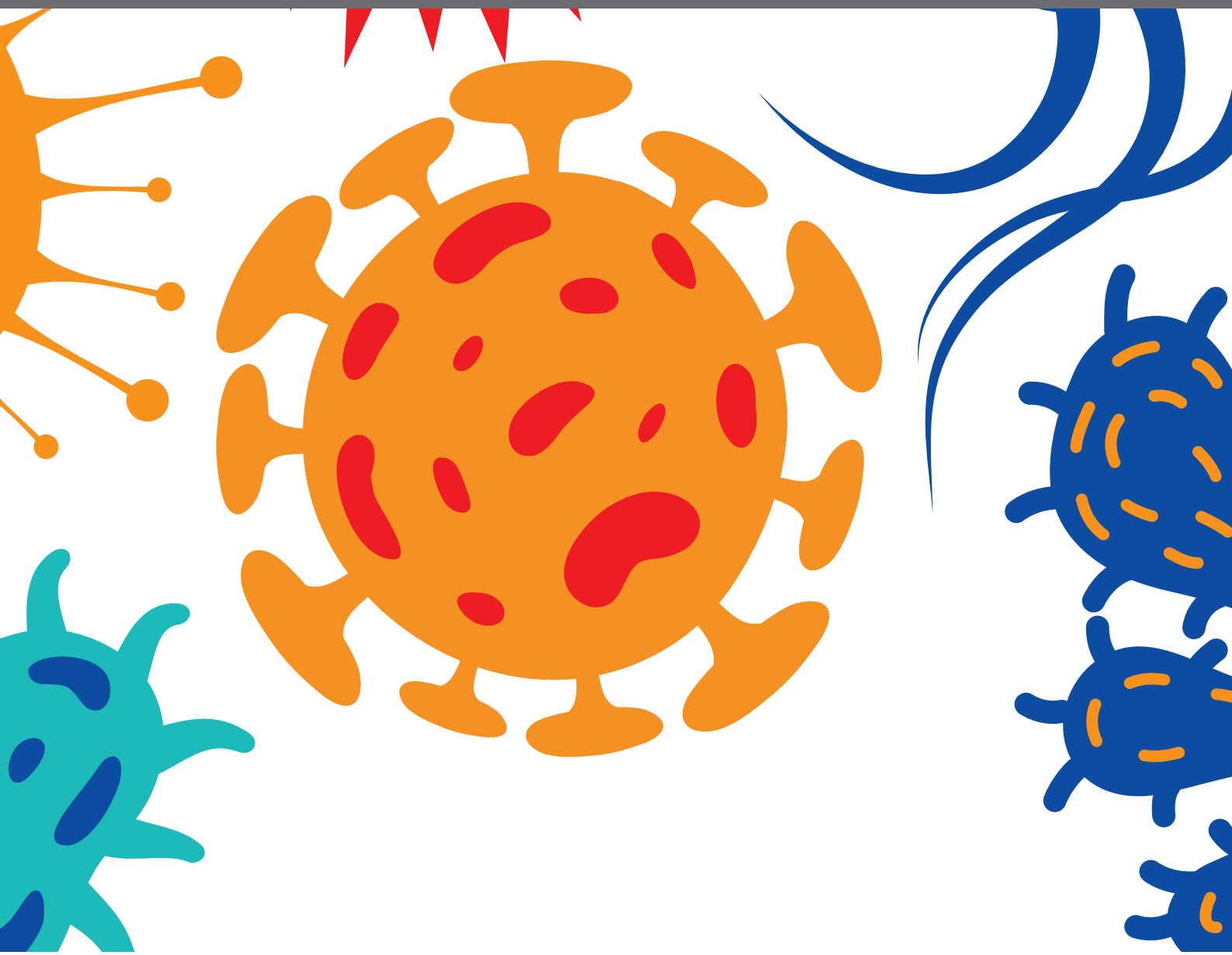
EXPLORING NOVEL APPROACHES TO ELIMINATE HIV RESERVOIRS TO ACHIEVE A CURE FOR HIV

EDITED BY: Renee Marije Van Der Sluis, Matthew Parsons and Andrés Finzi
PUBLISHED IN: Frontiers in Cellular and Infection Microbiology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88966-666-9
DOI 10.3389/978-2-88966-666-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

EXPLORING NOVEL APPROACHES TO ELIMINATE HIV RESERVOIRS TO ACHIEVE A CURE FOR HIV

Topic Editors:
Renee Marije Van Der Sluis, Aarhus Institute of Advanced Studies, Denmark
Matthew Parsons, Emory University, United States
Andrés Finzi, Université de Montréal, Canada

Citation: Van Der Sluis, R. M., Parsons, M., Finzi, A., eds. (2021). Exploring Novel Approaches to Eliminate HIV Reservoirs to Achieve a Cure for HIV. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-666-9

Table of Contents

04 ***Editorial: Exploring Novel Approaches to Eliminate HIV Reservoirs to Achieve a Cure for HIV***
Renée M. van der Sluis, Andrés Finzi and Matthew S. Parsons

07 ***Measuring the Success of HIV-1 Cure Strategies***
Jordan Thomas, Alessandra Ruggiero, William A. Paxton and Georgios Pollakis

27 ***Promise and Progress of an HIV-1 Cure by Adeno-Associated Virus Vector Delivery of Anti-HIV-1 Biologics***
Matthew R. Gardner

43 ***TIGIT Blockade: A Multipronged Approach to Target the HIV Reservoir***
Kayla A. Holder and Michael D. Grant

52 ***Pattern Recognition Receptor Ligands as an Emerging Therapeutic Agent for Latent HIV-1 Infection***
Shokichi Takahama and Takuya Yamamoto

61 ***What Can Gamma Delta T Cells Contribute to an HIV Cure?***
Jennifer A. Juno and Stephen J. Kent

70 ***Plasmacytoid Dendritic Cells as Cell-Based Therapeutics: A Novel Immunotherapy to Treat Human Immunodeficiency Virus Infection?***
Renée M. van der Sluis, Johanne H. Egedal and Martin R. Jakobsen

82 ***Engineering CAR T Cells to Target the HIV Reservoir***
Wenli Mu, Mayra A. Carrillo and Scott G. Kitchen

97 ***Block and Lock HIV Cure Strategies to Control the Latent Reservoir***
Chantelle L. Ahlenstiel, Geoff Symonds, Stephen J. Kent and Anthony D. Kelleher

frontiers in Cellular and Infection Microbiology

EDITORIAL
published: 25 February 2021
doi: 10.3389/fcimb.2021.658848

Editorial: Exploring Novel Approaches to Eliminate HIV Reservoirs to Achieve a Cure for HIV

Renée M. van der Sluis[1,2*], *Andrés Finzi*[3,4,5] *and Matthew S. Parsons*[6,7*]

[1] Aarhus Institute of Advanced Studies (AIAS), Aarhus University, Aarhus, Denmark, [2] Department of Biomedicine, Aarhus University, Aarhus, Denmark, [3] Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montreal, QC, Canada, [4] Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada, [5] Centre de Recherche du CHUM, Montreal, QC, Canada, [6] Yerkes National Primate Research Center, Emory University, Atlanta, GA, United States, [7] Department of Pathology and Laboratory Medicine, School of Medicine, Emory University, Atlanta, GA, United States

Keywords: human immunodeficiency virus (HIV), HIV cure, HIV cure research, HIV cure strategies, HIV latency

Editorial on the Research Topic

Exploring Novel Approaches to Eliminate HIV Reservoirs to Achieve a Cure for HIV

OPEN ACCESS

Edited and reviewed by:
Colleen B. Jonsson, University of Tennessee Health Science Center (UTHSC), United States

***Correspondence:**
Renée M. van der Sluis
renee@biomed.au.dk
Matthew S. Parsons
matthew.s.parsons@emory.edu

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *26 January 2021*
Accepted: *02 February 2021*
Published: *25 February 2021*

Citation:
van der Sluis RM, Finzi A and Parsons MS (2021) Editorial: Exploring Novel Approaches to Eliminate HIV Reservoirs to Achieve a Cure for HIV. Front. Cell. Infect. Microbiol. 11:658848. doi: 10.3389/fcimb.2021.658848

Antiretroviral therapy (ART) has revolutionized the treatment of HIV and has dramatically reduced morbidity and mortality among people living with HIV (PLWH) (Antiretroviral Therapy Cohort Collaboration, 2017). ART inhibits HIV replication, decreasing viral loads, halting disease progression and preventing sexual transmission of the virus (Attia et al., 2009; Volberding and Deeks, 2010; Cohen et al., 2011; Bavinton et al., 2018; Rodger et al., 2019). Cessation of ART, however, allows HIV to re-initiate replication (Davey et al., 1999; Colby et al., 2018). As such, ART is required throughout the lifespan of PLWH. Treatments that aim to cure HIV or induce viral remission are highly desirable to alleviate the need for lifelong ART.

The primary barrier to curing HIV is the persistence of a latent viral reservoir that predominantly resides in $CD4^+$ T cells (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997). Numerous strategies have been proposed to target this reservoir (Pitman et al., 2018). Currently, the predominant strategy is the `shock and kill' approach, which proposes employing latency reversing agents (LRAs) to induce virus expression and make virus harboring cells visible to the immune system. Subsequently, immune cells are engaged to eliminate cells harboring reactivated latent virus. Although clinical trials exploring the utility of the `shock and kill' strategy have shown LRAs to induce viral expression in ART-treated PLWH, evidence of the efficacy of this approach for decreasing the frequency of cells carrying integrated HIV DNA has yet to be generated (Dufour et al., 2020). The reason(s) that `shock and kill' strategies have not reduced the frequency of cells carrying integrated HIV DNA remains unclear. Novel LRAs and/or more potent anti-viral immune responses may be required to purge the virus (Kim et al., 2018; Dufour et al., 2020).

In this special issue of Frontiers in Cellular and Infection Microbiology, we invited early stage investigators to contribute their ideas as to how to tackle the important scientific problem of curing HIV infection. We received eight manuscripts, which we will briefly summarize to introduce this special issue.

Thomas et al. provide an excellent overview of recent advances towards understanding the latent viral reservoir and highlight various potential strategies for its eradication. To measure the success of these strategies, highly sensitive and accurate assays are required. Measuring the latent HIV

reservoir is made difficult by variation in the viral genome, the low frequency of cells carrying latent virus and the presence of defective proviruses. Thomas et al. discuss the different methods that are available and debate the advantages and disadvantages of these assays.

Takahama and Yamamoto review the potential of using ligands for pattern recognition receptors (PRRs) as a cure strategy. PRR ligands can be used as vaccine adjuvants to enhance activation of the innate immune system and promote antigen-specific immune responses. PRR ligands can also be used to enhance preexisting immune responses. PRR ligands fit the 'shock and kill' approach well, because they can be used to activate the latent virus (i.e. deliver the "shock") and boost the anti-viral immune response (i.e. the "kill"). Additionally, Takahama and Yamamoto discuss the possibility of combining PRR ligands with other immunotherapies, such as therapeutic vaccines, checkpoint inhibitors and broadly neutralizing antibodies (bNAbs).

Five of the articles in this special issue provide novel ideas as to how to enhance anti-viral immune responses through the use immunotherapeutic approaches.

Holder and Grant review the potential utility of immune checkpoint blockers targeting TIGIT to invigorate anti-viral immune responses. The advantage of using anti-TIGIT over other immune checkpoint blockers is that TIGIT is expressed on many HIV-specific $CD8^+$ T cells in PLWH (Chew et al., 2016; Tauriainen et al., 2017) and blocking TIGIT can reinvigorate exhausted T cell responses. Additionally, anti-TIGIT therapy may also enhance NK cell function.

Mu et al. summarize recent developments pertaining to HIV-specific CAR T cells. One of the challenges Mu et al. describe is that CAR T cells need to function under conditions where low amounts of HIV-antigen are present. They discuss how combining CAR T cell therapy with LRAs might increase the effectiveness of this therapeutic option.

Juno and Kent summarize the different subsets of gamma-delta-(γδ) T cells and review their immunotherapeutic potential in HIV cure strategies. A major clinical advantage of employing these cells is the lack of MHC restriction for γδ T cell-mediated killing. This provides an opportunity for an "off-the-shelf" allogeneic product, circumventing the MHC-restriction present for most other cell-based immunotherapies.

van der Sluis et al. describe the potential of using plasmacytoid dendritic cells (pDCs) as an immunotherapy. PDCs are mostly known for their type I interferon-producing capacity, which can enhance NK cell activation and killing. However, pDCs can also improve T cell immunity by delivering antigens or therapeutic peptides combined with interferons.

Gardner provides an extensive overview of the potential of using recombinant Adeno-Associated Virus Vectors (rAAVs) to deliver bNAbs and engineered HIV inhibitors. Using rAAVs to deliver bNAbs or inhibitors would overcome the need for continued passive administration to sustain expression of these molecules in PLWH.

Finally, Ahlenstiel et al. summarize the recent advances toward developing an alternative HIV cure strategy, termed "block and lock." This approach proposes to permanently silence HIV expression by "blocking" HIV transcription and "locking" the HIV promotor in a permanently latent state. Achievement of this premise would provide a functional cure, where a person still lives with HIV but the presence of the virus is not harmful in the absence of ART.

In summary, the articles presented in this special issue of Frontiers in Cellular and Infection Microbiology give an overview of the obstacles to developing successful HIV cure strategies. Additionally, the articles provide a collection of excellent novel ideas that could advance efforts to reduce the burden of integrated HIV DNA in ART-treated PLWH.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

FUNDING

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754513 and The Aarhus University Research Foundation (RMS).

REFERENCES

Antiretroviral Therapy Cohort Collaboration (2017). Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. *Lancet HIV* 4, e349–e356. doi: 10.1016/S2352-3018(17)30066-8

Attia, S., Egger, M., Muller, M., Zwahlen, M., and Low, N. (2009). Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. *AIDS* 23, 1397–1404. doi: 10.1097/QAD.0b013e32832b7dca

Bavinton, B. R., Pinto, A. N., Phanuphak, N., Grinsztejn, B., Prestage, G. P., Zablotska-Manos, I. B., et al. (2018). Viral suppression and HIV transmission in serodiscordant male couples: an international, prospective, observational, cohort study. *Lancet HIV* 5, e438–e447. doi: 10.1016/S2352-3018(18)30132-2

Chew, G. M., Fujita, T., Webb, G. M., Burwitz, B. J., Wu, H. L., Reed, J. S., et al. (2016). TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. *PLoS Pathog.* 12, e1005349. doi: 10.1371/journal.ppat.1005349

Chun, T. W., Carruth, L., Finzi, D., Shen, X., Digiuseppe, J. A., Taylor, H., et al. (1997). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387, 183–188. doi: 10.1038/387183a0

Cohen, M. S., Chen, Y. Q., Mccauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., et al. (2011). Prevention of HIV-1 infection with early antiretroviral therapy. *N. Engl. J. Med.* 365, 493–505. doi: 10.1056/NEJMoa1105243

Colby, D. J., Trautmann, L., Pinyakorn, S., Leyre, L., Pagliuzza, A., Kroon, E., et al. (2018). Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat. Med.* 24, 923–926. doi: 10.1038/s41591-018-0026-6

Davey, R.T. Jr., Bhat, N., Yoder, C., Chun, T. W., Metcalf, J. A., Dewar, R., et al. (1999). HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15109–15114. doi: 10.1073/pnas.96.26.15109

Dufour, C., Gantner, P., Fromentin, R., and Chomont, N. (2020). The multifaceted nature of HIV latency. *J. Clin. Invest.* 130, 3381–3390. doi: 10.1172/JCI136227

Finzi, D., Hermankova, M., Pierson, T., Carruth, L. M., Buck, C., Chaisson, R. E., et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295–1300. doi: 10.1126/science.278.5341.1295

Kim, Y., Anderson, J. L., and Lewin, S. R. (2018). Getting the "Kill" into "Shock and Kill": Strategies to Eliminate Latent HIV. *Cell Host Microbe* 23, 14–26. doi: 10.1016/j.chom.2017.12.004

Pitman, M. C., Lau, J. S. Y., Mcmahon, J. H., and Lewin, S. R. (2018). Barriers and strategies to achieve a cure for HIV. *Lancet HIV* 5, e317–e328. doi: 10.1016/S2352-3018(18)30039-0

Rodger, A. J., Cambiano, V., Bruun, T., Vernazza, P., Collins, S., Degen, O., et al. (2019). Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. *Lancet* 393, 2428–2438. doi: 10.1016/S0140-6736(19)30418-0

Tauriainen, J., Scharf, L., Frederiksen, J., Naji, A., Ljunggren, H. G., Sonnerborg, A., et al. (2017). Perturbed CD8(+) T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals. *Sci. Rep.* 7, 40354. doi: 10.1038/srep40354

Volberding, P. A., and Deeks, S. G. (2010). Antiretroviral therapy and management of HIV infection. *Lancet* 376, 49–62. doi: 10.1016/S0140-6736(10)60676-9

Wong, J. K., Hezareh, M., Gunthard, H. F., Havlir, D. V., Ignacio, C. C., Spina, C. A., et al. (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 278, 1291–1295. doi: 10.1126/science.278.5341.1291

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 07 April 2020
doi: 10.3389/fcimb.2020.00134

Measuring the Success of HIV-1 Cure Strategies

Jordan Thomas[1], *Alessandra Ruggiero*[1,2], *William A. Paxton*[1]* *and Georgios Pollakis*[1]*

[1] *Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom,* [2] *Immune and Infectious Disease Division, Academic Department of Pediatrics (DPUO), Bambino Gesù Children's Hospital, Rome, Italy*

HIV-1 eradication strategies aim to achieve viral remission in the absence of antiretroviral therapy (ART). The development of an HIV-1 cure remains challenging due to the latent reservoir (LR): long-lived CD4 T cells that harbor transcriptionally silent HIV-1 provirus. The LR is stable despite years of suppressive ART and is the source of rebound viremia following therapy interruption. Cure strategies such as "shock and kill" aim to eliminate or reduce the LR by reversing latency, exposing the infected cells to clearance via the immune response or the viral cytopathic effect. Alternative strategies include therapeutic vaccination, which aims to prime the immune response to facilitate control of the virus in the absence of ART. Despite promising advances, these strategies have been unable to significantly reduce the LR or increase the time to viral rebound but have provided invaluable insight in the field of HIV-1 eradication. The development and assessment of an HIV-1 cure requires robust assays that can measure the LR with sufficient sensitivity to detect changes that may occur following treatment. The viral outgrowth assay (VOA) is considered the gold standard method for LR quantification due to its ability to distinguish intact and defective provirus. However, the VOA is time consuming and resource intensive, therefore several alternative assays have been developed to bridge the gap between practicality and accuracy. Whilst a cure for HIV-1 infection remains elusive, recent advances in our understanding of the LR and methods for its eradication have offered renewed hope regarding achieving ART free viral remission.

