co-receptor CCR5 binds to HIV-1 gp120 (**a**), once bound gp120 undergoes a conformational change generating gp-41-mediated membrane fusion (**b**). Entry inhibitors block conformational changes post CD4 attachment (Fostemsavir and Ibalizumab) or bind and neutralize unattached virus (BiKEs). HIV co-receptor CCR5 can be blocked via a CCR5 inhibitor (Maraviroc and Pro140). Upon viral fusion, the virus is uncoated and the nucleocapsid is released into the cytosol (**c**). Fusion inhibitors bind to the prehairpin intermediate that bridges the viral and cell membranes and inhibits the formation of the viral six-helix bundle structure necessary for membrane fusion (Enfuvirtide). Reverse transcription is initiated producing linear cDNA products (**d**). NRTIs, NtRTIs and NNRTIs block reverse transcription preventing conversion of viral RNA into DNA. If reverse transcription is not blocked, a reverse transcription complex transforms into the pre-integration complex and translocates through a nuclear pore (**e**). HIV-1 provirus is integrated into host DNA (**f**). Integrase strand inhibitors (e.g. Raltegravir, Dolutegravir, and Elvitegravir) selectively interfere with viral strand transfer whereby preventing transfer of proviral DNA into host chromosomal DNA. Once proviral DNA is integrated, viral DNA is then expressed by host cell transcription and RNA processing (**g**). HIV Tat protein directs P-TEFb to nascent RNA polymerases stimulating transcription elongation. Transcripts are exported back to the cytosol through the nuclear pore via HIV Rev protein complexes (**h**). The HIV mRNA undergoes protein synthesis via translation (**i**). Viral proteins (Gag and GagPol precursor proteins) are led to the host cell plasma membrane for viral assembly (**j**). Protease inhibitors block HIV protease enzyme, ultimately blocking maturation of viral particles (**k**). ESCRT mediated budding releases the Gag polyprotein precursor (**l**)

membrane fusion (Duncan and Sattentau 2011; Kondo et al. 2015) (Fig. 1b). Viral fusion is believed to incorporate the macrophage's endocytic machinery as well as dynamin to aid with actin remodeling and membrane-bending protein associations. Use of the host's cell machinery allows the virus to optimize the host's energy to expand a fusion pore and allow uncoating of the HIV-1 virion into the macrophage core (Miyauchi et al. 2009) (Fig. 1c). Viral fusion takes place via an endocytic pathway; however, less common plasma membrane fusion can also occur (Jakobsdottir et al. 2017; Kondo et al. 2015). Cell-to-cell spread of virus allows for increased amounts of proviral DNA transferred to target cells, as well as a means to bypass the extracellular space avoiding interaction with antiretroviral therapeutics (Costiniuk and Jenabian 2014). Cell-to-cell viral spread can be both receptor-dependent or receptor-independent, with the latter suggested via nanotubules allowing for the intact virus to enter the cell cytoplasm and remain this way for undetermined lengths of time (Costiniuk and Jenabian 2014). Macrophages can also become infected via receptor-independent mechanisms such as cell to cell transfer via capture of infected CD4+ T cells (Baxter et al. 2014) (Fig. 1). It remains a hotly debated topic whether the presence of viral RNA and DNA identified within macrophages represents active infection or simply retained, compartmentalized HIV+, CD4+ T-cell debris.

HIV Reverse Transcription

Upon viral fusion, the nucleocapsid is released into the cytosol and a reverse transcription complex (RTC) is formed. The RTC consists of a capsid protein (CA) cone encompassing viral reverse transcriptase (RT), host tRNA, the primer binding site (PBS), viral nucleocapsid protein (NC), viral integrase (IN), viral matrix protein (MA), and Vpr (Puglisi and Puglisi 2011; Warren et al. 2009). Reverse transcription is initiated within hours of infection by viral RT binding to host tRNA;

HIV uses tRNA strand Lys3. Reverse transcription produces linear cDNA products with long-terminal repeats (LTR) on both ends of the DNA strand (Hamid et al. 2017) (Fig. 1d).

HIV Integration

Viral IN then engages the LTR regions of the newly formed linear cDNA product and processes them adjacent to an invariant CA dinucleotide (Krishnan and Engelman 2012). This yields CA_{OH} 3'-hydroxyl groups that will act as nucleophiles for DNA transfer, marking the transformation of the RTC into a pre-integration complex (PIC) (Hu and Hughes 2012; Krishnan and Engelman 2012; Warren et al. 2009) (Fig. 1e). PIC translocates into the cell nucleus through a nuclear pore. IN mediates 3' viral DNA cuts that are joined with host DNA 5'-ends to form a DNA recombination intermediate. Host cell enzymes repair the viral DNA 5'-ends that ultimately form the integrated provirus within the host DNA (Krishnan and Engelman 2012) (Fig. 1f).

Pre-Integration Latency

As mentioned above, the linear cDNA produced by RT can integrate and be transcribed into viral mRNA. The circular cDNA can undergo an abortive integration process and accumulate within the infected cell, predominantly within the nucleus (Hamid et al. 2017) (Fig. 1d). Circular cDNA can have multiple forms such as 2-LTR circles formed by non-homologous end joining (NHEJ), or 1-LTR circles formed by defective RT, re-arrangement, or homologous recombination (Farnet and Haseltine 1991; Hamid et al. 2017). Integration is a key step in the viral pathogenesis of HIV; however, the presence of unintegrated viral DNA in macrophages may also contribute significantly to viral pathogenesis. Macrophages do not divide and are able to maintain high levels of unintegrated HIV DNA for extended periods of time making them well-suited as viral reservoirs (Hamid et al. 2017; Kumar et al. 2015; 2014). While the role of unintegrated DNA remains to be determined, pre-integration virus is able to selectively express gene proteins and produce some rescuable virions acting as HIV reservoirs that are able to evade current antiviral therapies (Hamid et al. 2017; Zhang et al. 2017).

Post-Integration Latency

After integration has occurred and before or even during transcription events, post-integration latency can occur due to HIV-1 gene expression silencing including epigenetic gene silencing, transcription gene silencing, and post-transcriptional gene silencing (Kumar et al. 2015, 2014) (Fig. 1f and g). HIV-1 viral DNA preferentially integrates into active portions of the host genome (euchromatin); however, when the virus integrates into modified or suppressed areas of the host genome (heterochromatin) there is more frequent transcriptional interference and gene silencing (Kumar et al. 2015, 2014). Host transcription factors can also impact latency. Histone deacetylase (HDAC) 1 is recruited through an SP1/c-Myc pathway and induces chromatin remodeling leading to HIV-1 gene suppression (Kumar et al. 2015, 2014). Additionally, HDAC1 can also be recruited by other factors including C-promoter binding factor-1 (CBF-1), COUP-TF interacting protein 2 (CTIP2), YY1, and LSF (Kumar et al. 2015, 2014). Other chromatin-modifying enzymes include histone acetyltransferase (HAT), DNA methyltransferases (DNMT), and histone methyltransferases (HMT).

Viral components nuc-0 and nuc-1 also play an integral role in post-integration latency. Latency can occur when both nucleosomes are located in the HIV-1 5'-LTR region, with nuc-1 significantly contributing to blockage of RNA polymerase II (Pol II) and transcriptional elongation (Darcis et al. 2017)(Fig. 1g).