Keywords: HIV-1, persistence, latency, latent reservoir, DNA/RNA quantification

OPEN ACCESS

Edited by:
Renee Marije Van Der Sluis, Aarhus Institute of Advanced Studies, Denmark

Reviewed by:
Marta Massanella, IrsiCaixa, Spain
Michael Roche, Peter Doherty Institute for Infection and Immunity, Australia

***Correspondence:**
William A. Paxton
w.a.paxton@liverpool.ac.uk
Georgios Pollakis
g.pollakis@liverpool.ac.uk

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *05 February 2020*
Accepted: *13 March 2020*
Published: *07 April 2020*

Citation:
Thomas J, Ruggiero A, Paxton WA and Pollakis G (2020) Measuring the Success of HIV-1 Cure Strategies. Front. Cell. Infect. Microbiol. 10:134. doi: 10.3389/fcimb.2020.00134

INTRODUCTION

Infection with human immunodeficiency virus type-1 (HIV-1) requires life-long adherence to antiretroviral therapy (ART) due to the presence of latently infected cells that are central to viral persistence and rebound viremia following ART interruption (Chun et al., 1997, 1998, 1999; Finzi et al., 1997; Perelson et al., 1997; Wong et al., 1997; Davey et al., 1999; Rosenbloom et al., 2017). HIV-1 primarily infects activated CD4 T cells, where genomic RNA is reverse transcribed into DNA and stably integrated into the host genome. Integrated proviral DNA therein serves as the template for HIV-1 gene expression and genomic RNA production, driven by T cell activation induced transcription factors such as NF-κB (Liu et al., 1992; Kinoshita et al., 1998). The latent reservoir (LR) is established when a small subset of activated CD4 T cells, harboring proviral DNA, revert to a resting memory phenotype with reduced gene expression, rendering the cell non-permissive for HIV-1 production but providing a sanctuary to evade the immune response and ART

(Hermankova et al., 2003; Siliciano and Greene, 2011). The LR is stable over long periods in therapy suppressed individuals; the result of infection in naturally long-lived memory CD4 T cells that are continually replenished by clonal expansion and homeostatic proliferation (Finzi et al., 1999; Siliciano et al., 2003; Bailey et al., 2006; Chomont et al., 2009; Maldarelli et al., 2014; Wagner et al., 2014; Cohn et al., 2015; Lorenzi et al., 2016; Simonetti et al., 2016; Hosmane et al., 2017). Latently infected cells therefore represent the principle barrier to an HIV-1 cure and should be specifically targeted by novel treatment and eradication strategies.

To date, an effective cure for HIV-1 infection has been achieved twice via CCR5Δ32/Δ32 hematopoietic stem cell transplantation and in both cases latently infected cells were eliminated and replaced with HIV-1 resistant donor cells (Hutter et al., 2009; Gupta et al., 2019). Whilst this method is not feasible for widespread use, its repeated success proves the principle that HIV-1 cure strategies must either eliminate (sterilizing cure) or silence (functional cure) the LR. Proposed cure strategies such as "shock and kill" aim to eliminate the LR by utilizing latency reversing agents (LRAs) during ART mediated virus suppression to drive expression of HIV-1 from latently infected cells, exposing those cells to viral cytopathic effects or immune clearance whilst limiting *de novo* infections (Deeks, 2012). An alternative and conceptually opposing method, "block and lock," aims to reinforce viral latency and therefore maintain the provirus in an inactivate state in the absence of ART (Mousseau et al., 2015; Méndez et al., 2018). Additionally, therapeutic vaccination based approaches aim to silence the LR by inducing strong HIV-1 specific T cell responses to aid immune control of the infection following ART cessation (Mylvaganam et al., 2015; Pantaleo and Levy, 2016).

Measuring the success of HIV-1 cure and vaccine strategies requires highly sensitive and accurate assays and there is currently no consensus as to the most appropriate method to utilize. Several technical challenges limit the ability to measure accurately the size of the LR, including the paucity of cells infected with replication competent provirus and the vast heterogeneity of the HIV-1 genome. Culture based assays such as the viral outgrowth assay (VOA) are routinely used to measure the LR but are labor and resource intensive and invariably underestimate the size of the replication competent reservoir (Ho et al., 2013; Bruner et al., 2015). Conversely, PCR based assays offer a more practical approach to proviral quantification but overestimate the size of the LR by indiscriminately measuring defective viral genomes that predominate the *in vivo* landscape (Ho et al., 2013).

Despite the success of ART in reducing HIV-1 associated mortality, the global burden of the disease necessitates the urgent development of a cure or vaccine and both understanding and accurately measuring the LR is crucial in the path toward HIV-1 eradication. In this review, we will focus on the mechanisms that facilitate the establishment and maintenance of the HIV-1 LR, some of the prominent methods proposed to achieve a cure and the developments and challenges on the way to measuring their success.

THE LATENT RESERVOIR

Establishing Latency

The HIV-1 LR can be defined as the fraction of cells harboring transcriptionally silent proviral DNA that are capable of producing infectious virions following activation (Eisele and Siliciano, 2012). Resting memory CD4 T cells are the primary host of the LR but HIV-1 infection in these cells is inefficient due their low co-receptor expression and inherent restrictions to reverse transcription (Pierson et al., 2000; Baldauf et al., 2012). Nevertheless, there is evidence that HIV-1 can infect resting CD4 T cells directly or via cell-to-cell transmission, though infection in these cells is associated with slower replication kinetics (Swiggard et al., 2004, 2005; Agosto et al., 2007, 2018; Plesa et al., 2007; Vatakis et al., 2007; Lassen et al., 2012). Alternatively, latency is established when a subset of infected, activated CD4 T cells revert to a resting memory phenotype, effectively silencing viral gene expression whilst sustaining the proviral DNA long-term (Chun et al., 1995). The provirus is maintained in a quiescent state in these cells via host factors such as epigenetic suppression, depletion of transcription factors such as NF-κB and transcriptional interference due to integration into expressed genes, reviewed in more detail (Cary et al., 2016).

Amongst the pool of viral genomes integrated into host cells, only a small fraction are replication competent and therefore capable of producing infectious HIV-1 virions following T cell activation (Sanchez et al., 1997; Ho et al., 2013; Bruner et al., 2016; Imamichia et al., 2016). Instead, the majority of the reservoir exists as defective provirus, unable to support HIV-1 infection due to deletions, insertions and hypermutation introduced into the genome during reverse transcription (Ho et al., 2013; Bruner et al., 2016). Despite this, viral rebound from the LR following ART cessation is rapid, leading to detectable viremia within weeks of therapy interruption (Chun et al., 1999; Davey et al., 1999). Additionally, initiating ART early in infection is not sufficient to stop the formation of the LR, suggesting the LR is established and disseminated early (Chun et al., 1998; Whitney et al., 2014; Colby et al., 2018), even in vertically infected children that started ART soon after birth (Persaud et al., 2013; Ananworanich and Robb, 2014; Giacomet et al., 2014; Tagarro et al., 2018).

Maintaining the Reservoir

The half-life of the LR is estimated to be 3.6 years in patients with sustained viral suppression, meaning that eradication of the LR is not possible within a lifetime and adherence to ART must therefore be lifelong (Siliciano et al., 2003; Crooks et al., 2015). The natural longevity of memory T cells contributes to the persistence of the LR, however, its long-term stability indicates that this pool of cells is continually replenished notwithstanding effective ART. Two mechanisms have been proposed as drivers of LR maintenance: ongoing virus replication in anatomical compartments with sub-optimal drug concentrations and/or clonal expansion of latently infected cells (Sengupta and Siliciano, 2018). Ongoing replication of HIV-1 would lead to the accumulation of genetically diverse HIV-1 provirus, integrated into various positions of the host genome, therefore,

researchers have monitored viral evolution and integration sites in ART suppressed patients to determine the mechanism of LR propagation. Separate studies have demonstrated a high proportion of genetically indistinct viral genomes, as well as identical integration sites recovered from different cells, indicating that these cells must arise from proliferation as appose to subsequent HIV-1 replication (Josefsson et al., 2013; Wagner et al., 2013, 2014; Maldarelli et al., 2014; von Stockenstrom et al., 2015; Wang Z. et al., 2018). Indeed, memory T cells are maintained by homeostatic proliferation in response to IL-7, and several studies have shown that this process drives LR persistence without inducing HIV-1 gene expression (Agosto et al., 2007; Chomont et al., 2009; Archin et al., 2012). These studies, however, do not demonstrate that the expanded viral clones are replication competent and therefore, their contribution to HIV-1 persistence is unclear. One study, in fact, revealed that of a population of 75 expanded clones, none of the proviral sequences were found to be replication competent (Cohn et al., 2015). To address this, researchers have utilized full-length sequencing approaches to demonstrate that within the replication competent proviral pool, 55–60% of viral genomes had identical sequences in different cells (Lorenzi et al., 2016; Hosmane et al., 2017). Further, a recent longitudinal analysis revealed that rebound viremia matched archival provirus that was present prior to ART initiation and during long term ART suppression (De Scheerder et al., 2019). Taken together, these studies indicate that cellular expansions play a key role in the maintenance of the replication competent viral reservoir in long-term therapy suppressed patients, providing a clear mechanism for HIV-1 persistence and a source of rebound viremia following ART cessation.

On the other hand, the contribution of low-level virus replication in anatomical compartments with sub-optimal drug concentrations, such as lymph nodes (LN), to LR maintenance is a topic of continued debate (Fletcher et al., 2014; Fukazawa et al., 2015; Lorenzo-Redondo et al., 2016; Nolan et al., 2017; Bozzi et al., 2019). Generally, most studies demonstrate little evidence of provirus evolution in ART suppressed patients, refuting the likelihood that ongoing replication is continually seeding the reservoir (Bailey et al., 2006; Chomont et al., 2009; Josefsson et al., 2013; Hiener et al., 2017; Lee et al., 2017; Van Zyl et al., 2017; Bozzi et al., 2019; De Scheerder et al., 2019). Additionally, ART intensification studies have been unable to reduce low-level viremia, suggesting that this phenomena is a result of stochastic activation of latently infected cells, rather than continued rounds of replication (Dinoso et al., 2009; McMahon et al., 2010; Anderson et al., 2011; Gandhi et al., 2012). Nevertheless, evidence from various studies has supported the hypothesis that ongoing replication takes place notwithstanding suppressive ART. Intensification of the integrase inhibitor raltegravir, for example, led to transient increases in 2-LTR circular DNA which, as products of failed integration events, suggests inhibition of new infections (Buzón et al., 2010; Hatano et al., 2013; Puertas et al., 2018). Further, evidence of virus evolution within the LN of therapy suppressed patients was also suggested as an indication of ongoing replication (Lorenzo-Redondo et al., 2016). However, two groups have reported that this is instead an artifact of rapidly decaying viral species associated with early antiretroviral treatment (Kearney et al., 2017; Rosenbloom et al., 2017).

The Hosts of the Reservoir

Critical to the elimination of HIV-1 is the elucidation of the specific anatomical and cellular reservoirs of HIV-1. Various differentiation states of CD4 T cells appear to play important roles in the establishment and maintenance of the LR as well as viral recrudescence following ART interruption (Buzon et al., 2014; Kulpa and Chomont, 2015; Banga et al., 2016; De Scheerder et al., 2019; Falcinelli et al., 2019). As discussed above, the LR is primarily hosted in memory CD4 T cells, specifically, central (T_{CM}), transitional (T_{TM}), effector memory (T_{EM}), and memory stem (T_{SCM}) cells, although the exact contribution of each cell type to the replication competent reservoir is still to be determined (Chomont et al., 2009; Buzon et al., 2014; Soriano-Sarabia et al., 2014; Banga et al., 2016, 2018; Kwon et al., 2020). Recently, CD32+ CD4 T cells have been proposed to be a major host of the LR, whereby selection of this cell population resulted in significant enrichment of inducible provirus (Descours et al., 2017; Darcis et al., 2020). Conflicting reports, however, have failed to replicate this finding and the contribution of CD32+ CD4 T cells to HIV-1 persistence and rebound remains controversial (Abdel-Mohsen et al., 2018; Badia et al., 2018; Bertagnolli et al., 2018; Martin et al., 2018; Osuna et al., 2018; Pérez et al., 2018). Nonetheless, the use of CD32 as a marker of latent infection is a topic of particular interest and may provide a mechanism by which the LR can be specifically targeted.

As well as categorizing cells based on their differentiation state, these cells can also be subdivided based on their functional properties. Accordingly, specific CD4 functional sub-sets, such as regulatory T cells (T_{reg}), Th17 cells and follicular T helper cells (T_{fh}) are now being characterized in more detail to determine which cells are the primary contributor to HIV-1 latency. T_{reg} cells modulate the immune response through regulation of T cell proliferation and differentiation whilst Th17 cells are critical to maintaining mucosal immunity via secretion of IL-17 and the balance of these two cell subsets is therefore critical in providing effective immune function (Valverde-Villegas et al., 2015). Both T_{reg} and Th17 cells have been shown to harbor a high proportion of the LR in therapy suppressed patients and as such, may be an important target in HIV-1 cure efforts (Tran et al., 2008; Alvarez et al., 2013; Sun et al., 2015; Christensen-Quick et al., 2016; Caruso et al., 2019).

Due to the inherent difficulty of sampling from tissues, most LR studies are based on the analysis of peripheral blood. In recent years, more research has focused on studying anatomical reservoirs such as lymph nodes (LN) and gut associated lymphoid tissue (GALT), as these sites are enriched in activated CD4 T cells (Chun et al., 2008; Di Mascio et al., 2009; Yukl et al., 2010; Churchill et al., 2016). Follicular T helper cells (T_{fh}), resident within the B cell follicle of LN have recently been identified as a major host of the replication competent viral reservoir (Buzon et al., 2014; Banga et al., 2016, 2019). These studies demonstrate the importance of individual anatomical and cellular hosts of the LR to HIV-1 persistence and highlight that HIV-1 eradication

studies will need to not only target these sites, but also efficiently and specifically measure the LR within these compartments.

In addition to lymphocytes, a number of other cells types such as macrophages and plasmacytoid dendritic cells (pDCs) are potential hosts of the LR, and despite being infected at a lower frequency, may play an important role in viral persistence (Centlivre et al., 2011). Studies suggest that macrophages infected with HIV-1 are resistant to cell mediated immune clearance as well as virus induced cell death and may therefore represent a significant hurdle to cure (Swingler et al., 2007; Clayton et al., 2018). Further, replication competent provirus has recently been recovered from macrophages in long-term ART suppressed patients, indicating that cure strategies targeting only lymphocytes may not be sufficient (Ganor et al., 2019). Infection of macrophages with HIV-1 can facilitate entry of virus into anatomical sanctuary sites such as the brain and central nervous system (CNS), therefore providing an additional barrier to HIV-1 eradication (Castellano et al., 2017; Wong et al., 2019).

HIV-1 CURE STRATEGIES

Progress Toward a Cure

The progress toward the development of a functional or sterilizing cure for HIV-1 has been significantly hindered by the presence of the LR. Currently, two people have been cured of HIV-1 infection, the so-called Berlin and London patients, who since receiving allogenic stem cell transplantations from CCR5Δ32/Δ32 donors, have consistently tested negative for viral rebound for over 10 and 2 years, respectively, without ART (Hutter et al., 2009; Gupta et al., 2019). In these cases, the infected cell pool was significantly depleted during pre-transplant conditioning and replaced with donor cells that are resistant to infection with R5-tropic virus due a large deletion in the CCR5 co-receptor (Liu et al., 1996). Due to the relative paucity of CCR5Δ32/Δ32 donors and the unique circumstances predetermining these cases, this type of cure is not feasible for widespread use, it does however emphasize the basic principle of HIV-1 cure; silence or eradicate the HIV-1 LR.

Shock and Kill

One of the most prominent approaches to achieve HIV-1 cure is "shock and kill"; the use of latency reversing agents (LRAs) to induce viral gene expression and productive infection in latently infected cells, exposing those cells to immune clearance or the viral cytopathic effect with the aim of reducing the size of the LR and limiting viral rebound (**Figure 1**; Deeks, 2012). A major challenge in this approach is the ability to achieve broad and efficient latency reversal without eliciting toxic side effects or global immune activation. Early latency reversal studies that utilized interleukin-2 (IL-2) to induce HIV-1 activation produced a toxic "cytokine storm" response and did not sufficiently reduce the size of the LR when the dosage was lowered to safer levels (Prins et al., 1999; Lafeuillade et al., 2001). Instead, novel LRAs induce HIV-1 gene expression either by activating cellular transcription factors, such as NF-κB, or by altering the chromatin structure of the integrated provirus. In their review, Abner and Jordan extensively list published LRAs and categorize them into six groups based on their mechanism of action as follows: histone post-translational modification modulators, non-histone chromatin modulators, NF-κB stimulators, TLR agonists, extracellular stimulators, and a miscellaneous category of unique cellular mechanisms (Abner and Jordan, 2019).

Some of the prominent LRAs currently in use in ongoing clinical trials include histone deacetylate inhibitors (HDACi) and histone methyltransferases inhibitors (HMTi), which induce HIV-1 expression by reversing epigenetic silencing (Lehrman et al., 2005; Agosto et al., 2007; Archin et al., 2012, 2014a; Delagrèverie et al., 2016; Aid et al., 2018; Abner and Jordan, 2019). Alternatively, protein kinase C (PKC) agonists (Williams et al., 2004; Perez et al., 2010; Marsden et al., 2018) and CCR5 agonists (López-Huertas et al., 2017; Madrid-Elena et al., 2018) stimulate latent HIV-1 by activating NF-κB. The use of toll like receptor (TLR) agonists as LRAs has also been explored, as they stimulate immune signaling pathways, leading to HIV-1 expression (Thibault et al., 2009; Novis et al., 2013; Alvarez-Carbonell et al., 2017). As an alternative to conventional LRAs, the use of a polyvalent HIV-1 vaccine has been proposed as a potential candidate to initiate latency reversal, based on the rationale that latently infected CD4 T cells express HIV-1 specific T cell receptors (TCR) and are therefore activated by HIV-1 antigen presentation (Pankrac et al., 2017). These molecules have so far resulted in modest viral activation *in vivo,* however, two recent studies have demonstrated potent and persistent latency reversal in mouse and SIV models in multiple tissues as well as peripheral blood: one utilized a LRA that activates the non-canonical NF-κB pathway (Nixon et al., 2020) and the other combined CD8 T cell depletion with IL-15 stimulation (McBrien et al., 2020). Evidence suggests that the capacity of different LRAs to activate HIV-1 gene expression is varied amongst different CD4 T cell subsets due to the diversity of the mechanisms that drive viral latency across these subsets (Grau-Expósito et al., 2019; Pardons et al., 2019b). Combinations of LRAs could therefore conceivably elicit more global reactivation by acting on different mechanisms that enforce viral latency, and synergy between multiple combinations of LRAs has so far been identified *in vitro* (Darcis et al., 2015; Jiang et al., 2015; Albert et al., 2017; Zaikos et al., 2018; Abner and Jordan, 2019; McBrien et al., 2020; van der Sluis et al., 2020). Nevertheless, achieving global reactivation of HIV-1 from latently infected cells is only part of the challenge; these cells must also be efficiently killed, either by the viral cytopathic effect or by cytotoxic T lymphocyte (CTL) mediated immune clearance. Currently, studies that have achieved latency reversal *in vivo* have failed to reduce the LR or increase the time to viral rebound (Xing et al., 2011; Doyon et al., 2013; Archin et al., 2014a,b, 2017; Elliott et al., 2015), indicating a deficiency in the clearance of infected cells. This impairment of the "kill" response may be due, in part, to loss of HIV-1 specific CTL responses in long-term suppressed patients (Chomont et al., 2018) that may need to be restored in order to achieve sufficient clearance of infected cells (Shan et al., 2012). Importantly, LRAs that activate HIV-1 mRNA expression may not be sufficient to induce the production of viral proteins or infectious virions, and therefore the presentation of viral antigens to CTLs via major histocompatibility complex class 1 (MHC-1)

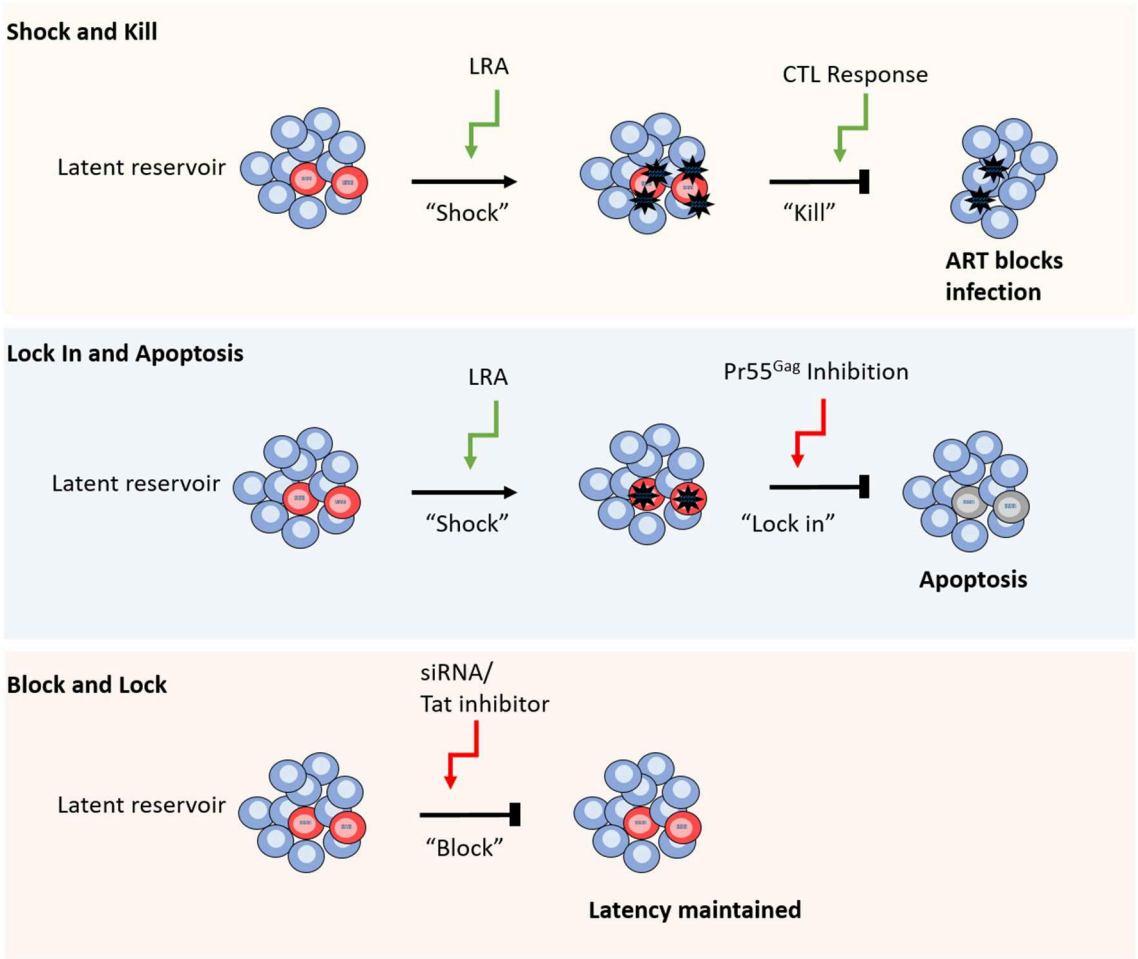


FIGURE 1 | Different strategies for HIV-1 cure. From top to bottom. Shock and kill relies on reversal of latency using a range of different compounds including TLR agonists and HDACis, followed by CTL mediated cell clearance, whilst ART blocks new infections caused by virus release. Lock in and apoptosis utilizes latency reversal agents, as well as a Pr55Gag inhibitor to block virus budding from the cell. The build-up of viral RNA and proteins leads to apoptosis of the infected cell. Block and lock approaches aim to reinforce latency mechanisms by using siRNAs or Tat inhibitors to disrupt cellular epigenetic regulators or viral replication, respectively (red cells represent HIV-1 latently infected cells).

may be limited (Clutton and Jones, 2018; Grau-Expósito et al., 2019) Additionally, treatment with LRAs may specifically inhibit the clearance of infected cells, for example, HDACis have been shown to impair CTL function and the LRA, disulfiram, may induce an anti-apoptotic state that promotes cell survival despite productive viral infection (Jones et al., 2014; Knights, 2017). Interestingly, the recent finding that CD8 T depletion could significantly enhance latency reversal indicates that CD8 T cells may block HIV-1 reactivation by LRAs (McBrien et al., 2020).

These findings emphasize the need for a more specific and potent "kill" function, such as LRAs that enhance the clearance of infected cells or combinations of treatment strategies to aid CTL function. To this end, TLR agonists offer promise due to their ability to induce a broad anti-viral response, simultaneously activating virus production and priming immune clearance of HIV-1 infected cells (Borducchi et al., 2016; Tsai et al., 2017; Lim et al., 2018; Macedo et al., 2018). To circumvent the need for CTL mediated cell clearance altogether, an alternative approach is to block the release of virions and induce apoptosis of the infected cell (Tateishi et al., 2017). In this method, a novel compound is used to inhibit HIV-1 $Pr55^{Gag}$, blocking virus budding and leading to a build-up of viral products and subsequent apoptosis of the infected cell (**Figure 1**; Tateishi et al., 2017).

Block and Lock

Recently, a novel cure strategy has been proposed that, rather than inducing latency reversal, aims to reinforce latency to prevent viral rebound following ART interruption (**Figure 1**; Mousseau et al., 2015; Méndez et al., 2018). The so called "block and lock" approach utilizes small interfering RNAs (siRNAs) to induce transcriptional gene silencing (TGS) by disrupting the regulation of chromatin structure, thereby preserving the epigenetic mechanisms that maintain HIV-1 latency (Suzuki et al., 2008; Ahlenstiel et al., 2015; Méndez et al., 2018).

Alternatively, latency may be enforced by the targeted inhibition of the HIV-1 positive regulator, Tat, to lock the viral replication cycle at transcription (Mousseau et al., 2015). Whilst these approaches offer a conceptual alternative cure mechanism to "shock and kill," their development is still in preliminary stages and is yet to be tested in human trials.

Gene Editing

The rise to prominence of gene editing tools such as CRISPR-Cas9 and zinc-finger nucleases (ZFN) has led to increased hope of a HIV-1 cure by targeting various host or viral genes to induce host resistance, enforce viral latency or silence integrated provirus. Gene editing approaches have the advantage of highly specific gene targeting, so unlike LRAs, can produce the desired outcome without global physiological impact. Nevertheless, off-target effects have been observed in a number of studies and may affect the safety of these methods (Kimberland et al., 2018). So far, the potential of ZFN targeted editing of host CCR5, to induce partial genetic resistance to HIV-1, has been tested in a clinical trial (Tebas et al., 2014). Most research, however, has focused on the use of CRISPR-Cas9 for its relatively simple approach and a number of studies have demonstrated its use in CCR5 or CXCR4 gene editing to induce host cell resistance to HIV-1 (Wang et al., 2014, 2017; Xu et al., 2017). This approach may also be used to specifically knockout or attenuate the HIV-1 provirus, for example, by targeting the LTR to disrupt viral gene expression or excise the integrated genome (Ebina et al., 2013; Hu et al., 2014; Kaminski et al., 2016; Lebbink et al., 2017; Yin et al., 2017; Bella et al., 2018; Wang Q. et al., 2018). Alternatively, various positions of the latent provirus could be targeted by CRISPR-Cas9 to induce multiple non-homologous end joining (NHEJ) associated indels that deactivate the virus through frame shift mutation (Liao et al., 2015; Ueda et al., 2016; Wang et al., 2016; Ophinni et al., 2018). Additionally, recent work has shown that, in combination with a novel drug delivery system, CRISPR-Cas9 directed editing of proviral DNA could effectively eliminate HIV-1 infection in mouse models (Dash et al., 2019). This technology could feasibly be used to target myriad steps in the viral replication cycle, however, its major limitation is its delivery, requiring viral vectors or lipid compounds, as reviewed (Xiao et al., 2019). To achieve clinically significant effects, the majority, if not all of the LR will need to be affected, which is a major challenge considering the array of anatomical compartments which host a significant proportion of latently infected cells.

Therapeutic Vaccination

Rebound viremia from latently infected cells is detectable within weeks of ART interruption, though the exact cellular and anatomical source of this rebound varies between patients (De Scheerder et al., 2019). Therefore, rather than targeting this elusive source, therapeutic vaccination aims to eliminate or significantly diminish rebound viremia by priming the host immune response to HIV-1, thereby achieving a "functional cure." In therapeutic vaccine trials, the vaccine regimen is administered during sustained ART mediated viral suppression, followed by a period of ART interruption, during which vaccine efficacy can be assessed by measuring time to viral rebound, size of the LR and the profile of the host immune response.

Therapeutic vaccines may aim to elicit narrow CTL responses to specific HIV-1 proteins, such as Gag, though the success of these approaches may be impeded by the re-emergence of CTL escape mutants that were established during primary infection (Schooley et al., 2010; Pollard et al., 2014; Deng et al., 2015). Alternatively, vaccines designed to generate a broader anti-HIV-1 immune response may be more effective. To this end, several studies have used a dendritic cell (DC) based vaccine, in which autologous DCs are pulsed with inactivated HIV-1, or transfected to produce viral proteins, with the aim of generating DCs that can efficiently stimulate T cell responses (García et al., 2011; Gandhi et al., 2016; Gay et al., 2017b). Further, a vaccine that expresses multiple HIV-1 proteins may be used to induce a multivalent immune response, and previous studies combining such vaccines with IL-2 to boost T cell survival have demonstrated moderate success, with increased time to viral rebound associated with HIV-1 specific T cell responses in vaccinated participants (Lévy et al., 2005, 2006). Of note, a recent report has demonstrated continual decreases in the proviral reservoir as well as recovery of immune function following Tat based immunization, signifying that therapeutic vaccination can improve the immune response to HIV-1 (Sgadari et al., 2019).

Despite the promise of vaccine-based approaches, no study has yet induced sustained viral remission in vaccinated patients and in their recent analysis, Davenport et al. suggest that, even with highly efficacious vaccines that block 80% of viral reactivations, rebound viremia would likely emerge within 5 weeks following ART interruption (Davenport et al., 2019). This suggests that therapeutic vaccination alone may not be sufficient to cure HIV-1 infection and that instead, combinations of cure strategies may be more effective. For example, considering that "shock and kill" strategies have so far failed to achieve meaningful reduction in the LR, combining these strategies with therapeutic vaccination may increase the efficacy of each treatment. Indeed, this principle was tested in a clinical trial where Gag based vaccination was followed by HDCAi latency reversal and though this study was able to significantly reduce the LR, rebound viremia was measured within 2 weeks (Leth et al., 2016; Tapia et al., 2017).