HIV Transcription and Translation

Once viral DNA is integrated into the host chromatin, it is expressed by host cell transcription, RNA processing, and translation. Two HIV viral proteins, Tat and Rev, control HIV-1 gene expression. HIV-1 Tat protein directs P-TEFb to nascent RNA polymerases stimulating transcription elongation (Karn and Stoltzfus 2012) (Fig. 1g). Spliced viral mRNAs are processed similarly to host mRNA with 5'cap structure and 3' poly(A) tails (Burugu et al. 2014; Ohlmann et al. 2014) (Fig. 1g). Alternative splicing produces various groups of transcripts including full-length transcripts, singly spliced transcripts, and fully spliced transcripts. HIV-1 Rev protein binds to mRNAs forming a protein complex necessary for transport of viral mRNAs from the nucleus back to the cytoplasm for protein translation (Karn and Stoltzfus 2012) (Fig. 1h). Using host cell machinery, HIV mRNA undergoes protein synthesis (Fig. 1i). Technological advancements have allowed researchers to better evaluate post-integration processing on a single cell basis, showing that not all cells contribute to infection/persistence in the same capacity (Baxter et al. 2018). Subsets of infected cells have been identified including i) silent reservoirs containing integrated HIV-1 proviral DNA ii) transcription competent virus producing viral mRNA iii) translation competent virus producing viral proteins iv) replication competent virus producing infectious viral particles (Baxter et al. 2018). Understanding the interactions between the various subsets of cellular infection and persistence will be crucial to viral eradication.

HIV Assembly

Once Gag precursor protein and GagPol precursor protein are synthesized, the MA domain leads Gag to the host cell plasma

membrane for viral incorporation of Env glycoproteins (Fig. 1j) (Lippincott-Schwartz et al. 2017). The cellular Endosomal Sorting Complex Required for Transport (ESCRT) apparatus is recruited to the plasma membrane inner leaflet (assembly site) via p6 domain which drives membrane fission for particle release (Lippincott-Schwartz et al. 2017).

HIV Budding

ESCRT mediated budding releases the Gag polyprotein precursor (Fig. 1k). Viral protease then cleaves sites at both the Gag and GagPol polyprotein precursors initiating HIV-1 maturation and CA morphological shifting into a hexameric lattice composed of 12 pentamers (Lippincott-Schwartz et al. 2017). The newly produced mature virion is now able to continue productive infection.

Cell Fate Post-Infection

Cellular response to HIV-1 infection is largely determined by cell origin. Additionally, viral accessory factors such as *tat, rev, vif, vpr, vpu, and nef* are essential for viral replication. These accessory factors manipulate several cellular pathways, counteract innate and adaptive immune defenses, and antagonize antiviral factors both by degradation and downregulation of expression (Sauter and Kirchhoff 2018). Soluble proteins such as Gp120, Tat, Nef, and Vpr have a direct cytotoxic effect on T-cells (Cummins and Badley 2014). In particular, Vpr has been shown to cause G_2 cell cycle arrest and apoptosis in T cells (Andersen et al. 2008). T-cell death can also occur via "bystander" effect in which aberrant apoptosis is induced by over-expression of death ligands as well as activation-induced death due to a chronic state of hyperimmune activation in response to HIV-1 infection (Cummins and Badley 2014). A hallmark of the immunosuppressive lentiviruses (HIV, SIV, and FIV) is CD4 T cell apoptosis and CD4/CD8 T cell inversion (Sahay and Yamamoto 2018).

Similar to T-cells, Vpr protein induces cell cycle arrest at G_2 in macrophages including microglia (Casey Klockow et al. 2013; Chen et al. 2018; Igarashi 2001; Pujantell et al. 2016). Vpr also binds to Dicer and Cul4 forming a protein complex leading to Dicer depletion (Casey Klockow et al. 2013). Dicer plays a role in the formation of miRNAs to restrict HIV replication. When Dicer is depleted, this removes the miRNA restriction and enhances HIV-1 infection (Casey Klockow et al. 2013). In contrast to T-cells, macrophages are refractory to the cytopathic effects of HIV-1 viral components (Igarashi 2001). Therefore, macrophage machinery becomes "hijacked" by the virus, but remains a long-lived, viral reservoir. It has also been shown that Vpr and Vif proteins are able to block IFN I/III gene expression via a TBK1 pathway (Harman et al. 2015). Production of type I IFNs stimulate IFN-stimulated genes that have a potent antiviral response (Soper et al. 2018). HIV-1 blockage of IFN pathways ultimately dampens that host's ability to identify and remove virally infected cells.

Current Antiretroviral Therapies (Reference Fig. 1 and Table 2)

The discovery of Antiretroviral Therapies (ART) in the 1980s revolutionized HIV treatment and created a large subpopulation of HIV infected individuals that would need lifelong intensive medical therapy. The goal of ART is to maximally suppress plasma HIV RNA, restore and preserve immunologic function, reduce HIV-associated morbidity, prolong duration and quality of life, and prevent HIV transmission (Panel on Antiretroviral Guidelines for Adults and Adolescents 2018). Monotherapy of any retroviral drug should not be used as it increases the risk of treatment failure and drug resistance. The current ART recommendation is combination of two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) with an added third active ARV drug from the following drug classes: Integrase Strand Transfer Inhibitor (ISTI), Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) or Protease Inhibitor (PI) with a boosting agent (Panel on Antiretroviral Guidelines for Adults and Adolescents 2018). Some of the currently effective ART drugs, as well as those in the clinical trial pipeline, will be briefly summarized.

Entry Inhibitors

Entry inhibitors, targeting the gp120-CD4+ receptor interaction, are being developed for treatment-experienced HIV-1 infected patient populations (Meanwell et al. 2018). Fostemsavir (BMS-663068), a drug currently in phase III clinical trials, binds viral gp120 protein at the inner/outer domain interface and stabilizes the gp120 conformation, ultimately blocking the conformational shift necessary for binding of the CD4 receptor (Meanwell et al. 2018) (Fig. 1a). Fostemsavir is believed to have great potential considering the limited possibility of resistance development due to the highly conserved and integral nature of the gp120 viral protein (Meanwell et al. 2018).

Another type of attachment inhibitor is an anti-CD4 IgG4 monoclonal antibody. Currently in phase III clinical trial, Ibalizumab (iMab, TNX-355) binds the CD4+ receptor at the interface of Domain 1 and 2 (Fig. 1a). The exact mechanism in which iMab is able to neutralize HIV-1 is poorly understood; however, it is believed that the drug's binding site is in close proximity with the binding site of viral protein gp120 thereby inhibiting the second binding interface between gp120 and CD4+ (Duan et al. 2017; Kaplon and Reichert 2018; Pace and Markowitz 2015; Reichert 2017; Xu et al. 2017; Zhang et al. 2016). iMab's binding site does not inhibit major histocompatibility complex (MHC) class II molecules thus it does