Novel Cure Strategies

Several novel approaches to induce sustained viral remission in treated patients have been proposed. One such method utilizes the relatively new discovery that exhausted CD4 T cells expressing immune checkpoint (IC) makers such as PD1 and CTLA-4, are a major reservoir of replication competent provirus (Banga et al., 2016; Fromentin et al., 2016; Castellano et al., 2017; McGary et al., 2017). IC markers are inhibitory receptors expressed by T cells in response to chronic viral infection to attenuate their effector function and limit tissue damage associated with long term immune activation (Boyer and Palmer, 2018). Cells expressing these markers, that are enriched in latent provirus, could therefore be specifically targeted for drug delivery or clearance using PD1, CTLA-4, or PD-L1 antibodies (Pantaleo and Levy, 2016; Gay et al., 2017a; Boyer and Palmer, 2018). To

this end, several studies have demonstrated that IC blockade can inhibit the establishment of latency *in vitro* and aid latency reversal *in vivo*, revealing its potential as an HIV-1 therapeutic (McManamy et al., 2014; Gay et al., 2017a; Evans et al., 2018; Fromentin et al., 2019; van der Sluis et al., 2020).

Alternatively, following the success of chimeric antigen receptor T cells (CAR-T) in cancer therapy, their potential to treat HIV-1 is the subject of ongoing research. CAR-T cells are autologous T cells genetically engineered to express disease specific antibodies linked to an intracellular T cell receptor domain; therefore, when re-administered to the patient can direct the CTL response to cells expressing the disease epitope (Wagner, 2018). As such, this technology could be used to direct CTL mediated clearance of HIV-1 infected cells, aiding control of the virus in the absence of therapy. Currently, several studies using anti-HIV-1 CAR-T cells have demonstrated virus-clearing function *in vitro* (Sahu et al., 2013; Liu et al., 2015; Ali et al., 2016; Hale et al., 2017; Sung et al., 2018). More recently, a multi-specific CAR-T cell demonstrated potent clearance of HIV-1 infected cells in a humanized mouse model (Anthony-Gonda et al., 2019). The use of CAR-T cells is therefore an exciting new prospect in HIV-1 therapeutics and may work synergistically with LRAs to add more killing power into the "shock and kill" approach.

As discussed earlier, myeloid cells such as macrophages are known to support virus replication and may represent an additional barrier to HIV-1 cure. The use of "shock and kill" may not be effective against these cellular reservoir as they are refractory to CTL mediated immune clearance and the viral cytopathic effect (Swingler et al., 2007; Clayton et al., 2018). To address this, researchers have demonstrated differential expression of an anti-apoptotic, long non-coding RNA (lncRNA) that promotes survival of HIV-1 infected macrophages (Boliar et al., 2019). This study also showed that inhibition of this lncRNA with small interfering RNAs (siRNAs) could induce apoptosis in HIV-1 infected macrophages, indicating the potential of targeting lncRNAs as a novel therapeutic approach to aid the clearance of the LR in all cell types (Boliar et al., 2019).

ASSAYS TO MEASURE THE SUCCESS OF HIV-1 CURE

Viral Outgrowth Assay

Assessing the efficacy of HIV-1 cure and vaccine trials requires assays that reproducibly measure different virological markers to estimate the size of the LR with limited error. This is inherently challenging because of the relatively low abundance of latently infected cells and the heterogeneity of the HIV-1 genome, though several assays have been developed to this end (**Table 1**). Additionally, very few proviruses can generate infectious virions following activation and it is difficult to quantify specifically the replication competent reservoir. The standard assay used to measure intact provirus is the functional viral outgrowth assay (VOA) (**Figure 2**; Finzi et al., 1997, 1999; Siliciano and Siliciano, 2005). In this assay, limiting dilutions of CD4 T cells are stimulated to reverse latency and drive HIV-1 expression from integrated provirus. Activation of CD4 T cells is most commonly achieved via the addition of phytohemagglutinin (PHA) and CD8 T cell depleted PBMCs or by incubation with anti-CD28/CD3 antibodies (Wong et al., 1997; Finzi et al., 1999; Siliciano and Siliciano, 2005; Laird et al., 2013; Bruner et al., 2015). Following activation, viral outgrowth is supported by incubation with CD4 T cells from HIV-1 negative donors for 2–3 weeks and measured via the detection of p24 capsid antigen ELISA. Cell positive for exponential viral replication are quantified and the frequency of cells latently infected with intact provirus is determined based on Poisson distribution and expressed as infectious units per million (IUPM) cells (Siliciano and Siliciano, 2005; Rosenbloom et al., 2015).

The original VOA provides high specificity for intact provirus but is limited by the large sample volume required, high resource cost and is susceptible to donor variation due to virus propagation in primary CD4 T cells (Bruner et al., 2015; Massanella and Richman, 2016). Several improvements of the VOA have attempted to overcome these limitations, including the use of continuous cell lines to improve reproducibility (Laird et al., 2013; Fun et al., 2017; Badia et al., 2018; Massanella et al., 2018) the use of RT PCR to detect HIV-1 RNA reducing time to read out (Laird et al., 2013) or utilizing improved p24 ELISA to increase sensitivity (Passaes et al., 2017). Recently, a novel improvement of the VOA has been described in which CD4 T cells are differentiated into effector cells to promote expression of HIV-1, enhancing cell activation and thereby increasing the sensitivity of the assay (Wonderlich et al., 2019). Additionally, an *in vivo* VOA, whereby humanized mouse models are used to support viral outgrowth, has been shown to increase sensitivity and detect virus replication in samples that were previously negative when quantified using traditional VOA (Metcalf Pate et al., 2015; Charlins et al., 2017).

The ability to distinguish intact and defective provirus has made the VOA assay the gold standard method to measure the LR, thought this assay underestimates the size of the intact LR by ∼25 to 60-fold (Ho et al., 2013; Bruner et al., 2016). Genetic characterization of cells negative for viral outgrowth has revealed the presence of intact provirus, within active transcription units that is capable of generating replication competent virions following successive rounds of PHA stimulation (Ho et al., 2013; Hosmane et al., 2017). The mechanism underpinning the initial failure of these cells to generate viral outgrowth is likely the result of the stochastic nature of virus activation (Weinberger and Weinberger, 2013), nevertheless, their presence indicates an additional hurdle in both eradicating the LR and assessing the efficacy of eradication strategies. Of note, an extensive analysis of VOA performance using the same samples across different labs has indicated significant variability of results both within batches and between labs that is more pronounced in lower IUPM samples (Rosenbloom et al., 2019). This finding may have significant implications for HIV-1 cure research, where small differences in the replication competent reservoir must be accurately and reproducibly measured to assess the efficacy of therapeutic interventions.

TABLE 1 | Different methods used to measure the latent reservoir.

	Assay	Advantages	Disadvantages	Examples
Viral outgrowth assay (VOA)	Stimulated patient CD4 T cells in limiting dilution grown with donor cells and outgrowth measured	-Only measures replication competent provirus	-Time consuming -Requires large volumes of patient material -Underestimate size of the reservoir	Finzi et al., 1997 Siliciano and Siliciano, 2005 Laird et al., 2013 Bruner et al., 2015 Fun et al., 2017 Badia et al., 2018 Massanella et al., 2018 Wonderlich et al., 2019
Total HIV-1 DNA qPCR	Measures proviral DNA from cell extracts using primers/probes in conserved regions, primarily within the LTR	-Fast time from sample collection to result -Relatively inexpensive -Small sample volume -Can be used to detect different DNA forms (2-LTR, integrated)	-Cannot distinguish between intact and defective provirus so overestimates the reservoir -Quantification relative to a standard so prone to bias -Highly specific and prone to error from primer/template mismatches	Kostrikis et al., 2002 Beloukas et al., 2009 van der Sluis et al., 2013 Munir et al., 2013 Casabianca et al., 2014 Rouzioux et al., 2014 Vandergeeten et al., 2014 Thomas et al., 2019
Integrated HIV-1 DNA	Specifically measures only integrated provirus using a primer specific to HIV-1 and to *Alu* sequences randomly dispersed in the human genome	-Measures the LR by excluding unintegrated DNA forms -Fast and relatively inexpensive	-Distances between *Alu* and HIV-1 means ∼10% of integrated provirus is measured -Heterogeneous nature of integration sites means standard design is complex	Brussel et al., 2005 Yu et al., 2008 Liszewski et al., 2009 Brady et al., 2013 Agosto et al., 2007 De Spiegelaere et al., 2014 Vandergeeten et al., 2014 Lada et al., 2018
Digital PCR	Measures frequency of proviral DNA (integrated, total or circular) by partitioning sample into limiting dilutions and assigning partitions either positive or negative	-Eliminates the need for a standard and so reduced bias (especially useful for integrated and 2-LTR circular DNA quantifications)	-More expensive and less widely available than standard qPCR methods -Suffers from false-positives inherent to the method -Setting thresholds to determine distinguish truly positive and negative partitions is difficult	De Spiegelaere et al., 2014 Henrich et al., 2012 Strain et al., 2013 Malatinkova et al., 2015 Henrich et al., 2017 Lada et al., 2018
Cell associated RNA	Measures all or different forms of cell associated RNA with the rationale that it is more likely to measure replication competent provirus than defective	-More sensitivity for replication competent provirus	-Cannot distinguish transcripts that arise from replication competent cells and defective cells	Archin et al., 2012 Pasternak et al., 2012 Shan et al., 2013 Cillo et al., 2014 Yucha et al., 2017 Massanella et al., 2018 Yukl et al., 2018
TILDA	Measures multiply spliced tat/rev transcripts following stimulation of CD4 T cells plated in limiting dilution	-Higher sensitivity for replication competent provirus -Faster, cheaper and less resources needed than VOA	Measured transcripts may arise from defective proviral genomes	Procopio et al., 2015 Frank et al., 2019 Bertoldi et al., 2020
ISH and flow cytometry	Measures mRNA and viral proteins measured following T cell activation	-Higher sensitivity for replication competent provirus -Simultaneously phenotype the cells that host the reservoir	-Does not confirm that RNA or proteins produced arise from replication competent provirus	Graf et al., 2013 Baxter et al., 2016, 2017 Martrus et al., 2016 Grau-Expósito et al., 2017 Deleage et al., 2018 Pardons et al., 2019a
IPDA	Multiplex digital PCR based assay to measure intact provirus based on the presence of two regions that are frequently mutated in the viral genome	-Enables distinction between intact and defective provirus -Faster readout than viral outgrowth assay	-Does not screen the whole genome and may therefore miss other deleterious mutations	Bruner et al., 2019
Q4PCR	Multiplex qPCR assay to assign replication competency based on presence of 4 genomic regions, confirmed by next generation sequencing if 2/4 are present	-Able to accurately distinguish intact and defective provirus -Filters out most defective provirus before using expensive sequencing	-Relatively expensive method	Gaebler et al., 2019

The advantages and disadvantages of each approach as well as prominent examples.

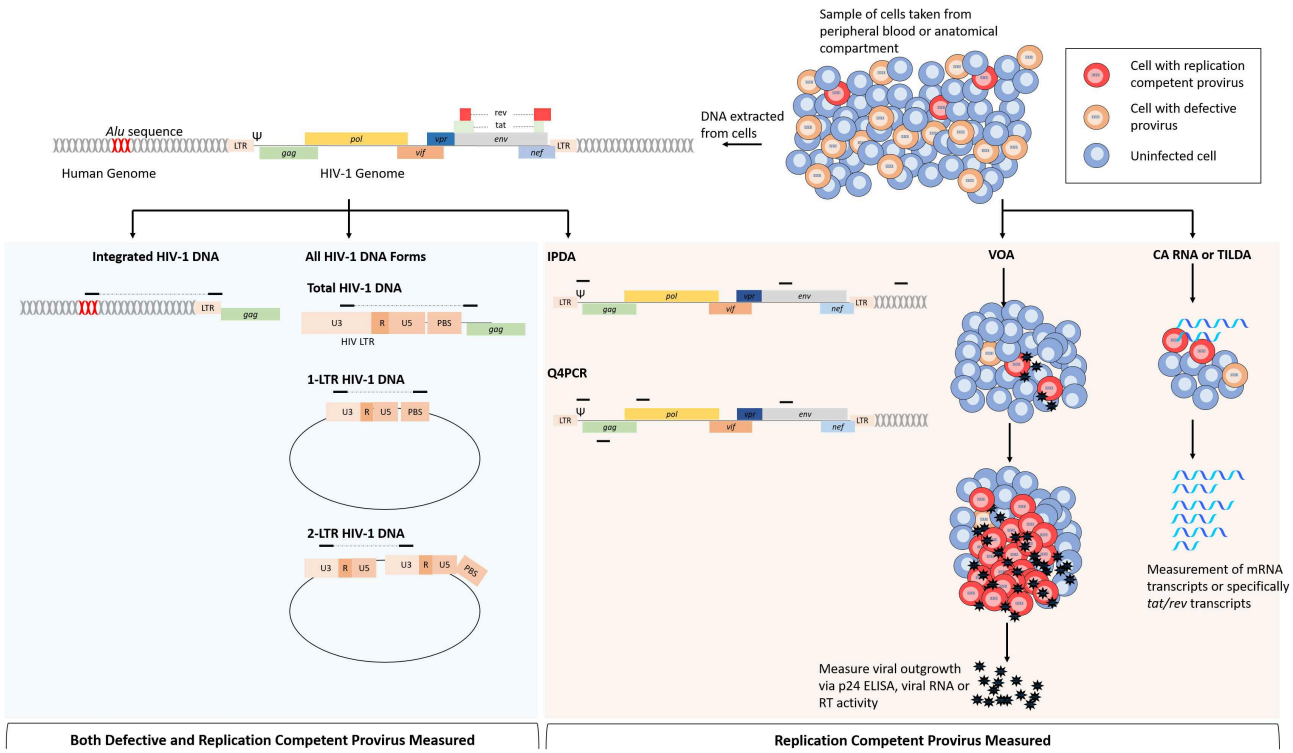


FIGURE 2 | Comparison of assays that measure replication competent provirus specifically or all provirus. Cells for analysis come from either peripheral blood or from anatomical compartments. From left to right: following DNA extraction, multiple HIV-1 DNA forms can be assayed by PCR based on the primer position. For integrated HIV-1 DNA assays, a primer targeting repeated *Alu* sequences within the human genome are paired with a HIV-1 specific primer. Total HIV-1 DNA can be measured by primers specific for regions within the viral genome, this is most commonly performed with primers targeting conserved regions within the LTR. Non-integrated HIV-1 DNA forms such as 2-LTR and 1-LTR circular DNA can be measured by primers specific that will amplify junctions that are only present in these DNA forms. The intact proviral DNA assay (IPDA) uses primers within the packaging signal (Ψ) and *env* to determine replication competence. This assay also uses primers targeting regions within the human genome to measure cell numbers and correct for DNA shearing. Replication competence is determined when both sequences are present from ddPCR. The quadruplex PCR (Q4PCR) uses primers within Ψ, *env*, *gag,* and *pol* to quantify provirus in limiting dilutions, and NGS is uses to confirm replication competence in reactions with 2/4 of the sequences present. Cell based assays use purified cell samples to measure virus or RNA production following stimulation. The viral outgrowth assay (VOA) uses limiting dilutions of CD4 T cells that are stimulated with PMA and irradiated PBMCs to induce viral gene expression; viral outgrowth is supported by incubation with HIV-1 negative donor cells and measured by p24 ELISA, viral RNA or reverse transcriptase activity. Cell associated (CA) RNA or *tat/rev* induced limiting dilution assays measure viral RNAs following HIV-1 activation, reducing time to read out when compared to the VOA. Assays in blue shaded area are not specific for cells infected with replication competent provirus because viral DNA is measured indiscriminately. Assays in shaded orange area are more specific for replication competent provirus, or in the case of the VOA, only measure replication competent provirus.

qPCR Based HIV-1 Quantification

Quantification of cell associated DNA by PCR provides a fast and relatively inexpensive marker to measure the size of the viral reservoir. HIV-1 DNA quantification methods rely on amplification of short genomic regions and so cannot distinguish intact and defective provirus and therefore vastly overestimate the size of the LR (**Figure 2**; Eriksson et al., 2013). Despite this limitation, HIV-1 DNA quantification has been shown to predict viral rebound (Williams et al., 2014) and offers the potential to identify different DNA forms, such as integrated HIV-1 DNA, non-integrated HIV-1 DNA (2-LTR and 1-LTR circular forms) or both (total HIV-1 DNA) (Mexas et al., 2012; Rouzioux and Avettand-Fenoël, 2018). Several factors affect the specificity, accuracy and reproducibly of HIV-1 DNA assays and as there is no standard method, meaningful comparison between different studies is limited. Currently, most HIV-1 DNA quantification assays utilize real-time quantitative PCR (qPCR) to measure the abundance of HIV-1 DNA relative to a calibration standard derived from cell lines harboring HIV-1 provirus. Cell lines such as 8E5 and ACH2 are widely used as the source of calibration DNA, though recent work has demonstrated that HIV-1 integration into these cell lines is unstable, likely due to ongoing replication, and their use may confound accurate quantification and reproducibility between labs (Sunshine et al., 2016; Wilburn et al., 2016; Busby et al., 2017; Symons et al., 2017; Rutsaert et al., 2018b; Thomas et al., 2019). Recent analysis of HIV-1 quantification methods has demonstrated the stability of HIV-1 integration into J-Lat 10.6, a Jurkat cell latently infected with full length, *env* deficient provirus, and suggested the use of this cell line as the gold standard for HIV-1 DNA quantification by qPCR (Sunshine et al., 2016; Thomas et al., 2019).

Another key determinant of the accuracy and specificity of HIV-1 DNA quantification assays is the genomic location at which the primers and probes anneal. The vast genetic

variation of HIV-1 both within patients and across the epidemic necessitates appropriate selection of oligonucleotides that can efficiently amplify patient samples from a variety of sub-types and circulating recombinant forms (CRFs). Prominent assays have targeted various, highly conserved regions in the HIV-1 genome including *gag* (Kabamba-Mukadi et al., 2005; Kondo et al., 2009; Li et al., 2010) and *pol* (Désiré et al., 2001; Vitone et al., 2005). Nevertheless, the LTR region has been increasingly favored for HIV-1 DNA quantification because it is both highly conserved and facilitates the distinction between all of the various HIV-1 DNA forms (Kostrikis et al., 2002; Beloukas et al., 2009; Munir et al., 2013; van der Sluis et al., 2013; Casabianca et al., 2014; Rouzioux et al., 2014; Vandergeeten et al., 2014). Recently, an extensive *in silico* analysis of published HIV-1 DNA assays revealed substantial variation between different methods, especially when comparing quantification of different HIV-1 subtypes, and indicated the best performing assays for quantification of diverse patient cohorts (Rutsaert et al., 2018b).

As discussed above, LTR based DNA assays can distinguish different HIV-1 DNA forms. During HIV-1 replication, linear unintegrated cDNA accumulates in the cell as well as abortive DNA forms such as 1-LTR and 2-LTR circular DNA, which are products of recombination events and interaction with host DNA repair mechanisms (Sloan and Wainberg, 2011; Munir et al., 2013). Because 2-LTR circular forms arise from failed integration, they are considered markers of recent infection and their quantification may therefore provide insight into the replication competent reservoir (Buzón et al., 2010; Hatano et al., 2013; Kiselinova et al., 2016). Conflicting evidence, however, suggests that these DNA forms may be persistent for long periods in latently infected cells and the clinical relevance of 2-LTR quantification remains controversial (Pierson et al., 2002).

To exclude unintegrated DNA forms from quantification, it is possible to amplify specifically integrated provirus by targeting an endogenous *Alu* sequence that are found randomly across the human genome (**Figure 2**). Generally, *Alu* PCR assays utilize a nested approach in which the junction between an HIV-1 sequence and a human *Alu* sequence is amplified, followed by qPCR with primers specific to HIV-1 (Brussel et al., 2005; Agosto et al., 2007; Liszewski et al., 2009; Brady et al., 2013; De Spiegelaere et al., 2014; Vandergeeten et al., 2014; Ruggiero et al., 2017). *Alu* PCR remains the most common approach to measure integrated HIV-1 DNA, though alternative methods have been developed as reviewed here (Liszewski et al., 2009; Ruggiero et al., 2017). Whilst the *Alu* PCR assay has been shown to correlate well with the VOA (Eriksson et al., 2013), it is hindered by limitations in accuracy and sensitivity that are inherent to the method. The random dispersion of human *Alu* sequences, as well as the heterogeneity of HIV-1 integration sites, means that the sequence length between the *Alu* and HIV-1 specific primers is unknown and variable; presenting several technical challenges that may confound accurate quantification of proviral DNA (Brady et al., 2013). Cell lines used as quantification standards, for example, are generally derived from clonal, latently infected cells and therefore do not represent the random nature of integration within a patient sample (Ruggiero et al., 2017). To overcome this issue, researchers have developed a calibration standard containing multiple integration sites to resemble more closely the sample population (Agosto et al., 2007). Alternatively, the reliance on a standard may be circumvented by the use of repetitive sampling and absolute quantification based on Poisson distribution (De Spiegelaere et al., 2014). Additionally, only 10% of integrated HIV-1 is detected by this assay because 90% of integrated provirus is too far from an *Alu* sequence to be exponentially amplified and a correction factor must therefore be applied to the quantification (Agosto et al., 2007; Yu et al., 2008; Liszewski et al., 2009; De Spiegelaere et al., 2014). Accuracy is further limited by linear amplification of unintegrated HIV-1 DNA, though the effect of this can be partially negated by simultaneous pre-amplification with only the HIV-1 specific primer to enable distinction between integrated and unintegrated DNA (O'Doherty et al., 2002; Yu et al., 2008) or by pulsed-field gel electrophoresis (PFGE) prior to amplification to remove low molecular weight DNA (Lada et al., 2018). Despite its limitations and owing to the various improvements made, quantification integrated HIV-1 via *Alu* PCR is a powerful and high-throughput method to quantify the LR. An improved *Alu* PCR assay, where the HIV-1 LTR primer is closer to the integration junction and therefore detects more integration events, is currently in development (Personal Communication).

Digital Droplet PCR Based HIV-1 Quantification

As discussed above, the selection of an appropriate calibration standard is required for quantification of HIV-1 DNA, however, quantification relative to a standard is inherently biased. Amplification efficiencies between the standard and the sample must be equal to limit bias when quantifying relative to a standard curve (Rutsaert et al., 2018a). Amplification efficiency is affected by the DNA input per reaction, the presence of inhibitory contaminants and, crucially for HIV-1 quantification, recent work has shown that small mismatches between the primer and target sequence significantly impair sample quantification (Rutsaert et al., 2018b; Thomas et al., 2019). Digital droplet PCR (ddPCR) platforms mitigate these issues by facilitating absolute quantification of a sample and as such, are becoming increasingly popular in HIV-1 research and clinical trials. In ddPCR, samples are randomly divided into multiple partitions and separately amplified, after which each partition is deemed positive or negative based on fluorescence above or below a threshold and absolute quantification is determined based on Poisson distribution (Hindson et al., 2011). In principle, the use of ddPCR to eliminate the need for a standard reduces these biases because each partition only needs to accumulate enough fluorescence to be deemed positive, so factors that reduce PCR efficiency should not impair the accuracy of quantification. The major limitation of ddPCR, however, is the difficulty to accurately determine the threshold above which a partition can be deemed positive (Rutsaert et al., 2018a). Partitions in which intermediate fluorescence is observed may be incorrectly assigned as positive or negative if the threshold selection is not sufficiently robust and a number of approaches to determine the threshold have been developed to overcome this issue, reviewed in detail here

(Rutsaert et al., 2018a). Additionally, even with robust threshold selection, ddPCR is known to suffer from a high frequency of false-positive results (Henrich et al., 2012; Strain et al., 2013; Kiselinova et al., 2014; Bosman et al., 2015; Trypsteen et al., 2015). False-positives are likely the result of combined droplets resulting in increased fluorescence or from DNA contamination that is difficult to distinguish from truly positive samples (Henrich et al., 2012; Strain et al., 2013; Kiselinova et al., 2014; Bosman et al., 2015; Trypsteen et al., 2015). Despite these limitations, the use of ddPCR has proven an invaluable tool for measuring HIV-1 DNA and has been used successfully in various studies (De Spiegelaere et al., 2014; Malatinkova et al., 2015; Henrich et al., 2017).

Bridging the Gap Between Culture and PCR Based Assays

Given that the majority of HIV-1 DNA is replication deficient, PCR based assays vastly overestimate the size of the latent reservoir (Eriksson et al., 2013; Ho et al., 2013). Conversely, the VOA is known to underestimate the size of the LR due to the presence of intact non-induced proviruses and so both methods may confound the assessment of treatment and cure strategies (Eriksson et al., 2013; Ho et al., 2013). Several assays have been developed with the aim to bridge the gap between these two types of analyses by providing a fast and relatively inexpensive method to specifically quantify only replication competent provirus. In a method conceptually similar to the VOA, cell associated (CA) HIV-1 RNA quantification following CD4 T cell activation has been used to measure the size of the inducible LR (**Figure 2**; Archin et al., 2012; Pasternak et al., 2012; Shan et al., 2013; Cillo et al., 2014; Yucha et al., 2017; Massanella et al., 2018; Yukl et al., 2018). The measurement of CA RNA provides the opportunity to quantify different transcripts and therefore, different stages of the replication cycle that may be used as a surrogate for measuring the size of the intact LR (Cillo et al., 2014; Massanella et al., 2018; Pasternak and Berkhout, 2018; Yukl et al., 2018). However, cells harboring defective provirus are still capable of producing HIV-1 mRNA following T cell activation despite being unable to generate infectious virions, and so these methods are prone to false positive results (Hermankova et al., 2003; Pasternak et al., 2009; Schmid et al., 2010; Cillo et al., 2014). By measuring cell-free HIV-1 RNA from culture supernatant, indicative of virus release from cells, as well as CA RNA, it is possible to more closely predict replication competence (Cillo et al., 2014; Massanella et al., 2018). In addition, a novel assay has addressed this issue by specifically measuring *tat/rev* multiply spliced mRNAs with the rationale that these transcripts are rarely produced in cells with defective HIV-1 provirus (**Figure 2**; Procopio et al., 2015; Frank et al., 2019; Bertoldi et al., 2020). The *tat/rev* induced limiting dilution assay (TILDA) relies on measurement of *tat/rev* transcripts from cells plated in limiting dilution, following activation with phorbol 12-myristate 13-acetate (PMA) and ionomycin (Procopio et al., 2015). Results obtained from TILDA quantification correlated well with HIV-1 DNA quantification and measures the LR close to levels predicted by Ho et al. (2013) and Procopio et al. (2015). This method, however, did not significantly correlate with results obtained from VOA and is still susceptible to overestimating the size of the LR due to the possibility that these transcripts arise from cells with defective HIV-1 genomes (Procopio et al., 2015).

Other groups have sought to quantify the replication competent reservoir using *in situ* hybridization (ISH) and flow cytometry to measure CA RNA or capsid p24 protein (Graf et al., 2013; Baxter et al., 2016, 2017; Martrus et al., 2016; Grau-Expósito et al., 2017; Deleage et al., 2018; Pardons et al., 2019a). By combining flow cytometry based quantification of CA RNA and p24 capsid protein, it is possible to measure provirus that is capable of transcription as well as protein production, providing a close surrogate for the measurement of the intact LR (Baxter et al., 2016, 2017, 2018; Martrus et al., 2016; Grau-Expósito et al., 2017; Puray-Chavez et al., 2017). An additional benefit of flow cytometry based approaches is the opportunity to simultaneously infer phenotypic characteristics of the cell populations that host the replication competent reservoir, as reviewed (Baxter et al., 2018).

More recently, a novel assay known as the intact proviral DNA assay (IPDA) has demonstrated the use of a multiplexed ddPCR approach to measure the size of the intact LR based on the presence of regions that are frequently mutated in defective genomes (**Figure 2**; Bruner et al., 2019). In this assay, intact and defective proviruses are separately quantified by amplifying regions within the HIV-1 packaging signal (Ψ) and *env* and the presence or absence of these regions is sufficient to distinguish 90% of defective genomes (Bruner et al., 2019). By determining replication competence based on DNA composition, this assay is not dependent on T cell stimulation and is therefore not impaired by the presence of non-inducible, intact proviruses that contribute to LR underestimation in the VOA (Bruner et al., 2015, 2016). Despite this, the IPDA is still only able to distinguish 90% of defective proviruses, with mutations that occur in non-amplified regions counting toward the quantification. Additionally, like all PCR based HIV-1 assays, primer mismatches in target regions may result in false negative quantifications. Similarly, Gaebler et al., recently described an approach (Q4PCR) that uses multiplexed qPCR measurement of four proviral regions; *gag, pol, env,* and Ψ, followed by next generation sequencing (NGS) of samples that are positive for two out of four regions to confirm replication competence (**Figure 2**; Gaebler et al., 2019). In comparison with IPDA, the Q4PCR method offers increased accuracy to predict replication competence due to a higher percentage of the viral genome being interrogated and likely positive samples being validated via NGS (Gaebler et al., 2019). Nevertheless, this increased sensitivity does come with the increased cost and lower throughput associated with NGS.

Previously, full-length sequencing of proviral DNA has provided invaluable insight into the composition of the LR (Ho et al., 2013) but the methods used are time consuming and technically challenging. The advent of various NGS technologies, however, has also paved the way for novel methods to measure the HIV-1 LR with relative ease and high throughput (Lambrechts et al., 2020). The use of Illumina based sequencing techniques has so far been used in LR studies to measure full-length, individual proviral sequences, helping to elucidate the

driving force of LR persistence and latency maintenance (Hiener et al., 2017; Lee et al., 2017; Einkauf et al., 2019). Further, the emergence of NGS technologies that can sequence long-reads, such as PacBio's SMRT Sequencing and Oxford Nanopore's MinION, may be employed to measure full-length proviral genomes or variant transcript forms from patient samples and are likely to lead to advances in our understanding of the LR.

CONCLUSIONS

The use of antiretroviral therapy has succeeded in reducing HIV-1 mortality but cannot eliminate the virus due to the persistent and stable LR. The global disease burden, equating to ∼36 million infected individuals of which ∼22 million have access to ART, warrants the continued search for a therapeutic approach that can either eliminate the virus or induce sustained viral remission in the absence of therapy (Sung et al., 2018). Recent advances in our understanding of the LR, its cellular and anatomical hosts and the mechanisms that facilitate its long-term persistence have contributed to renewed hope of a curative intervention for HIV-1 infection. Generally, an HIV-1 cure should eliminate the possibility of viral rebound following treatment interruption, and this relies on drastic reduction in the LR and efficient immune mediated clearance of HIV-1 infected cells.

Currently, several approaches for HIV-1 cure have been proposed and trialed to varying degrees of success. One of the most prominent cure strategies, "shock and kill," has demonstrated virus reactivation *in vivo*, but has been unable to lead to a meaningful increase in the time to viral rebound; suggesting improvement is required to aid the "killing" of infected cells. Alternative approaches, such as therapeutic vaccination, aim to prime the immune response to HIV-1 infection with the rationale that upon treatment interruption, immune mediated control of the virus will be improved. Several new technologies and approaches, such as immune checkpoint inhibitors, gene editing and CAR-T cells may offer an alternative method for cure, though currently their assessment in clinical trials is limited. An added complication in the search for an HIV-1 cure is the difficulty in accurately measuring the success of such trials. The inherent variability of the HIV-1 genome, the low frequency of latently infected cells as well as the abundance of defective provirus contribute to the complexity of LR quantification.

Rather than an improvement in the current strategies leading to a cure, it is likely that synergistic combinations of different approaches, such as the use of LRAs following therapeutic vaccination, will lead to more drastic reductions in the LR and may aid the ultimate goal of long term ART free viral remission.

AUTHOR CONTRIBUTIONS

JT conceptualized and outlined the manuscript and wrote the first draft. AR, WP, and GP contributed to editing the manuscript. All authors approved the final version.

FUNDING

This work was partly funded under EU Horizon 2020 program under grant agreement number 681032 (EHVA).