Table 2 Antiretroviral drugs

Drug	Mode of action	Current status	References
Entry Inhibitors			
Fostemsavir (BMS-663068)	Blocks entry via stabilization of conformation	Phase III Clinical Trial	(Meanwell et al. 2018)
Ibalizumab (TNX-355)	Inhibits normal binding of virus to receptor/coreceptor	Phase III Clinical Trial	(Duan et al. 2017; Kaplon and Reichert 2018; Pace and Markowitz 2015; Reichert 2017; Xu et al. 2017; Zhang et al. 2016)
LSEVh-LS-F	Binds and neutralizes infected cells; ADCC	*In vitro*	(Bardhi et al. 2017; Li et al. 2017)
Maraviroc	Inhibits CCR5 receptor binding	FDA Approved	(Elsa et al. 2018; Shah and Savjani 2018)
B07	CCR5 coreceptor inhibitor/spermicidal activity	*In vivo* (Animal Model: Rabbit)/*Ex vivo* human sperm	(Yang et al. 2018)
Pro140 (PA14)	Monoclonal antibody entry inhibitor	Phase IIb/III Clinical Trial	(Kaplon and Reichert 2018; Olson and Jacobson 2009; Pace and Markowitz 2015; Xu et al. 2017)
Fusion Inhibitors			
Enfuvirtide (T20)	Inhibits formation of viral six-helix bundle structure necessary for membrane fusion	FDA Approved	(Ding et al. 2017)
LP-40 and LP-46	Inhibits formation of viral six-helix bundle structure necessary for membrane fusion	*In vitro*	(Zhu et al. 2018)
ISTIs			
Dolutegravir	Binds to active site of HIV integrase and blocks strand transfer step	FDA Approved	(Meintjes et al. 2017; Messiaen et al. 2013; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018)
Elvitegravir and Raltegravir	Inhibits HIV-1 integrase enzyme	FDA Approved	(Meintjes et al. 2017; Messiaen et al. 2013; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018)
NRTIs/NtRTIs			
Tenofovir and Lamivudine	Competitively inhibits HIV-1 reverse transcriptase	FDA Approved	(Panel on Antiretroviral Guidelines for Adults and Adolescents 2018)
Emtricitabine	Inhibits HIV-1 reverse transcriptase	FDA Approved	(Panel on Antiretroviral Guidelines for Adults and Adolescents 2018)
Abacavir	Inhibits HIV-1 reverse transcriptase and DNA transcription	FDA Approved	(Panel on Antiretroviral Guidelines for Adults and Adolescents 2018)
NNRTIs			
Delavirdine, Efavirenz, Etravirine, Nevirapine, Rilpivirine	Binds to reverse transcriptase and blocks transcription	FDA Approved	(Panel on Antiretroviral Guidelines for Adults and Adolescents 2018)
Protease Inhibitors			
Darunavir	Inhibits HIV protease enzyme by forming a complex that prevents cleavage of gap-pol polyproteins	FDA Approved	(Meintjes et al. 2017)
Atazanavir	Binds to protease active site and inhibits the protease enzyme	FDA Approved	(Meintjes et al. 2017)

not interfere with normal immune function (Duan et al. 2017; Kaplon and Reichert 2018; Pace and Markowitz 2015; Reichert 2017; Xu et al. 2017; Zhang et al. 2016).

Bispecific Killer Cell Engagers (BiKEs), while still in the developmental stage, have been shown to bind and neutralize HIV-1 infected cells (Fig. 1a). Additionally,

BiKEs are also able to guide natural killer (NK) cells to HIV-1 infected cells and mediate killing, termed Antibody-Dependent Cellular Cytotoxicity (ADCC) (Li et al. 2017). BiKEs have the unique feature of one-domain mediated CD4 binding, therefore suggesting the potential to neutralize and kill all HIV-1 isolates, reducing the risk of resistance via escape viruses (Li et al. 2017). LSEVh-LS-F, a BiKE therapeutic has been tested *in vitro,* using human peripheral blood mononuclear cells (PBMC), as well as *in vivo*, both in humanized mice and macaque animal models. Studies showed LSEVh-LS-F has a potent antiretroviral activity to both neutralize and eliminate HIV-1 infected cells (Bardhi et al. 2017). The major drawback of this drug is a short half-life, pharmacodynamic evaluation showed a drug concentration above 100ug/ml at day 2 and below 1ug/ml at day 7, which dictates its use as a short, intensive course of therapy in conjunction with other therapeutic modalities such as latency-reversal agents (Bardhi et al. 2017).

Viruses using the CCR5 co-receptor have high transmission success (Keele et al. 2008; Elsa et al. 2018; Salazar-Gonzalez et al. 2009). A mutation of the CCR5 chemokine receptor, consisting of a 32-base pair deletion within the coding region was discovered in 1996 (Liu et al. 2017a, b; Martin 1998; Samson et al. 1996). This mutation generates severely truncated proteins that cannot be detected at the cell surface (Liu et al. 2017a, b). Patients that are homozygous for the CCR5 delta 32 mutation do not support membrane fusion of M-tropic strains of HIV-1 (Samson et al. 1996). Interestingly, there are no obvious phenotypic manifestations of the mutation other than resistance to pathogens using the CCR5 receptor for entry. The discovery of CCR5 delta 32 revealed the importance of the CCR5 chemokine receptor for macrophage infection, making it a potential target for therapeutic development against HIV-1. CCR5 inhibitors, anti-CCR5 monoclonal antibodies, and an antibody-like CCR5 inhibitor have been developed to block HIV entry into permissible cells. Nucleic acid-based approaches to block the CCR5 receptor are still in the development stage.

CCR5 inhibitors interact with amino acid residues within the CCR5 binding pockets (Shah and Savjani 2018). Five CCR5 inhibitors have been developed and are listed in Table 2. To date only one, Maraviroc, has been licensed for use treating patients with HIV-1 infection (Elsa et al. 2018; Shah and Savjani 2018). Maraviroc interacts with amino acid residues Lys26 and Lys191, as well as a negatively charged carboxyl group of a Glu283 residue to inhibit HIV-1 from binding with the CCR5 co-receptor (Fig. 1a). The five different CCR5 antagonist drugs use different amino acid residues to achieve CCR5 inhibition. The major drawback of CCR5 antagonists is the development of resistance to the therapeutic. Resistance can develop due to clinical bypass of specific receptor binding by the virus (Shah and Savjani 2018). Specific CCR5 point mutations have been shown to have variable effects on the efficacy of CCR5 antagonists. Additional CCR5 antagonists continue to be developed to circumvent the issue of resistance (Shah and Savjani 2018) Table 3.

One of these drugs currently being developed, B07, is a dual action CCR5 inhibitor that protects from HIV-1 CCR5 co-receptor mediated cell entry as well as possessing potent spermicidal activity (Yang et al. 2018). Initial studies show B07 has potent CCR5 antagonism as well as the ability to irreversibly immobilize sperm. While lacking efficacy to reduce fertilization or implantation in female rabbits, B07 did effectively block sperm mobilization to reach and fertilize oocytes (Yang et al. 2018). B07 would serve as both a vaginal spermicide and microbicide conferring protecting against both unplanned pregnancy and HIV-1 infection (Yang et al. 2018).

Several monoclonal CCR5 co-receptor antibodies have been discovered. The most promising monoclonal antibody (mAb) entry inhibitor is Pro140 (PA14), a humanized IgG4 mAb with two ongoing phase IIb/III clinical trials (Kaplon and Reichert 2018; Olson and Jacobson 2009; Xu et al. 2017). Pro140 attaches to an epitope of the CCR5 co-receptor spanning ECL2 and N-terminus to prevent viral entry (Pace and Markowitz 2015; Xu et al. 2017) (Fig. 1a). The drug acts in a non-competitive, allosteric manner (Pace and Markowitz 2015). Once available for use, monoclonal antibodies should improve current therapeutic regimens based on their broad, potent neutralizing abilities, infrequent dosing requirements (e.g. weekly to monthly), favorable patient tolerability and lack of interactions with drugs and food (Olson and Jacobson 2009).

Fusion Inhibitors

Enfuvirtide (T20) is the only fusion inhibitor currently available for HIV-1 treatment; however, antiviral activity is low and drug resistance is easily induced. T20 is thought to work by binding to the pre-hairpin intermediate that bridges the viral and cell membranes (Fig. 1b). By binding at this site, it inhibits the formation of the viral six-helix bundle structure necessary for membrane fusion, ultimately inhibiting membrane fusion. Due to lack of efficacy of this drug other fusion inhibitors are being developed with hopes of improved antiviral activity and higher genetic barriers to drug resistance (Ding et al. 2017). Using the same or similar lipopeptide base of T20 as a template, other fusion inhibitors are being developed such as LP-40 and LP-46, which have shown promising preliminary results (Zhu et al. 2018). Currently, fusion inhibitors are not included in the first-line regimen guidelines for ART (Meintjes et al. 2017; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018).