REFERENCES

Abdel-Mohsen, M., Kuri-Cervantes, L., Grau-Exposito, J., Spivak, A. M., Nell, R. A., Tomescu, C., et al. (2018). CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells. *Sci. Transl. Med.* 10:eaar6759. doi: 10.1126/scitranslmed.aar6759

Abner, E., and Jordan, A. (2019). HIV "shock and kill" therapy: in need of revision. *Antiviral Res.* 166, 19–34. doi: 10.1016/j.antiviral.2019.03.008

Agosto, L. M., Herring, M. B., Mothes, W., and Henderson, A. J. (2018). HIV-1-infected CD4+ T cells facilitate latent infection of resting CD4+ T cells through cell-cell contact. *Cell Rep.* 24, 2088–2100. doi: 10.1016/j.celrep.2018.07.079

Agosto, L. M., Yu, J. J., Dai, J., Kaletsky, R., Monie, D., and O'Doherty, U. (2007). HIV-1 integrates into resting CD4+ T cells even at low inoculums as demonstrated with an improved assay for HIV-1 integration. *Virology* 368, 60–72. doi: 10.1016/j.virol.2007.06.001

Ahlenstiel, C., Mendez, C., Lim, S. T. H., Marks, K., Turville, S., Cooper, D. A., et al. (2015). Novel RNA duplex locks HIV-1 in a latent state via chromatin-mediated transcriptional silencing. *Mol. Ther. Nucleic Acids* 4:e261. doi: 10.1038/mtna.2015.31

Aid, M., Dupuy, F. P., Moysi, E., Moir, S., Haddad, E. K., Estes, J. D., et al. (2018). Follicular CD4 T helper cells as a major HIV reservoir compartment: a molecular perspective. *Front. Immunol.* 9:895. doi: 10.3389/fimmu.2018.00895

Albert, B. J., Niu, A., Ramani, R., Marshall, G. R., Wender, P. A., Williams, R. M., et al. (2017). Combinations of isoform-targeted histone deacetylase inhibitors and bryostatin analogues display remarkable potency to activate latent HIV without global T-cell activation. *Sci. Rep.* 7:7456. doi: 10.1038/s41598-017-07814-4

Ali, A., Kitchen, S. G., Chen, I. S. Y., Ng, H. L., Zack, J. A., and Yang, O. O. (2016). HIV-1-specific chimeric antigen receptors based on broadly neutralizing antibodies. *J. Virol.* 90, 6999–7006. doi: 10.1128/JVI.00805-16

Alvarez, Y., Tuen, M., Shen, G., Nawaz, F., Arthos, J., Wolff, M. J., et al. (2013). Preferential HIV infection of CCR6+ Th17 cells is associated with higher levels of virus receptor expression and lack of CCR5 ligands. *J. Virol.* 87, 10843–10854. doi: 10.1128/JVI.01838-13

Alvarez-Carbonell, D., Garcia-Mesa, Y., Milne, S., Das, B., Dobrowolski, C., Rojas, R., et al. (2017). Toll-like receptor 3 activation selectively reverses HIV latency in microglial cells. *Retrovirology* 14:9. doi: 10.1186/s12977-017-0335-8

Ananworanich, J., and Robb, M. L. (2014). The transient HIV remission in the Mississippi baby: why is this good news? *J. Int. AIDS Soc.* 17:19859. doi: 10.7448/IAS.17.1.19859

Anderson, J. A., Archin, N. M., Ince, W., Parker, D., Wiegand, A., Coffin, J. M., et al. (2011). Clonal sequences recovered from plasma from patients with residual HIV-1 viremia and on intensified antiretroviral therapy are identical to replicating viral RNAs recovered from circulating resting CD4+ T cells. *J. Virol.* 85, 5220–5223. doi: 10.1128/JVI.00284-11

Anthony-Gonda, K., Bardhi, A., Ray, A., Flerin, N., Li, M., Chen, W., et al. (2019). Multispecific anti-HIV duoCAR-T cells display broad *in vitro* antiviral activity and potent *in vivo* elimination of HIV-infected cells in a humanized mouse model. *Sci. Transl. Med.* 11:eaav5685. doi: 10.1126/scitranslmed.aav5685

Archin, N. M., Bateson, R., Tripathy, M. K., Crooks, A. M., Yang, K.-H., Dahl, N. P., et al. (2014a). HIV-1 expression within resting CD4+ T cells after multiple doses of vorinostat. *J. Infect. Dis.* 210, 728–735. doi: 10.1093/infdis/jiu155

Archin, N. M., Kirchherr, J. L., Sung, J. A., Clutton, G., Sholtis, K., Xu, Y., et al. (2017). Interval dosing with the HDAC inhibitor vorinostat effectively reverses HIV latency. *J. Clin. Invest.* 127, 3126–3135. doi: 10.1172/JCI92684

Archin, N. M., Liberty, A. L., Kashuba, A. D., Choudhary, S. K., Kuruc, J. D., Crooks, A. M., et al. (2012). Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 487, 482–485. doi: 10.1038/nature11286

Archin, N. M., Sung, J. M., Garrido, C., Soriano-Sarabia, N., and Margolis, D. M. (2014b). Eradicating HIV-1 infection: seeking to clear a persistent pathogen. *Nat. Rev. Microbiol.* 12, 750–764. doi: 10.1038/nrmicro3352

Badia, R., Ballana, E., Castellví, M., García-Vidal, E., Pujantell, M., Clotet, B., et al. (2018). CD32 expression is associated to T-cell activation and is not a marker of the HIV-1 reservoir. *Nat. Commun.* 9:2739. doi: 10.1038/s41467-018-05157-w

Bailey, J. R., Sedaghat, A. R., Kieffer, T., Brennan, T., Lee, P. K., Wind-Rotolo, M., et al. (2006). Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating CD4+ T Cells. *J. Virol.* 80, 6441–6457. doi: 10.1128/JVI.00591-06

Baldauf, H.-M., Pan, X., Erikson, E., Schmidt, S., Daddacha, W., Burggraf, M., et al. (2012). SAMHD1 restricts HIV-1 infection in resting CD4+ T cells. *Nat. Med.* 18, 1682–1688. doi: 10.1038/nm.2964

Banga, R., Procopio, F. A., Noto, A., Pollakis, G., Cavassini, M., Ohmiti, K., et al. (2016). PD-1 (+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat. Med.* 22, 754–761. doi: 10.1038/nm.4113

Banga, R., Procopio, F. A., Ruggiero, A., Noto, A., Ohmiti, K., Cavassini, M., et al. (2018). Blood CXCR3+ CD4 T Cells are enriched in inducible replication competent HIV in aviremic antiretroviral therapy-treated individuals. *Front. Immunol.* 9:144. doi: 10.3389/fimmu.2018.00144

Banga, R., Rebecchini, C., Procopio, F. A., Noto, A., Munoz, O., Ioannidou, K., et al. (2019). Lymph node migratory dendritic cells modulate HIV-1 transcription through PD-1 engagement. *PLoS Pathog.* 15:e1007918. doi: 10.1371/journal.ppat.1007918

Baxter, A. E., Niessl, J., Fromentin, R., Richard, J., Porichis, F., Charlebois, R., et al. (2016). Single-cell characterization of viral translation-competent reservoirs in HIV-infected individuals. *Cell Host Microbe* 20, 368–380. doi: 10.1016/j.chom.2016.07.015

Baxter, A. E., Niessl, J., Fromentin, R., Richard, J., Porichis, F., Massanella, M., et al. (2017). Multiparametric characterization of rare HIV-infected cells using an RNA-flow FISH technique. *Nat. Protoc.* 12, 2029–2049. doi: 10.1038/nprot.2017.079

Baxter, A. E., O'Doherty, U., and Kaufmann, D. E. (2018). Beyond the replication-competent HIV reservoir: transcription and translation-competent reservoirs. *Retrovirology* 15:18. doi: 10.1186/s12977-018-0392-7

Bella, R., Kaminski, R., Mancuso, P., Young, W.-B., Chen, C., Sariyer, R., et al. (2018). Removal of HIV DNA by CRISPR from patient blood engrafts in humanized mice. *Mol. Ther. Nucleic Acids* 12, 275–282. doi: 10.1016/j.omtn.2018.05.021

Beloukas, A., Paraskevis, D., Haida, C., Sypsa, V., and Hatzakis, A. (2009). Development and assessment of a multiplex real-time PCR assay for quantification of human immunodeficiency virus type 1 DNA. *J. Clin. Microbiol.* 47, 2194–2199. doi: 10.1128/JCM.01264-08

Bertagnolli, L. N., White, J. A., Simonetti, F. R., Beg, S. A., Lai, J., Tomescu, C., et al. (2018). The role of CD32 during HIV-1 infection. *Nature* 561, E17–E19. doi: 10.1038/s41586-018-0494-3

Bertoldi, A., D'Urbano, V., Bon, I., Verbon, A., Rokx, C., Boucher, C., et al. (2020). Development of C-TILDA: a modified TILDA method for reservoir quantification in long term treated patients infected with subtype C HIV-1. *J. Virol. Methods* 276:113778. doi: 10.1016/j.jviromet.2019.113778

Boliar, S., Gludish, D. W., Jambo, K. C., Kamng'ona, R., Mvaya, L., Mwandumba, H. C., et al. (2019). Inhibition of the lncRNA SAF drives activation of apoptotic effector caspases in HIV-1–infected human macrophages. *Proc. Natl. Acad. Sci. U.S.A.* 116, 7431–7438. doi: 10.1073/pnas.1818662116

Borducchi, E. N., Cabral, C., Stephenson, K. E., Liu, J., Abbink, P., Ng'ang'a, D., et al. (2016). Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 540, 284–287. doi: 10.1038/nature20583

Bosman, K. J., Nijhuis, M., van Ham, P. M., Wensing, A. M. J., Vervisch, K., Vandekerckhove, L., et al. (2015). Comparison of digital PCR platforms and semi-nested qPCR as a tool to determine the size of the HIV reservoir. *Sci. Rep.* 5:13811. doi: 10.1038/srep13811

Boyer, Z., and Palmer, S. (2018). Targeting immune checkpoint molecules to eliminate latent HIV. *Front. Immunol.* 9:2339. doi: 10.3389/fimmu.2018.02339

Bozzi, G., Simonetti, F. R., Watters, S. A., Anderson, E. M., Gouzoulis, M., Kearney, M. F., et al. (2019). No evidence of ongoing HIV replication or compartmentalization in tissues during combination antiretroviral therapy: implications for HIV eradication. *Sci. Adv.* 5:eaav2045. doi: 10.1126/sciadv.aav2045

Brady, T., Kelly, B. J., Male, F., Roth, S., Bailey, A., Malani, N., et al. (2013). Quantitation of HIV DNA integration: effects of differential integration site distributions on Alu-PCR assays. *J. Virol. Methods* 189, 53–57. doi: 10.1016/j.jviromet.2013.01.004

Bruner, K. M., Hosmane, N. N., and Siliciano, R. F. (2015). Towards an HIV-1 cure: measuring the latent reservoir. *Trends Microbiol.* 23, 192–203. doi: 10.1016/j.tim.2015.01.013

Bruner, K. M., Murray, A. J., Pollack, R. A., Soliman, M. G., Laskey, S. B., Capoferri, A. A., et al. (2016). Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat. Med.* 22, 1043–1049. doi: 10.1038/nm.4156

Bruner, K. M., Wang, Z., Simonetti, F. R., Bender, A. M., Kwon, K. J., Sengupta, S., et al. (2019). A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566, 120–125. doi: 10.1038/s41586-019-0898-8

Brussel, A., Delelis, O., and Sonigo, P. (2005). "Alu-LTR real-time nested PCR assay for quantifying integrated HIV-1 DNA," in *Methods in Molecular Biology (Clifton, N.J.)*, ed T. Zhu (Totowa, NJ: Humana Press), 139–154. doi: 10.1385/1-59259-907-9:139

Busby, E., Whale, A. S., Bridget Ferns, R., Grant, P. R., Morley, G., Campbell, J., et al. (2017). Instability of 8E5 calibration standard revealed by digital PCR risks inaccurate quantification of HIV DNA in clinical samples by qPCR. *Sci. Rep.* 7:1209. doi: 10.1038/s41598-017-01221-5

Buzón, M. J., Massanella, M., Llibre, J. M., Esteve, A., Dahl, V., Puertas, M. C., et al. (2010). HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat. Med.* 16, 460–465. doi: 10.1038/nm.2111

Buzon, M. J., Sun, H., Li, C., Shaw, A., Seiss, K., Ouyang, Z., et al. (2014). HIV-1 persistence in CD4+ T cells with stem cell-like properties. *Nat. Med.* 20, 139–142. doi: 10.1038/nm.3445

Caruso, M. P., Falivene, J., Holgado, M. P., Zurita, D. H., Laufer, N., Castro, C., et al. (2019). Impact of HIV-ART on the restoration of Th17 and Treg cells in blood and female genital mucosa. *Sci. Rep.* 9:1978. doi: 10.1038/s41598-019-38547-1

Cary, D. C., Fujinaga, K., and Peterlin, B. M. (2016). Molecular mechanisms of HIV latency. *J. Clin. Invest.* 126, 448–454. doi: 10.1172/JCI80565

Casabianca, A., Orlandi, C., Canovari, B., Scotti, M., Acetoso, M., Valentini, M., et al. (2014). A real time PCR platform for the simultaneous quantification of total and extrachromosomal HIV DNA forms in blood of HIV-1 infected patients. *PLoS ONE* 9:e111919. doi: 10.1371/journal.pone.0111919

Castellano, P., Prevedel, L., and Eugenin, E. A. (2017). HIV-infected macrophages and microglia that survive acute infection become viral reservoirs by a mechanism involving Bim. *Sci. Rep.* 7:12866. doi: 10.1038/s41598-017-12758-w

Centlivre, M., Legrand, N., Steingrover, R., van der Sluis, R., Grijsen, M. L., Bakker, M., et al. (2011). Altered dynamics and differential infection profiles of lymphoid and myeloid cell subsets during acute and chronic HIV-1 infection. *J. Leukoc. Biol.* 89, 785–795. doi: 10.1189/jlb.0410231

Charlins, P., Schmitt, K., Remling-Mulder, L., Hogan, L. E., Hanhauser, E., Hobbs, K. S., et al. (2017). A humanized mouse-based HIV-1 viral outgrowth assay with higher sensitivity than *in vitro* qVOA in detecting latently infected cells from individuals on ART with undetectable viral loads. *Virology* 507, 135–139. doi: 10.1016/j.virol.2017.04.011

Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F. A., Yassine-Diab, B., et al. (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat. Med.* 15, 893–900. doi: 10.1038/nm.1972

Chomont, N., Okoye, A. A., Favre, D., and Trautmann, L. (2018). Wake me up before you go: a strategy to reduce the latent HIV reservoir. *AIDS* 32, 293–298. doi: 10.1097/QAD.0000000000001695

Christensen-Quick, A., Lafferty, M., Sun, L., Marchionni, L., DeVico, A., and Garzino-Demo, A. (2016). Human Th17 cells lack HIV-inhibitory RNases and are highly permissive to productive HIV infection. *J. Virol.* 90, 7833–7847. doi: 10.1128/JVI.02869-15

Chun, T.-W., Davey, R. T., Engel, D., Lane, H. C., and Fauci, A. S. (1999). Re-emergence of HIV after stopping therapy. *Nature* 401, 874–875. doi: 10.1038/44755

Chun, T.-W., Finzi, D., Margolick, J., Chadwick, K., Schwartz, D., and Siliciano, R. F. (1995). *In vivo* fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat. Med.* 1, 1284–1290. doi: 10.1038/nm1295-1284

Chun, T.-W., Nickle, D. C., Justement, J. S., Meyers, J. H., Roby, G., Hallahan, C. W., et al. (2008). Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. *J. Infect. Dis.* 197, 714–720. doi: 10.1086/527324

Chun, T. W., Engel, D., Berrey, M. M., Shea, T., Corey, L., and Fauci, A. S. (1998). Early establishment of a pool of latently infected, resting CD4+ T cells during primary HIV-1 infection. *Proc. Natl. Acad. Sci. U.S.A.* 95, 8869–8873. doi: 10.1073/pnas.95.15.8869

Chun, T. W., Stuyver, L., Mizell, S. B., Ehler, L. A., Mican, J. A. M., Baseler, M., et al. (1997). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13193–13197. doi: 10.1073/pnas.94.24.13193

Churchill, M. J., Deeks, S. G., Margolis, D. M., Siliciano, R. F., and Swanstrom, R. (2016). HIV reservoirs: what, where and how to target them. *Nat. Rev. Microbiol.* 14, 55–60. doi: 10.1038/nrmicro.2015.5

Cillo, A. R., Sobolewski, M. D., Bosch, R. J., Fyne, E., Piatak, M., Coffin, J. M., et al. (2014). Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7078–7083. doi: 10.1073/pnas.1402873111

Clayton, K. L., Collins, D. R., Lengieza, J., Ghebremichael, M., Dotiwala, F., Lieberman, J., et al. (2018). Resistance of HIV-infected macrophages to CD8+ T lymphocyte–mediated killing drives activation of the immune system. *Nat. Immunol.* 19, 475–486. doi: 10.1038/s41590-018-0085-3

Clutton, G. T., and Jones, R. B. (2018). Diverse impacts of HIV latency-reversing agents on CD8+ T-cell function: implications for HIV cure. *Front. Immunol.* 9:1452. doi: 10.3389/fimmu.2018.01452