Table 3 Latency reversal and autophagy inducing agents

Drug	Mode of action	Status	Reference
HDACIs			
Valproic Acid	Not fully understood, however, shows efficacy at inhibiting HIV-1 transcription and promoting viral degradation in MΦ	*In vitro/Ex vivo*	(Archin et al. 2017; Campbell et al. 2015; Pham and Mesplède 2018)
Vorinostat (Suberoylanilide hydroxamic acid)	Inhibits HDAC activity resulting in inhibition of HIV-1 transcription and induction of viral degradation in MΦ	*In vitro/Ex vivo*	(Archin et al. 2012; Archin et al. 2009, 2017; Campbell et al. 2015; Pham and Mesplède 2018)
Panobinostat (LBH589)	Inhibits HDAC activity resulting in inhibition of HIV-1 transcription and induction of viral degradation in MΦ	*In vitro/Ex vivo*	(Campbell et al. 2015; Pham and Mesplède 2018)
Romidepsin (FK228)	Reduction of the prodrugs disulfide bond releases zinc-binding thiol which blocks HDAC	*In vitro/Ex vivo*	(Campbell et al. 2015; Pham and Mesplède 2018; Wei et al. 2014)
PKC Agonists			
Ingenol-B	Upregulates NF-κB and pTEFb	*In vivo* (Animal Model: *Macaca nemestrina*)	(Darcis et al. 2015; Gama et al. 2017)
PICATO (PEP0005)	Upregulates NF-κB and pTEFb	*In vitro*	(Jiang et al. 2015)
Bryostatin-1	Modulates protein kinase C	*In vitro/Ex vivo*	(Darcis et al. 2015; Laird et al. 2015)
Maraviroc	Blocks CCR5 coreceptor binding/Induces NF-κB activation	*Ex vivo*	(Madrid-Elena et al. 2018)
BETis			
JQ-1	Blocks interaction of BRD4 and pTEFb to favor Tat recruitment of pTEFb to the HIV-1 promoter	*In vitro/Ex vivo*	(Bartholomeeusen et al. 2012; Brass et al. 2008; Bullen et al. 2014; Darcis et al. 2015; Huang et al. 2017; Jiang et al. 2015; Laird et al. 2015; Li et al. 2013; Zhu et al. 2012)
PI3K/MTOR inhibitors			
Dactolisib (BEZ-235)	Enhances maturation of autolysosomes	*In vitro*	(Campbell et al. 2018)
SF2523	Enhances HIV-1 reactivation and induces atuophagy	*In vitro*	(Campbell et al. 2018)
CSF-1R antagonists			
PLX647, PLX3397, PLX5622	Allows MΦ to regain susceptibility to TRAIL-mediated apoptotic cell death	*Ex vivo*	(Cunyat et al. 2016)

Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)

NRTIs and NtRTIs prevent viral RNA and DNA transcription by acting as mimetics of nucleic acid analogs (Fig. 1d). Current first-line regimen NRTI/NtRTIs include Tenofovir (TDF), Lamivudine (3TC), Emtricitabine (FTC), Abacavir (ABC). The most common combination is TDF/TFC or TDF/3TC added to a third ARV drug (ISTI, NNRTI, or PI with boosting agent) (Meintjes et al. 2017; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018). An extensive review of clinical trials of NRTI/NtRTI combinations can be found in the Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV (Panel on Antiretroviral Guidelines for Adults and Adolescents 2018).

Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)

NNRTIs block HIV replication during the final maturation stage leading to the formation of non-infective viral particles (Meintjes et al. 2017) (Fig. 1d). Five NNRTIs are available: delavirdine (DLV), efavirenz (EFV), etravirine (ETR), nevirapine (NVP), and rilpivirine (RPV). Two NNRTIs (EFC or RPV) are recommended for use in combination therapy when patients require concomitant treatment for tuberculosis due to their minimal interaction with rifamycins (Panel on Antiretroviral Guidelines for Adults and Adolescents 2018).

Integrase Strand Transfer Inhibitors (ISTIs)

ISTIs selectively interfere with the strand transfer step of integration by chelating two magnesium ions present at the

integrase active site preventing the transfer of proviral DNA into host chromosomal DNA (Boyer et al. 2018; Meintjes et al. 2017) (Fig. 1f). Three ISTIs are currently available; Dolutegravir (DTG), Elvitegravir (EVG/c), and Raltegravir (RAL) (Meintjes et al. 2017; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018). DTG is recommended in combination with two NRTI/NtRTIs for first-line regimen guidelines. DTG is a second-generation ISTI that has the highest genetic barrier to resistance of the available ISTIs (Meintjes et al. 2017; Messiaen et al. 2013; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018). Clinical trials of DTG efficacy are nicely summarized in the Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV (Panel on Antiretroviral Guidelines for Adults and Adolescents 2018).

To improve CNS delivery of ART as well as patient adherence, experimental ART dosing methods have been explored. A rodent model was used to determine neurotoxicity of dolutegravir if given as a single, high-level dose. Brain metabolic activities were measured via mass spectrometry-based metabolomics postmortem. This study confirmed that single high-level dosing of dolutegravir was neurotoxic; however, these negative alterations of neural homeostasis were completely attenuated if a nanoformulation was administered (Montenegro-Burke et al. 2018). This study highlights the importance of continued research to discover improved drug formulation and dosing of some currently approved ARDs.

Protease Inhibitors (PI)

PIs block HIV's protease enzyme from snipping newly translated protein chains of viral proteins (Fig. 1k). When protein snipping is blocked, short viral proteins are no longer able to mature into new viral particles. Two PIs are available; Darunavir (DRV) and Atazanavir (ATV). Both PIs require a boosting agent described below. PIs are recommended for use in first-line regimens when ART needs to be starting prior to resistance testing results. For information regarding clinical trials refer to the Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV (Panel on Antiretroviral Guidelines for Adults and Adolescents 2018).

Boosting Agents

Boosting agents are drugs that are combined with multiple antiretroviral drugs to improve the level of drug in the bloodstream and ultimately enhance ART efficiency. Current boosting agents are ritonavir and cobicistat (Meintjes et al. 2017; Panel on Antiretroviral Guidelines for Adults and Adolescents 2018).

Advancements in ART

Long Acting Slow Effective Release ART (LASER-ART)

The most recent advancement in regards to ART is LASER-ART. Essentially this system utilizes the phagocytic capacity of macrophages to serve as therapeutic drug carriers. Current ART therapeutics have been modified into hydrophobic prodrugs, encased in lipophilic excipients allowing for better drug-crystal formation and passage across cell membranes (Herskovitz and Gendelman 2018). LASER-ART equates to improved drug uptake and optimization of mononuclear phagocytes to carry and release drug throughout the body. Along with improved uptake and release, LASER-ART can be combined with the emerging field of theranostics (image-guided therapy) to aid in the pharmacodynamic evaluation of drugs *in vivo* (Herskovitz and Gendelman 2018; Tomitaka et al. 2017). Theranostics have already shown the ability to confirm successful reduction of viral replication in HIV-1 infected microglia in an *in vitro* blood brain barrier model (Tomitaka et al. 2017). For additional information regarding advancements in LASER-ART please refer to the review, "*HIV and the Macrophage: From Cell Reservoirs to Drug Delivery to Viral Eradication*" published in the Journal of Neuroimmune Pharmacology in May 2018 (Herskovitz and Gendelman 2018).

Even with the current efficacy of first- and second-line ART guidelines and future efficacy of LASER-ART; ART alone does not lead to a cure. Furthermore, if ART is discontinued, viral rebound occurs and if left uncontrolled, progression to AIDS is inevitable. Additional ART drawbacks include drug toxicities, negative off-target effects, and development of drug resistance, therefore the goal must be viral eradication.

HIV Latency and Cellular Reservoirs

The major challenge of HIV eradication is viral latency. As mentioned above two forms of HIV latency have been identified; pre-integration latency and post-integration latency, with post-integration latency the main contributor to cellular reservoirs throughout the body. True HIV latency within the macrophage lineage is hotly debated; however, a definition for cellular reservoir rather than latent reservoir has been proposed which includes all infected cells and tissues that contain any form of HIV persistence which participates in HIV pathogenesis (Avettand-Fènoël et al. 2016; Darcis et al. 2017). Some key viral sanctuaries have become well documented including peripheral blood, genitourinary tract, adipose tissue (stromal vascular fraction), gastrointestinal tract, gastrointestinal-associated lymphatic tissue (GALT), lymph nodes, parenchymal organs (lungs, liver, spleen) and the

central nervous system (CNS) (Avettand-Fènoël et al. 2016; Damouche et al. 2015; Herskovitz and Gendelman 2018; Igarashi 2001). Figure 2 shows HIV anatomical and cellular reservoirs in the context of ART-mediated long-term viral suppression. Four different types of macrophages (microglia, perivascular macrophages, meningeal macrophages, and choroid plexus macrophages) reside within the CNS, making it presumably one of the most important viral sanctuaries (Kumar et al. 2014).

To complicate understanding of the CNS as a viral reservoir, the CNS is an immune privileged site making it difficult to assess drug penetration and effectiveness. One major hurdle is confirming ART reaches the brain and at concentrations that confer effective viral suppression. Ongoing direct testing of brain tissue is difficult while patients are still alive. Therefore, evaluation of a drug's efficiency in the brain must be measured via cerebrospinal fluid (CSF), which may not offer an accurate assessment of viral load within the brain compartment itself. Longitudinal studies have shown that CSF biomarkers are not reliable to assess levels of immune activation and neuroinflammation within the brain. Functional imaging techniques such as PET-CT to measure translocator protein (TSPO) expression offer a more accurate picture of CNS activation. Use of [11C]-PK11195 PET in HIV chronically infected patients, who lacked any neurologic or cognitive symptoms, was able to identify microglial activation in spite of undetectable CSF and plasma viral loads (Calcagno et al. 2018; Garvey et al. 2014).

An important clinical feature of HIV-1 infection is long-term HIV-Associated Neurocognitive Disorders (HAND), ranging in severity from asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), HIV encephalopathy, HIV-1 Associated Dementia (HAD) to most severe AIDS Dementia Complex (ADC). The ability of

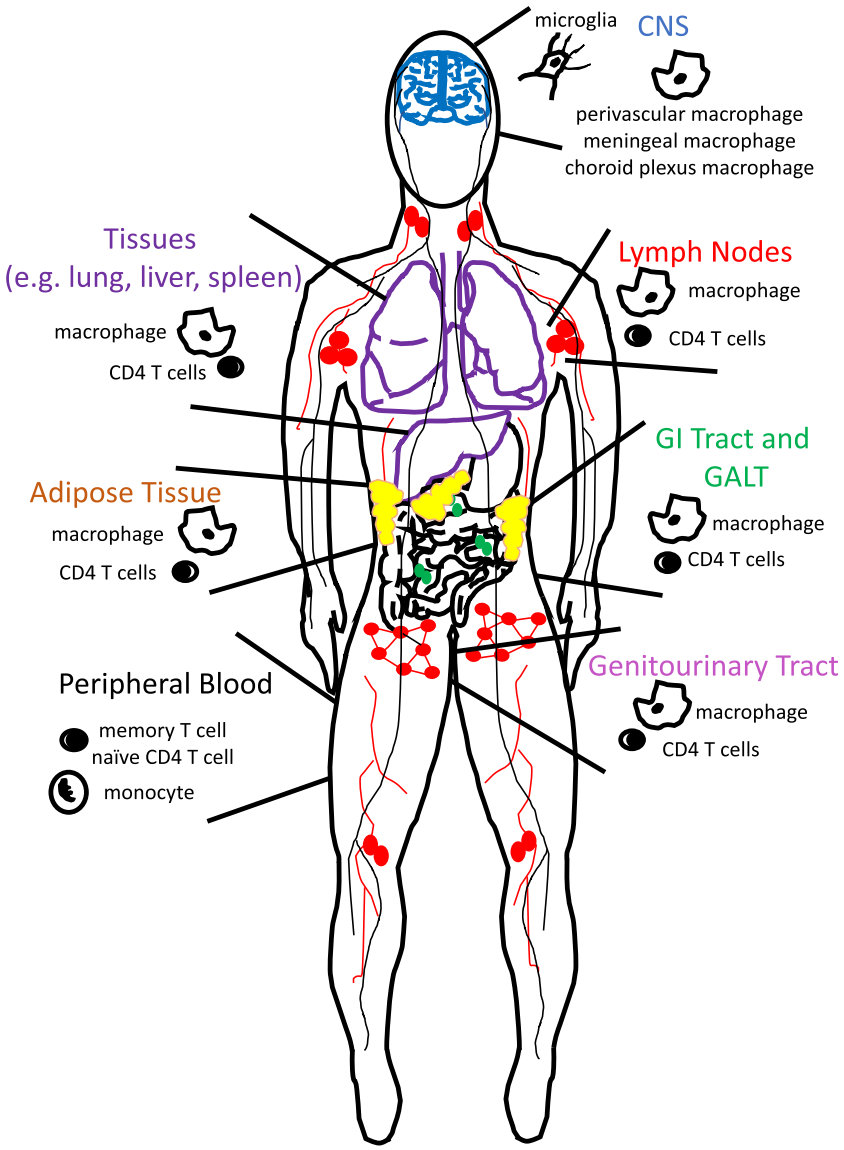

Fig. 2 HIV Anatomical and Cellular Reservoirs During Long-term Viral Suppressive ART. ART can reduce viral burden to undetectable levels within the plasma and CSF; however, HIV is able to persist as integrated provirus within CD4 T-cells (memory and naïve), monocytes, and macrophages throughout the body. Key anatomical sites containing HIV reservoir cells include the CNS (microglia, perivascular macrophages, meningeal macrophages and choroid plexus macrophages), lymph nodes (lymphocytes and macrophages), gastrointestinal tract (GI tract; CD4 T cells and macrophages within the lamina propria), Gut-Associated Lymphoid Tissue (GALT; CD4 T cells and macrophages), genitourinary tract (CD4 T cells and macrophages), parenchymal organs (lung, liver, spleen; T cells and macrophages), adipose tissue (CD4 T cells and macrophages within the stromal vascular fraction), and peripheral blood (memory and naïve CD4 T cells and monocytes)

macrophages to harbor virus for longs periods of time due to their low turnover rate and resistance to viral cytotoxic effects, specifically in microglia and perivascular macrophages, produce significant clinically relevant pathology within the CNS (Grant et al. 2014; Kumar et al. 2014).

It has been recently identified using brain magnetic resonance spectroscopy that intensification of maraviroc therapy can lead to improved neuronal integrity markers and reduction of CSF inflammation. Additionally, a similar drug still under development, Cenicriviroc, also showed potential for the reduction of CNS activation (Calcagno et al. 2018).

As previously discussed in the FIV section, intranasal IGF-1, was able to decrease FIV-related neuroinflammation improving neurobehavioral outcomes in FIV infected cats. Similar results were noted when intranasal insulin therapy was administered in an HIV/HAND model. Results showed that intranasal insulin reduced the expression of neuroinflammatory genes, inhibited HIV replication, and improved neurological outcomes (Mamik et al. 2016). A phase II clinical trial is currently underway to assess the ability of intranasal insulin to effectively treat HAND.

Understanding of HIV CNS pathology remains incomplete. There has been marked variability in experimental outcomes correlating viral loads within the brain with neuroinflammation and subsequent cognitive dysfunction. Even in the absence of productive infection, areas of neurodegeneration are noted. Two possible explanations for continued and slowly progressive cognitive decline include the release of neurotoxic secretions by infected macrophages as well as direct or indirect toxicity from antiretroviral therapy. One specific NNRTI, efavirenz, has been linked with lower neurocognitive performance in several studies (Calcagno et al. 2018).