Cohn, L. B., Silva, I. T., Oliveira, T. Y., Rosales, R. A., Parrish, E. H., Learn, G. H., et al. (2015). HIV-1 integration landscape during latent and active infection. *Cell* 160, 420–432. doi: 10.1016/j.cell.2015.01.020

Colby, D. J., Trautmann, L., Pinyakorn, S., Leyre, L., Pagliuzza, A., Kroon, E., et al. (2018). Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat. Med.* 24, 923–926. doi: 10.1038/s41591-018-0026-6

Crooks, A. M., Bateson, R., Cope, A. B., Dahl, N. P., Griggs, M. K., Kuruc, J. D., et al. (2015). Precise quantitation of the latent HIV-1 reservoir: implications for eradication strategies. *J. Infect. Dis.* 212, 1361–1365. doi: 10.1093/infdis/jiv218

Darcis, G., Kootstra, N. A., Hooibrink, B., van Montfort, T., Maurer, I., Groen, K., et al. (2020). CD32+CD4+ T cells are highly enriched for HIV DNA and can support transcriptional latency. *Cell Rep.* 30, 2284–2296.e3. doi: 10.1016/j.celrep.2020.01.071

Darcis, G., Kula, A., Bouchat, S., Fujinaga, K., Corazza, F., Ait-Ammar, A., et al. (2015). An in-depth comparison of latency-reversing agent combinations in various *in vitro* and *ex vivo* HIV-1 latency models identified bryostatin-1+JQ1 and ingenol-B+JQ1 to potently reactivate viral gene expression. *PLoS Pathog.* 11:e1005063. doi: 10.1371/journal.ppat.1005063

Dash, P. K., Kaminski, R., Bella, R., Su, H., Mathews, S., Ahooyi, T. M., et al. (2019). Sequential LASER ART and CRISPR treatments eliminate HIV-1 in a subset of infected humanized mice. *Nat. Commun.* 10:2753. doi: 10.1038/s41467-019-10366-y

Davenport, M. P., Khoury, D. S., Cromer, D., Lewin, S. R., Kelleher, A. D., and Kent, S. J. (2019). Functional cure of HIV: the scale of the challenge. *Nat. Rev. Immunol.* 19, 45–54. doi: 10.1038/s41577-018-0085-4

Davey, R. T., Bhat, N., Yoder, C., Chun, T. W., Metcalf, J. A., Dewar, R., et al. (1999). HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15109–15114. doi: 10.1073/pnas.96.26.15109

De Scheerder, M.-A., Vrancken, B., Dellicour, S., Schlub, T., Lee, E., Shao, W., et al. (2019). HIV rebound is predominantly fueled by genetically identical viral expansions from diverse reservoirs. *Cell Host Microbe* 26, 347–358.e7. doi: 10.1016/j.chom.2019.08.003

De Spiegelaere, W., Malatinkova, E., Lynch, L., van Nieuwerburgh, F., Messiaen, P., O'Doherty, U., et al. (2014). Quantification of integrated HIV DNA by repetitive-sampling Alu-HIV PCR on the basis of poisson statistics. *Clin. Chem.* 60, 886–895. doi: 10.1373/clinchem.2013.219378

Deeks, S. G. (2012). Shock and kill. *Nature* 487, 439–440. doi: 10.1038/487439a

Delagrèverie, H. M., Delaugerre, C., Lewin, S. R., Deeks, S. G., and Li, J. Z. (2016). Ongoing clinical trials of human immunodeficiency virus latency-reversing and immunomodulatory agents. *Open Forum Infect. Dis.* 3:ofw189. doi: 10.1093/ofid/ofw189

Deleage, C., Chan, C. N., Busman-Sahay, K., and Estes, J. D. (2018). Next-generation *in situ* hybridization approaches to define and quantify HIV and SIV reservoirs in tissue microenvironments. *Retrovirology* 15:4. doi: 10.1186/s12977-017-0387-9

Deng, K., Pertea, M., Rongvaux, A., Wang, L., Durand, C. M., Ghiaur, G., et al. (2015). Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. *Nature* 517, 381–385. doi: 10.1038/nature14053

Descours, B., Petitjean, G., López-Zaragoza, J.-L., Bruel, T., Raffel, R., Psomas, C., et al. (2017). CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature* 543, 564–567. doi: 10.1038/nature21710

Désiré, N., Dehée, A., Schneider, V., Jacomet, C., Goujon, C., Girard, P. M., et al. (2001). Quantification of human immunodeficiency virus type 1 proviral load by a TaqMan real-time PCR assay. *J. Clin. Microbiol.* 39, 1303–1310. doi: 10.1128/JCM.39.4.1303-1310.2001

Di Mascio, M., Paik, C. H., Carrasquillo, J. A., Maeng, J.-S., Jang, B.-S., Shin, I. S., et al. (2009). Noninvasive *in vivo* imaging of CD4 cells in simian-human immunodeficiency virus (SHIV)–infected nonhuman primates. *Blood* 114, 328–337. doi: 10.1182/blood-2008-12-192203

Dinoso, J. B., Kim, S. Y., Wiegand, A. M., Palmer, S. E., Gange, S. J., Cranmer, L., et al. (2009). Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9403–9408. doi: 10.1073/pnas.0903107106

Doyon, G., Zerbato, J., Mellors, J. W., and Sluis-Cremer, N. (2013). Disulfiram reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog. *AIDS* 27, F7–F11. doi: 10.1097/QAD.0b013e32835 70620

Ebina, H., Misawa, N., Kanemura, Y., and Koyanagi, Y. (2013). Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus. *Sci. Rep.* 3:2510. doi: 10.1038/srep02510

Einkauf, K. B., Lee, G. Q., Gao, C., Sharaf, R., Sun, X., Hua, S., et al. (2019). Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. *J. Clin. Invest.* 129, 988–998. doi: 10.1172/JCI124291

Eisele, E., and Siliciano, R. F. (2012). Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity* 37, 377–388. doi: 10.1016/j.immuni.2012.08.010

Elliott, J. H., McMahon, J. H., Chang, C. C., Lee, S. A., Hartogensis, W., Bumpus, N., et al. (2015). Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* 2, e520–e529. doi: 10.1016/S2352-3018(15)00226-X

Eriksson, S., Graf, E. H., Dahl, V., Strain, M. C., Yukl, S. A., Lysenko, E. S., et al. (2013). Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. *PLoS Pathog.* 9:e1003174. doi: 10.1371/journal.ppat.1003174

Evans, V. A., van der Sluis, R. M., Solomon, A., Dantanarayana, A., McNeil, C., Garsia, R., et al. (2018). Programmed cell death-1 contributes to the establishment and maintenance of HIV-1 latency. *AIDS* 32, 1491–1497. doi: 10.1097/QAD.0000000000001849

Falcinelli, S. D., Ceriani, C., Margolis, D. M., and Archin, N. M. (2019). New frontiers in measuring and characterizing the HIV reservoir. *Front. Microbiol.* 10:2878. doi: 10.3389/fmicb.2019.02878

Finzi, D., Blankson, J., Siliciano, J. D., Margolick, J. B., Chadwick, K., Pierson, T., et al. (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.* 5, 512–517. doi: 10.1038/8394

Finzi, D., Hermankova, M., Pierson, T., Carruth, L. M., Buck, C., Chaisson, R. E., et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295–1300. doi: 10.1126/science.278.5341.1295

Fletcher, C. V., Staskus, K., Wietgrefe, S. W., Rothenberger, M., Reilly, C., Chipman, J. G., et al. (2014). Persistent HIV-1 replication is associated with lower

antiretroviral drug concentrations in lymphatic tissues. *Proc. Natl. Acad. Sci. U.S.A.* 111, 2307–2312. doi: 10.1073/pnas.1318249111

Frank, I., Acharya, A., Routhu, N. K., Aravantinou, M., Harper, J. L., Maldonado, S., et al. (2019). A Tat/Rev induced limiting dilution assay to measure viral reservoirs in non-human primate models of HIV infection. *Sci. Rep.* 9:12078. doi: 10.1038/s41598-019-48354-3

Fromentin, R., Bakeman, W., Lawani, M. B., Khoury, G., Hartogensis, W., DaFonseca, S., et al. (2016). CD4+ T cells expressing PD-1, TIGIT and LAG-3 contribute to HIV persistence during ART. *PLoS Pathog.* 12:e1005761. doi: 10.1371/journal.ppat.1005761

Fromentin, R., DaFonseca, S., Costiniuk, C. T., El-Far, M., Procopio, F. A., Hecht, F. M., et al. (2019). PD-1 blockade potentiates HIV latency reversal *ex vivo* in CD4+ T cells from ART-suppressed individuals. *Nat. Commun.* 10:814. doi: 10.1038/s41467-019-08798-7

Fukazawa, Y., Lum, R., Okoye, A. A., Park, H., Matsuda, K., Bae, J. Y., et al. (2015). B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. *Nat. Med.* 21, 132–139. doi: 10.1038/nm.3781

Fun, A., Mok, H. P., Wills, M. R., and Lever, A. M. (2017). A highly reproducible quantitative viral outgrowth assay for the measurement of the replication-competent latent HIV-1 reservoir. *Sci. Rep.* 7:43231. doi: 10.1038/srep43231

Gaebler, C., Lorenzi, J. C. C., Oliveira, T. Y., Nogueira, L., Ramos, V., Lu, C.-L., et al. (2019). Combination of quadruplex qPCR and next-generation sequencing for qualitative and quantitative analysis of the HIV-1 latent reservoir. *J. Exp. Med.* 216, 2253–2264. doi: 10.1084/jem.20190896

Gandhi, R. T., Coombs, R. W., Chan, E. S., Bosch, R. J., Zheng, L., Margolis, D. M., et al. (2012). No effect of raltegravir intensification on viral replication markers in the blood of HIV-1-infected patients receiving antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* 59, 229–235. doi: 10.1097/QAI.0b013e31823fd1f2

Gandhi, R. T., Kwon, D. S., Macklin, E. A., Shopis, J. R., McLean, A. P., McBrine, N., et al. (2016). Immunization of HIV-1-infected persons with autologous dendritic cells transfected with mRNA encoding HIV-1 Gag and Nef: results of a randomized, placebo-controlled clinical Trial. *JAIDS J. Acquir. Immune Defic. Syndr.* 71, 246–53 doi: 10.1097/QAI.0000000000000852

Ganor, Y., Real, F., Sennepin, A., Dutertre, C.-A., Prevedel, L., Xu, L., et al. (2019). HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. *Nat. Microbiol.* 4, 633–644. doi: 10.1038/s41564-018-0335-z

García, F., Climent, N., Assoumou, L., Gil, C., González, N., Alcam,í, J., et al. (2011). A therapeutic dendritic cell-based vaccine for HIV-1 infection. *J. Infect. Dis.* 203, 473–478. doi: 10.1093/infdis/jiq077

Gay, C. L., Bosch, R. J., Ritz, J., Hataye, J. M., Aga, E., Tressler, R. L., et al. (2017a). Clinical trial of the anti-PD-L1 antibody BMS-936559 in HIV-1 infected participants on suppressive antiretroviral therapy. *J. Infect. Dis.* 215, 1725–1733. doi: 10.1093/infdis/jix191

Gay, C. L., DeBenedette, M. A., Tcherepanova, I. Y., Gamble, A., Lewis, W. E., Cope, A. B., et al. (2017b). Immunogenicity of AGS-004 dendritic cell therapy in patients treated during Acute HIV infection. *AIDS Res. Hum. Retroviruses* 34, 111–122. doi: 10.1089/aid.2017.0071

Giacomet, V., Trabattoni, D., Zanchetta, N., Biasin, M., Gismondo, M., Clerici, M., et al. (2014). No cure of HIV infection in a child despite early treatment and apparent viral clearance. *Lancet* 384:1320. doi: 10.1016/S0140-6736(14)61405-7

Graf, E. H., Pace, M. J., Peterson, B. A., Lynch, L. J., Chukwulebe, S. B., Mexas, A. M., et al. (2013). Gag-positive reservoir cells are susceptible to HIV-specific cytotoxic T lymphocyte mediated clearance *in vitro* and can be detected *in vivo*. *PLoS ONE* 8:e71879. doi: 10.1371/journal.pone.0071879

Grau-Expósito, J., Luque-Ballesteros, L., Navarro, J., Curran, A., Burgos, J., Ribera, E., et al. (2019). Latency reversal agents affect differently the latent reservoir present in distinct CD4+ T subpopulations. *PLoS Pathog.* 15:e1007991. doi: 10.1371/journal.ppat.1007991

Grau-Expósito, J., Serra-Peinado, C., Miguel, L., Navarro, J., Curran, A., Burgos, J., et al. (2017). A novel single-cell FISH-flow assay identifies effector memory CD4+ T cells as a major niche for HIV-1 transcription in HIV-infected patients. *MBio* 8, e00876–e00817. doi: 10.1128/mBio.00876-17

Gupta, R. K., Abdul-Jawad, S., McCoy, L. E., Mok, H. P., Peppa, D., Salgado, M., et al. (2019). HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. *Nature* 568, 244–248. doi: 10.1038/s41586-019-1027-4

Hale, M., Mesojednik, T., Romano Ibarra, G. S., Sahni, J., Bernard, A., Sommer, K., et al. (2017). Engineering HIV-resistant, anti-HIV chimeric antigen receptor T cells. *Mol. Ther.* 25, 570–579. doi: 10.1016/j.ymthe.2016.12.023

Hatano, H., Strain, M. C., Scherzer, R., Bacchetti, P., Wentworth, D., Hoh, R., et al. (2013). Increase in 2-long terminal repeat circles and decrease in D-dimer after raltegravir intensification in patients with treated HIV infection: a randomized, placebo-controlled trial. *J. Infect. Dis.* 208, 1436–1442. doi: 10.1093/infdis/jit453

Henrich, T. J., Gallien, S., Li, J. Z., Pereyra, F., and Kuritzkes, D. R. (2012). Low-level detection and quantitation of cellular HIV-1 DNA and 2-LTR circles using droplet digital PCR. *J. Virol. Methods* 186, 68–72. doi: 10.1016/j.jviromet.2012.08.019

Henrich, T. J., Hatano, H., Bacon, O., Hogan, L. E., Rutishauser, R., Hill, A., et al. (2017). HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: an observational study. *PLoS Med.* 14:e1002417. doi: 10.1371/journal.pmed.1002417

Hermankova, M., Siliciano, J. D. D., Zhou, Y., Monie, D., Chadwick, K., Margolick, J. B. B., et al. (2003). Analysis of human immunodeficiency virus type 1 gene expression in latently infected resting CD4+ T lymphocytes *in vivo*. *J. Virol.* 77, 7383–7392. doi: 10.1128/JVI.77.13.7383-7392.2003

Hiener, B., Horsburgh, B. A., Eden, J.-S., Barton, K., Schlub, T. E., Lee, E., et al. (2017). Identification of genetically intact HIV-1 proviruses in specific CD4+ T cells from effectively treated participants. *Cell Rep.* 21, 813–822. doi: 10.1016/j.celrep.2017.09.081

Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., et al. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Anal. Chem.* 83, 8604–8610. doi: 10.1021/ac202028g

Ho, Y. C., Shan, L., Hosmane, N. N., Wang, J., Laskey, S. B., Rosenbloom, D. I. S., et al. (2013). Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 155, 540–551. doi: 10.1016/j.cell.2013.09.020

Hosmane, N. N., Kwon, K. J., Bruner, K. M., Capoferri, A. A., Beg, S., Rosenbloom, D. I. S., et al. (2017). Proliferation of latently infected CD4+ T cells carrying replication-competent HIV-1: potential role in latent reservoir dynamics. *J. Exp. Med.* 214, 959–972. doi: 10.1084/jem.20170193

Hu, W., Kaminski, R., Yang, F., Zhang, Y., Cosentino, L., Li, F., et al. (2014). RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. *Proc. Natl. Acad. Sci. U.S.A.* 111, 11461–11466. doi: 10.1073/pnas.1405186111

Hutter, G., Nowak, D., Mossner, M., Ganepola, S., Müß, A., Allers, K., et al. (2009). Long-term control of HIV by CCR5 delta32/delta32 stem-cell transplantation. *N. Engl. J. Med.* 360, 692–698. doi: 10.1056/NEJMoa0802905

Imamichia, H., Dewar, R. L., Adelsberger, J. W., Rehm, C. A., O'doherty, U., Paxinos, E. E., et al. (2016). Defective HIV-1 proviruses produce novel proteincoding RNA species in HIV-infected patients on combination antiretroviral therapy. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8783–8788. doi: 10.1073/pnas.1609057113