Extensive research has been conducted utilizing the SIV-macaque model to address latency within the CNS. Heterogeneity of molecular mechanisms that control HIV-1 transcriptional repression and ultimately viral latency make viral eradication a complex task.

Latency Reversal Agents (LRAs)

Two types of "cures" have been delineated; sterilizing and functional (Knights 2017). A sterilizing cure is described as a complete eradication of all virus, active and latent, from the body. A functional cure is described as long-term, host-mediated control of viral replication with lasting remission in the absence of ongoing therapeutic treatment; however, competent virus remains within the body. While the ideal goal is to achieve a sterilizing cure, a functional cure may be a more reasonable goal utilizing the LRA "shock and kill" method.

The concept of "shock and kill" is to "shock" infected cells into producing viral proteins. Once the cells have become activated, they can then be recognized and removed ("killed") by the immune system. LRAs are used in conjunction with cART to block *de novo* virus from entering virus naïve cells and propagating continued infection (Gray et al. 2016). Several different types of LRAs are currently undergoing clinical trials including HDAC inhibitors (HDACi), Protein kinase C (PKC) agonists, HMT inhibitors (HMTi), PI3K/Akt signalers, Tat mimetics, and Bromodomain inhibitors (BRDi).

Efficacy of LRAs have been extensively studied within blood compartments; particularly the effect on memory CD4+ T cell populations; in-depth discussion can be found in the following reviews (Bashiri et al. 2018; Gupta and Dixit 2018; Spivak and Planelles 2018; 2016). In contrast, the efficacy regarding macrophages and CNS reservoirs is just beginning to be thoroughly explored. Additional considerations are necessary when evaluating LRA efficacy regarding macrophage reservoirs within the CNS. These considerations include: i) reservoir cells located in the CNS are protected by the blood-brain barrier and choroid plexus which limits access of most drugs, ii) macrophages have different receptors and cellular mechanisms that affect drug efficacy, iii) the CNS is an immune-privileged area, therefore, reactivation of virus could pose a potential issue if subsequent immune clearance and blockage of *de novo* virus is not achieved, iv) post-reactivation inflammation would have marked negative effects on the surrounding neuropil (Marban et al. 2016; Schwartz et al. 2017). Additionally, the topic of true HIV latency within macrophages remains hotly debated, which largely impacts the foreseeable efficacy of latency reversal agents in this cell type. However, Gama et al. recently confirmed that the use of LRAs, in a well-established macaque AIDS/HAND model, is able to increase viral load within the CSF; these findings further support the brain as an important viral reservoir deserving particular consideration regarding viral eradication (Gama et al. 2017).

For this review, the focus will be placed on LRAs directed at viral eradication from the CNS macrophage lineage

Current LRA Therapeutics

Upregulation of histone deacetylases (HDAC) to the HIV 5' LTR induce chromatin condensation leading to hypoacetylation of LTR *nuc-1* nucleosome and transcription inhibition. HDACis have been shown to induce hyperacetylation of *nuc-1* leading to activation and virus production within CD4+ T cells (Campbell et al. 2015; Pham and Mesplède 2018). A few drugs previously approved for cancer treatment are being investigated as potential HDACi latency reversal agents. HDACis currently being evaluated include valproic acid, vorinostat, panobinostat, and romidepsin. The mechanism of latency within macrophages varies from that of memory CD4+ T cells. HDACis that have shown efficacy *in vitro* and *ex vivo* in T-cells may have different effects on

macrophages as well as CNS-type cells. This difference is well represented in a study by Campbell et al. (2015) where HDACi's were shown to induce autophagy within macrophage *in vitro*, thereby inhibiting HIV replication and achieving viral degradation without cell death (Campbell et al. 2015). Inhibition was induced via the degradative autophagy pathway through autophagosome formation and maturation into autolysosomes. This is in stark contrast to HDACi's ability to reactivate viral transcription as described above in CD4+ T cells. While the mechanism is quite variable, use of HDACi's for viral eradication from macrophages still may prove beneficial.

Combination strategies using cooperation between HDAC inhibitors themselves as well as with other classes of LRA drugs show increased efficacy at latency reversal. One such combination is the use of HDACi with NF-kB inducing agents. NF-kB inducing agents modulate the protein kinase C pathway by upregulating NF-kB and pTEFb expression that leads to activation of latent HIV-1 (Darcis et al. 2015). The PKC pathway is involved in several integral cellular functions including cytoskeleton remodeling and regulation of transcription which ultimately control cellular growth, attachment, differentiation, maturation, and death. Upregulation or downregulation of the PKC pathway are typically controlled by alternative phosphorylation of serine and threonine residues on specific singling factors leading to specific effector functions (McKernan et al. 2012). In addition to activation of latent virus, PKC agonists have also been shown to downregulate expression of cell surface receptors, CD4, CXCR4, and CCR5, ultimately preventing infection of additional cells from reactivated virus (Jiang et al. 2015).

Gama et al. (2017) showed that combination therapy with vorinostat and Ingenol-B, not only induced latency reversal in the CNS compartment but also prevented cell-to-cell viral spread, in one pigtailed macaque, *in vivo* (Gama et al. 2017). This study highlighted two very important points, 1) that LRAs have the potential to reach the CNS compartment and reactivate reservoirs that harbor unique viral variants not seen peripherally throughout the body, and 2) that reactivating virus within the CNS compartment also has the potential to initiate detrimental inflammatory processes leading to increased CNS damage and subsequent poor clinical outcomes (Gama et al. 2017).

Another study combined PKC agonists with a Bromodomain and Extraterminal (BET) bromodomain inhibitors (BETi) and exhibited beneficial effects on NF-κB and DNA-binding (Darcis et al. 2015). BETis target the BET bromodomain by blocking interaction with acetylated lysine residues (Darcis et al. 2015). The most specific target of BETi to date is the BRD4 protein. By BETis inhibiting the interaction between BRD4 and P-PTEFb, it favors Tat recruitment of P-PTEFb to the HIV-1 promoter (Bartholomeeusen et al. 2012; Brass et al. 2008; Darcis et al. 2015; Li et al. 2013). Additionally, both BETi and PKC agonists increase available P-PTEFb for Tat recruitment by increasing release from 7SK snRNP complex. In this study two different PKC agonists were evaluated; Bryostatin-1 and Ingenol-B, with the BETi; JQ1. Of note, Bryostatin-1 has undergone clinical trials for the treatment of a number of other CNS-related diseases including cancer and Alzheimer's disease and shown minimal toxicity (Darcis et al. 2015; Kollár et al. 2014). JQ1 alone shows weak activity in reactivating latent virus in J-Lat and primary CD4+ T cells (Bullen et al. 2014; Huang et al. 2017; Zhu et al. 2012). It also exerts severe cytotoxicity, preventing long-term or high dosage treatment; therefore, use as an acute, combination therapy could optimize JQ1's safety and efficacy. The combinations, Bryostatin-1 plus JQ1 and Ingenol-B plus JQ1, showed strong synergistic activation of HIV-1 in both lymphocytic and monocytic cells *in vitro* as well as *ex vivo* (Darcis et al. 2015). This study is the first time the combination of a PKC agonist and BETi has shown such potent reactivating effects.

In agreement with these findings, the Siliciano group reported similar synergism in CD4+ T-cells (Laird et al. 2015). This study concluded that PKC agonists, when combined with JQ1 or other HDACis potently induced viral transcription in rCD4+ T cells from patients on ART (Laird et al. 2015).

Recently, the currently approved CCR antagonist, Maraviroc, was evaluated for potential use as an LRA. In addition to blocking viral entry, Maraviroc can induce NF-κB activation as a result of CCR5 binding, ultimately activating latent virus (Madrid-Elena et al. 2018).

A few other studies have evaluated the use of JQ1 combined with either HDACis or PKC agonists particularly focusing on *in vitro* models of CNS-type cells (macrophages and astrocyte). One study used an ingenol ester derivative, PEP0005 (Jiang et al. 2015). PEP0005 has been approved by the FDA for the topical treatment of precancerous actinic keratosis with the drug name PICATO. Jiang et al. (2015) showed that a combination of JQ1 and PEP0005 have a potent synergistic effect to reactivate latent HIV-1 within the U1 cell line (well-studied promonocyte used as an HIV latency cell culture model). Additional studies have shown that LRAs should be well-tolerated by CNS-type cells based on *in vitro* testing (Gray et al. 2016).

Several of the LRAs listed above have potential to "shock" infected macrophages into an activated state; however, use of LRAs alone is unlikely to accomplish complete elimination of infected cells. To complete the "shock and kill" method, additional therapeutics for the "kill" portion will be needed. One of the large barriers to the elimination of macrophage viral reservoirs is their inherent resistance to viral cytopathicity (Cunyat et al. 2016). Once infected, macrophages have increased levels of myeloid cell prosurvival cytokine Monocyte Colony-Stimulating Factor (M-CSF) (Cunyat et al. 2016). Additionally, if CSF-1R phosphorylation is inhibited by CSF-1R antagonists, macrophages regain

susceptibility to TRAIL-mediated apoptotic cell death (Cunyat et al. 2016). Using three different M-CSF antagonists *in vitro*, PLX647, PLX3397, and PLX5622, the Stevenson group was able to achieve a reduction in virus output as well as reduction of macrophage viability. Ultimately, combinations of cART, with M-CSF antagonists and TRAIL could be a potential option for blockade of viral replication and clearance of HIV-1 infected macrophages.

Latency reversal agents show promise as a possible means to eradicate HIV-1 from the body; however, it is not without risk. A few key issues continue to require extensive evaluation such as 1) drug access to all viral containing cells, not only CD4+ T cells, but also macrophage-type cells, especially those in the CNS compartment, 2) efficient clearing of reactivated HIV-1 infected cells in various reservoir sanctuaries, and 3) the possible neurotoxic effects of inflammation necessary to clear the reactivated cells in the brain.

Autophagy Inducing Agents

Within macrophages, HIV-1 can utilize cell machinery to inhibit macroautophagy. Inhibiting macroautophagy allows virus-infected macrophages to evade the innate immune system. Numerous human autophagy-associated genes are utilized by the virus by using autophagosomal membranes as scaffolds for Gag processing, an essential component for HIV replication (Campbell et al. 2018). Once replication is completed, the virus must then block phagosome maturation to avoid degradation (Campbell et al. 2018; Campbell and Spector 2013). Ten human autophagy inducers, also essential to HIV replication, have been elucidated and include 1α,25-dihydroxycholecalciferol, amino acid starvation, hydroxamate histone deacetylase inhibitors, sirolimus, toll-like receptor 8 ligands, romidepsin, and Tat-beclin. These autophagy inducers are potential therapeutic targets to achieve viral degradation. Upregulating these targets should override downregulation of autophagy by the virus and ultimately lead to clearance of HIV from infected macrophages (Campbell et al. 2018).

The phosphatidylinositol 3-kinase (PI3K) pathway involves key factors AKT serine/threonine kinase 1 (AKT1) and mechanistic target of rapamycin (mTOR). Dactolisib, a dual PI3K/mTOR inhibitor, has shown efficacy in enhancing maturation of autolysosomes necessary to promote the degradative autophagy pathway. Combination therapy of autophagy inducers, as seen with SF2523; a PI3K/mTOR/BRD4 inhibitor, can enhance reactivation of HIV-1 virus and induce autophagy within the cell, thus clearing the infection (Campbell et al. 2018). Drugs targeting the PI3K/AKT1/mTOR/ BRD4 pathway showed a low risk of toxicity as well as low risk of development of resistance (Campbell et al. 2018). Autophagy inducers, especially PI3K/AKT1/MTOR/ BRD4 inhibitors represent a potential therapeutic to target eradication of HIV-1 from macrophage reservoirs.

Gene Modifications

With recent advances regarding gene editing, "Cell Therapy" and "Gene Therapy" have become a realistic method to achieve a "cure" for various genetic and infectious diseases (Peterson and Kiem 2017). These eloquent technologies offer a variety of methods to remove HIV-1 from the host. "Cell Therapy" employs transplantation of various types of altered hematopoietic cells into an infected individual. "Gene Therapy" uses CRISPR/Cas9 AAV-vector systems to cut large sections of the HIV-1 provirus from cellular DNA rendering it replication incompetent, as well as, accomplish cellular manipulations regarding the CCR5Δ32 deletion ultimately blocking infection/re-infection of permissive cells.

"Cell and Gene Therapy"

Justifications for "Cell Therapy" arose when various cART suppressed HIV-1 patients were diagnosed with hematological malignancies (Peterson and Kiem 2017). With these diagnoses came the need for more aggressive treatment protocols, opening the door for transplantation trials using Hematopoietic Stem and Progenitor Cells (HSPC). A number of these trials have been well documented; however, with variable outcomes. Perhaps the most notable to date, known as the "Berlin Patient", involved an HIV-1 positive, 40-year-old man who was diagnosed with acute myelogenous leukemia (AML). Myeloablative therapy including chemotherapy and consolidation therapy were performed prior to receiving an MHC-matched, homozygous CCR5Δ32deletion, allogenic bone marrow transplant in 2007. Due to rebound of the AML, this patient received a second bone marrow transplant from the same donor a year later in 2008. To date, the "Berlin Patient" has been off cART for approximately 10 years with no replication-competent HIV-detected (Peterson and Kiem 2017). Of note, this patient received extremely aggressive and very specific transplant therapy that would be difficult and likely impossible to replicate in large patient populations. Comparing the success of the "Berlin Patient" to other notable HIV-1 allogenic transplant cases, the key factor that appears to have impacted the positive outcome of cure/remission is the use of CCRΔ32 deletion, therefore to achieve such an outcome the use of the CCRΔ32 deletion or gene-engineered autologous cell product may be necessary (Peterson and Kiem 2017).

Other cell-based therapies currently under investigation include chimeric antigen receptor (CAR) therapies. CAR therapies are currently, predominantly targeted at T cells (MacLean et al. 2014). While these therapies may prove to be beneficial for clearance of infected cells with active, replicating virus; they most likely will not aid in the eradication of virus from macrophages, particularly in a latent state.

Other strategies using ribozymes, aptamers, antisense, and RNA interference approaches showed promise *in vitro* and in mouse models; however, have failed to show effectiveness in non-human primates or clinical trials. While in theory, these methods are efficient at restricting and eliminating virus in a number of cell types, unfortunately, the ability to deliver these therapies to a sufficient number of cells throughout the body has remained a limiting factor (Peterson and Kiem 2017).

Viral Vectors

Viral vectors allow administration of therapeutic drugs, whether broadly neutralizing antibodies or gene modifying drugs, at a high quantity with a small size achieving distribution to all areas of the body while eliciting minimal immunologic response from the host. The most promising viral vectors to date are adeno-associated viral (AAV) vectors and lentiviral vectors (Khalili et al. 2015; Peterson and Kiem 2017). AAV vectors do not integrate into host cells and serve as an efficient vehicle to administer gene modifying drugs to various target cells. Previously used gammaretrovirus vectors, which integrate into host cells, have led to unrestricted cellular proliferation resulting in myelodysplasia and leukemias (Peterson and Kiem 2017). AAV vectors can transduce both dividing and non-dividing cells. AAV vector drawbacks include their extremely small size, which limits the size of cargo (Khalili et al. 2015). Lentiviral vectors are also capable of transducing both diving and non-dividing cells and can be self-inactivating, replication incompetent, or integrase-deficient (Khalili et al. 2015). Additionally, specific strains of AAV and lentiviral vectors can be used to target specific anatomical locations such as the brain as well as specific cell types.

Permanent Gene Editing

Gene editing offers the ability to alter host genomic DNA at specific loci, using a number of various site-specific nuclease platforms including Zinc Finger Nuclease (ZFN), Transcription Activator-Like Effector Nucleases (TALENS), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein-9 nuclease (Cas9) (Peterson and Kiem 2017). The most promising strategy to date is the CRISPR-cas9 platform. CRISPR-cas9 works via CRISPR RNA forming guide RNA that lead the Cas9 endonuclease to the target site. Cas9 creates double-strand breaks in the DNA that are then repaired by host mechanisms such as Non-Homologous End Joining (NHEJ) and homologous recombination (HR) (Imran et al. 2017). NHEJ typically results in the generation of insertions and deletions or base substitutions which lead to a frameshift and/or premature stop codons. Ultimately, disruption of the target gene open reading frame (ORF) renders the target gene replication incompetent. With regards to HIV-1, this means efficient and successful virus killing, as well as disruption of cellular genes necessary for HIV-1 infection (Khalili et al. 2017). Other uses for the CRISPR/Cas9 systems have also been explored such as catalytically-deficient Cas9-synergistic activation mediator (dCas9-SAM) technology used to induce provirus reactivation similar to the LRAs described above (Khalili et al. 2015; Limsirichai et al. 2016; Saayman et al. 2016). The CRISPR/Cas 9 system has also been used to modify cellular components such as inducing a CCR5Δ32 deletion to block infection of permissive cells (Hu et al. 2014; Kang et al. 2015). Figure 3 depicts the basic principles of the CRISPR/Cas9 system.

An AAV_9-based saCas9/gRNA gene editing delivery system demonstrated *in vivo*, in both a mouse and rat HIV-1 model, efficient excision of a segment of integrated HIV-1 genome and suppression of viral RNA production (Kaminski et al. 2016a). Of note, this AAV_9-based saCas9/gRNA gene editing delivery system was able to remove the largest reported segment of HIV-1 integrated proviral DNA spanning 978 base pairs (Kaminski et al. 2016a). A number of studies have demonstrated that utilizing the LTR promoter for Cas9 expression and U6 promoter for gRNAs enacts a high impact on disruption of HIV-1 expression in a number of cell lines, including those of myeloid lineage such as microglia and macrophages (Ebina et al. 2013; Hu et al. 2014; Kaminski et al. 2016a; Liao et al. 2015; Yin et al. 2018). It has also been shown that multiplexed CRISPR/Cas9 systems targeting more than one region achieved an elevated disruption of HIV-1 proviral DNA excision as well as decreased risk of viral escape (Lebbink et al. 2017; Liao et al. 2015; Yin et al. 2018). Use of a quadruplex sgRNAs/saCas9 strategy showed efficient gene delivery and HIV-1 excision *in vivo* in multiple mouse models (Tg26 transgenic, EchoHIV-eLuc, and humanized BLT) (Yin et al. 2016). Kaminski et al. implemented an elegant strategy using the HIV-1 viral production of Tat protein to stimulate expression of Cas9 and its associated gRNAs. This system created a suicide pathway only activated in the presence of HIV-1 generating a negative feedback loop. Once HIV-1 virus is completely removed the Cas9

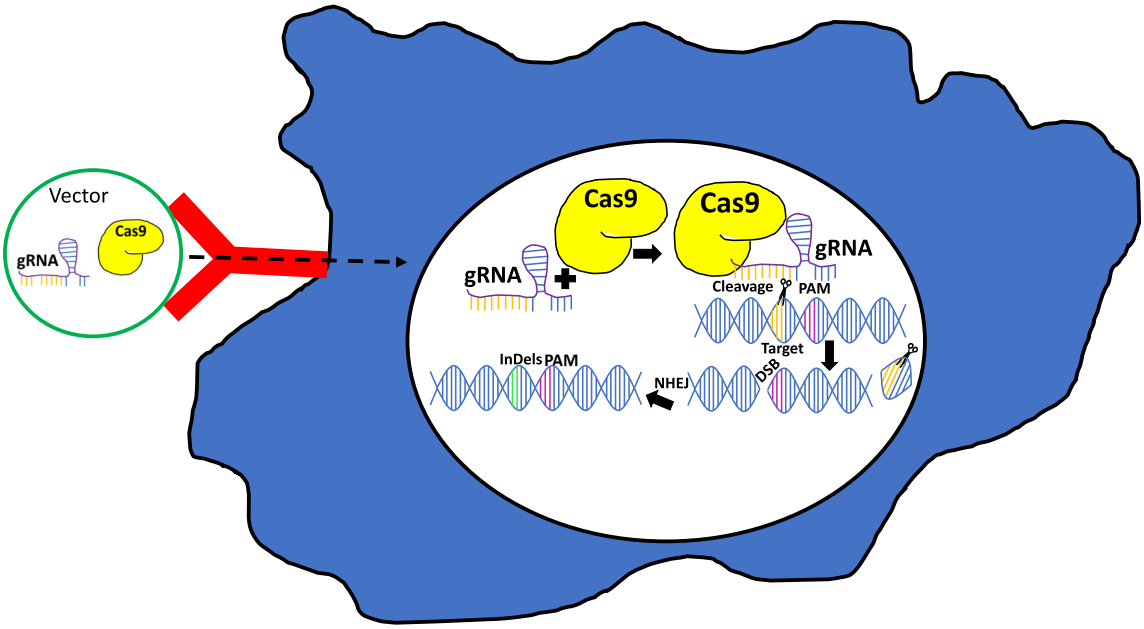

Fig. 3 Cleavage of HIV from reservoir cell (e.g. macrophage). This schematic depicts a viral vector carrying gRNA and Cas9 attaching to an entry receptor on an HIV infected cell. Cas9 and gRNA are transported to the nucleus. The gRNA directs the Cas9 endonuclease to target a specific segment of DNA adjacent to a protospacer adjacent motif (PAM). Cas9 cleaves viral DNA via endonucleolytic cleavage causing a double strand break (DSB). The DSB is repaired by non-homologous end-joining (NHEJ) associated with InDel mutations at the cut site.

gRNAs are no longer expressed allowing for the reduction of off-target effects (Kaminski et al. 2016b). Increasing selectivity of the CRISPR/Cas9 gRNAs to match HIV-1 sequences archived from reservoirs of individual patients showed improved safety and efficacy, specifically in reduction of viral escape (Wigdahl 2014; Zhu et al. 2015). Combination of selective gRNAs that are multiplexed to target multiple conserved regions of the viral genome provide a valid strategy to reach safe and effective gene editing (Dampier et al. 2017; Wang and Palmer 2018; Wigdahl 2014). The increasing ease of production of multiple sgRNA clones, improved reporter screening, PCR genotyping, and efficient vector production make personalized and precision gene editing an exciting reality in the near future (Yin et al. 2016).

Conclusion

HIV-1 remains a global health crisis; however, extensive research continues to make rapid progress towards finding a cure. It has become evident that looking at all HIV-1 permissive cells, including mononuclear phagocytes highlighted in this review, is necessary to achieve viral eradication. It may well be that complete eradication will need to include combinations of various effective therapeutic strategies such as LASER-ART, LRAs, and gene editing. The most promising combination appears to be LASER-ART in conjunction with viral and receptor gene modifications via the CRISPR/Cas9 system. Understanding the prominent role of macrophages in the inception and propagation of HIV infection is paramount regarding the possibility of complete viral eradication. It will be fascinating to watch the realistic possibility of a cure for HIV-1 unfold. After approximately 40 years of indefatigable research and scientific advancements, cautious excitement and hope may finally be warranted regarding complete eradication of HIV.

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Compliance with ethical standards

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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