Jiang, G., Mendes, E. A., Kaiser, P., Wong, D. P., Tang, Y., Cai, I., et al. (2015). Synergistic reactivation of latent HIV expression by ingenol-3-angelate, PEP005, targeted NF-kB signaling in combination with JQ1 induced p-TEFb activation. *PLoS Pathog.* 11:e1005066. doi: 10.1371/journal.ppat.1005066

Jones, R. B., O'Connor, R., Mueller, S., Foley, M., Szeto, G. L., Karel, D., et al. (2014). Histone deacetylase inhibitors impair the elimination of HIV-infected cells by cytotoxic T-lymphocytes. *PLoS Pathog.* 10:e1004287. doi: 10.1371/journal.ppat.1004287

Josefsson, L., von Stockenstrom, S., Faria, N. R., Sinclair, E., Bacchetti, P., Killian, M., et al. (2013). The HIV-1 reservoir in eight patients on long-term suppressive antiretroviral therapy is stable with few genetic changes over time. *Proc. Natl. Acad. Sci. U.S.A.* 110, E4987–E4996. doi: 10.1073/pnas.1308313110

Kabamba-Mukadi, B., Henrivaux, P., Ruelle, J., Delferrière, N., Bodéus, M., and Goubau, P. (2005). Human immunodeficiency virus type 1 (HIV-1) proviral DNA load in purified CD4+ cells by lightcycler® real-time PCR. *BMC Infect. Dis.* 5:15. doi: 10.1186/1471-2334-5-15

Kaminski, R., Bella, R., Yin, C., Otte, J., Ferrante, P., Gendelman, H. E., et al. (2016). Excision of HIV-1 DNA by gene editing: a proof-of-concept *in vivo* study. *Gene Ther.* 23, 690–695. doi: 10.1038/gt.2016.41

Kearney, M. F., Wiegand, A., Shao, W., McManus, W. R., Bale, M. J., Luke, B., et al. (2017). Ongoing HIV replication during ART reconsidered. *Open Forum Infect. Dis.* 4:ofx173. doi: 10.1093/ofid/ofx173

Kimberland, M. L., Hou, W., Alfonso-Pecchio, A., Wilson, S., Rao, Y., Zhang, S., et al. (2018). Strategies for controlling CRISPR/Cas9 off-target effects and biological variations in mammalian genome editing experiments. *J. Biotechnol.* 284, 91–101. doi: 10.1016/j.jbiotec.2018.08.007

Kinoshita, S., Chen, B. K., Kaneshima, H., and Nolan, G. P. (1998). Host control of HIV-1 parasitism in T cells by the nuclear factor of activated T cells. *Cell* 95, 595–604. doi: 10.1016/S0092-8674(00)81630-X

Kiselinova, M., de Spiegelaere, W., Buzon, M. J., Malatinkova, E., Lichterfeld, M., and Vandekerckhove, L. (2016). Integrated and total HIV-1 DNA predict *ex vivo* viral outgrowth. *PLoS Pathog.* 12:e1005472. doi: 10.1371/journal.ppat.1005472

Kiselinova, M., Pasternak, A. O., de Spiegelaere, W., Vogelaers, D., Berkhout, B., and Vandekerckhove, L. (2014). Comparison of droplet digital PCR and seminested real-time PCR for quantification of cell-associated HIV-1 RNA. *PLoS ONE* 9:e85999. doi: 10.1371/journal.pone.0085999

Knights, H. D. J. (2017). A critical review of the evidence concerning the HIV latency reversing effect of disulfiram, the possible explanations for its inability to reduce the size of the latent reservoir *in vivo,* and the caveats associated with its use in practice. *AIDS Res. Treat.* 2017:8239428. doi: 10.1155/2017/8239428

Kondo, M., Sudo, K., Tanaka, R., Sano, T., Sagara, H., Iwamuro, S., et al. (2009). Quantitation of HIV-1 group M proviral DNA using TaqMan MGB real-time PCR. *J. Virol. Methods* 157, 141–146. doi: 10.1016/j.jviromet.2008.12.006

Kostrikis, L. G., Touloumi, G., Karanicolas, R., Pantazis, N., Anastassopoulou, C., Karafoulidou, A., et al. (2002). Quantitation of human immunodeficiency virus type 1 DNA forms with the second template switch in peripheral blood cells predicts disease progression independently of plasma RNA load. *J. Virol.* 76, 10099–10108. doi: 10.1128/JVI.76.20.10099-10108.2002

Kulpa, D. A., and Chomont, N. (2015). HIV persistence in the setting of antiretroviral therapy: when, where and how does HIV hide? *J. Virus Erad.* 1, 59–66.

Kwon, K. J., Timmons, A. E., Sengupta, S., Simonetti, F. R., Zhang, H., Hoh, R., et al. (2020). Different human resting memory CD4+ T cell subsets show similar low inducibility of latent HIV-1 proviruses. *Sci. Transl. Med.* 12:eaax6795. doi: 10.1126/scitranslmed.aax6795

Lada, S. M., Huang, K., VanBelzen, D. J., Montaner, L. J., O'Doherty, U., and Richman, D. D. (2018). Quantitation of integrated HIV provirus by pulsed-field gel electrophoresis and droplet digital PCR. *J. Clin. Microbiol.* 56:e01158–e01118. doi: 10.1128/JCM.01158-18

Lafeuillade, A., Poggi, C., Chadapaud, S., Hittinger, G., Chouraqui, M., Pisapia, M., et al. (2001). Pilot study of a combination of highly active antiretroviral therapy and cytokines to induce HIV-1 remission. *J. Acquir. Immune Defic. Syndr.* 26, 44–55. doi: 10.1097/00126334-200101010-00006

Laird, G. M., Eisele, E. E., Rabi, S. A., Lai, J., Chioma, S., Blankson, J. N., et al. (2013). Rapid quantification of the latent reservoir for HIV-1 using a viral outgrowth assay. *PLoS Pathog.* 9:e1003398. doi: 10.1371/journal.ppat.1003398

Lambrechts, L., Cole, B., Rutsaert, S., Trypsteen, W., and Vandekerckhove, L. (2020). Emerging PCR-based techniques to study HIV-1 reservoir persistence. *Viruses* 12:149. doi: 10.3390/v12020149

Lassen, K. G., Hebbeler, A. M., Bhattacharyya, D., Lobritz, M. A., and Greene, W. C. (2012). A flexible model of HIV-1 latency permitting evaluation of many primary CD4 T-cell reservoirs. *PLoS ONE* 7:e30176. doi: 10.1371/journal.pone.0030176

Lebbink, R. J., de Jong, D. C. M., Wolters, F., Kruse, E. M., van Ham, P. M., Wiertz, E. J. H. J., et al. (2017). A combinational CRISPR/Cas9 gene-editing approach can halt HIV replication and prevent viral escape. *Sci. Rep.* 7:41968. doi: 10.1038/srep41968

Lee, G. Q., Orlova-Fink, N., Einkauf, K., Chowdhury, F. Z., Sun, X., Harrington, S., et al. (2017). Clonal expansion of genome-intact HIV-1 in functionally polarized Th1 CD4+ T cells. *J. Clin. Invest.* 127, 2689–2696. doi: 10.1172/JCI93289

Lehrman, G., Hogue, I. B., Palmer, S., Jennings, C., Spina, C. A., Wiegand, A., et al. (2005). Depletion of latent HIV-1 infection *in vivo*: a proof-of-concept study. *Lancet* 366, 549–555. doi: 10.1016/S0140-6736(05)67098-5

Leth, S., Schleimann, M. H., Nissen, S. K., Højen, J. F., Olesen, R., Graversen, M. E., et al. (2016). Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial. *Lancet HIV* 3, e463–e472. doi: 10.1016/S2352-3018(16)30055-8

Lévy, Y., Durier, C., Lascaux, A.-S., Meiffrédy, V., Gahéry-Ségard, H., Goujard, C., et al. (2006). Sustained control of viremia following therapeutic immunization in chronically HIV-1-infected individuals. *AIDS* 20, 405–413. doi: 10.1097/01.aids.0000206504.09159.d3

Lévy, Y., Gahéry-Ségard, H., Durier, C., Lascaux, A.-S., Goujard, C., Meiffrédy, V., et al. (2005). Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. *AIDS* 19, 279–286.

Li, P., Ruel, T., Fujimoto, K., Hatano, H., Yukl, S., Eller, L. A., et al. (2010). Novel application of locked nucleic acid chemistry for a Taqman assay for measuring diverse human immunodeficiency virus type 1 subtypes. *J. Virol. Methods* 170, 115–120. doi: 10.1016/j.jviromet.2010.09.011

Liao, H.-K., Gu, Y., Diaz, A., Marlett, J., Takahashi, Y., Li, M., et al. (2015). Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. *Nat. Commun.* 6:6413. doi: 10.1038/ncomms7413

Lim, S.-Y., Osuna, C. E., Hraber, P. T., Hesselgesser, J., Gerold, J. M., Barnes, T. L., et al. (2018). TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci. Transl. Med.* 10:eaao4521. doi: 10.1126/scitranslmed.aao4521

Liszewski, M. K., Yu, J. J., and O'Doherty, U. (2009). Detecting HIV-1 integration by repetitive-sampling Alu-gag PCR. *Methods* 47, 254–260. doi: 10.1016/j.ymeth.2009.01.002

Liu, J., Perkins, N. D., Schmid, R. M., and Nabel, G. J. (1992). Specific NF-kappa B subunits act in concert with Tat to stimulate human immunodeficiency virus type 1 transcription. *J. Virol.* 66, 3883–3887. doi: 10.1128/JVI.66.6.3883-3887.1992

Liu, L., Patel, B., Ghanem, M. H., Bundoc, V., Zheng, Z., Morgan, R. A., et al. (2015). Novel CD4-based bispecific chimeric antigen receptor designed for enhanced anti-HIV potency and absence of HIV entry receptor activity. *J. Virol.* 89, 6685–6694. doi: 10.1128/JVI.00474-15

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., et al. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86, 367–377. doi: 10.1016/S0092-8674(00)80110-5

López-Huertas, M. R., Jiménez-Tormo, L., Madrid-Elena, N., Gutiérrez, C., Rodríguez-Mora, S., Coiras, M., et al. (2017). The CCR5-antagonist maraviroc reverses HIV-1 latency *in vitro* alone or in combination with the PKC-agonist Bryostatin-1. *Sci. Rep.* 7:2385. doi: 10.1038/s41598-017-02634-y

Lorenzi, J. C. C., Cohen, Y. Z., Cohn, L. B., Kreider, E. F., Barton, J. P., Learn, G. H., et al. (2016). Paired quantitative and qualitative assessment of the replication-competent HIV-1 reservoir and comparison with integrated proviral DNA. *Proc. Natl. Acad. Sci. U.S.A.* 113, E7908–E7916. doi: 10.1073/pnas.1617789113

Lorenzo-Redondo, R., Fryer, H. R., Bedford, T., Kim, E.-Y., Archer, J., Kosakovsky Pond, S. L., et al. (2016). Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 530, 51–56. doi: 10.1038/nature16933

Macedo, A. B., Novis, C. L., de Assis, C. M., Sorensen, E. S., Moszczynski, P., Huang, S., et al. (2018). Dual TLR2 and TLR7 agonists as HIV latency-reversing agents. *JCI Insight* 3:e122673. doi: 10.1172/jci.insight.122673

Madrid-Elena, N., García-Bermejo, M. L., Serrano-Villar, S., Díaz-de Santiago, A., Sastre, B., Gutiérrez, C., et al. (2018). Maraviroc Is associated with latent HIV-1 reactivation through NF-κB activation in resting CD4+ T cells from HIV-infected individuals on suppressive antiretroviral therapy. *J. Virol.* 92:e01931-17. doi: 10.1128/JVI.01931-17

Malatinkova, E., de Spiegelaere, W., Bonczkowski, P., Kiselinova, M., Vervisch, K., Trypsteen, W., et al. (2015). Impact of a decade of successful antiretroviral therapy initiated at HIV-1 seroconversion on blood and rectal reservoirs. *Elife* 4:e09115. doi: 10.7554/eLife.09115

Maldarelli, F., Wu, X., Su, L., Simonetti, F. R., Shao, W., Hill, S., et al. (2014). Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. *Science* 345, 179–183. doi: 10.1126/science.1254194

Marsden, M. D., Wu, X., Navab, S. M., Loy, B. A., Schrier, A. J., DeChristopher, B. A., et al. (2018). Characterization of designed, synthetically accessible bryostatin analog HIV latency reversing agents. *Virology* 520, 83–93. doi: 10.1016/j.virol.2018.05.006

Martin, G. E., Pace, M., Thornhill, J. P., Phetsouphanh, C., Meyerowitz, J., Gossez, M., et al. (2018). CD32-expressing CD4 T cells are phenotypically

diverse and can contain proviral HIV DNA. *Front. Immunol.* 9:928. doi: 10.3389/fimmu.2018.00928

Martrus, G., Niehrs, A., Cornelis, R., Rechtien, A., García-Beltran, W., Lütgehetmann, M., et al. (2016). Kinetics of HIV-1 latency reversal quantified on the single-cell level using a novel flow-based technique. *J. Virol.* 90, 9018–9028. doi: 10.1128/JVI.01448-16

Massanella, M., and Richman, D. D. (2016). Measuring the latent reservoir *in vivo*. *J. Clin. Invest.* 126, 464–472. doi: 10.1172/JCI80567

Massanella, M., Yek, C., Lada, S. M., Nakazawa, M., Shefa, N., Huang, K., et al. (2018). Improved assays to measure and characterize the inducible HIV reservoir. *EBiomedicine* 36, 113–121. doi: 10.1016/j.ebiom.2018.09.036

McBrien, J. B., Mavigner, M., Franchitti, L., Smith, S. A., White, E., Tharp, G. K., et al. (2020). Robust and persistent reactivation of SIV and HIV by N-803 and depletion of CD8+ cells. *Nature* 578, 154–159. doi: 10.1038/s41586-020-1946-0

McGary, C. S., Deleage, C., Harper, J., Micci, L., Ribeiro, S. P., Paganini, S., et al. (2017). CTLA-4+PD-1– memory CD4+ T cells critically contribute to viral persistence in antiretroviral therapy-suppressed, siv-infected rhesus macaques. *Immunity* 47, 776–788.e5. doi: 10.1016/j.immuni.2017.09.018

McMahon, D., Jones, J., Wiegand, A., Gange, S. J., Kearney, M., Palmer, S., et al. (2010). Short-course Raltegravir intensification does not reduce persistent low-level viremia in patients with HIV-1 suppression during receipt of combination antiretroviral therapy. *Clin. Infect. Dis.* 50, 912–919. doi: 10.1086/650749

McManamy, M. E. M., Hakre, S., Verdin, E. M., and Margolis, D. M. (2014). Therapy for latent HIV-1 infection: the role of histone deacetylase inhibitors. *Antivir. Chem. Chemother.* 23, 145–149. doi: 10.3851/IMP2551

Méndez, C., Ledger, S., Petoumenos, K., Ahlenstiel, C., and Kelleher, A. D. (2018). RNA-induced epigenetic silencing inhibits HIV-1 reactivation from latency. *Retrovirology* 15:67. doi: 10.1186/s12977-018-0451-0

Metcalf Pate, K. A., Pohlmeyer, C. W., Walker-Sperling, V. E., Foote, J. B., Najarro, K. M., Cryer, C. G., et al. (2015). A murine viral outgrowth assay to detect residual HIV type 1 in patients with undetectable viral loads. *J. Infect. Dis.* 212, 1387–1396. doi: 10.1093/infdis/jiv230

Mexas, A. M., Graf, E. H., Pace, M. J., Yu, J. J., Papasavvas, E., Azzoni, L., et al. (2012). Concurrent measures of total and integrated HIV DNA monitor reservoirs and ongoing replication in eradication trials. *AIDS* 26, 2295–2306. doi: 10.1097/QAD.0b013e32835a5c2f

Mousseau, G., Kessing, C. F., Fromentin, R., Trautmann, L., Chomont, N., and Valente, S. T. (2015). The tat inhibitor didehydro-cortistatin a prevents HIV-1 reactivation from latency. *MBio* 6:e00465–e00415. doi: 10.1128/mBio.00465-15

Munir, S., Thierry, S., Subra, F., Deprez, E., and Delelis, O. (2013). Quantitative analysis of the time-course of viral DNA forms during the HIV-1 life cycle. *Retrovirology* 10:87. doi: 10.1186/1742-4690-10-87

Mylvaganam, G. H., Silvestri, G., and Amara, R. R. (2015). HIV therapeutic vaccines: moving towards a functional cure. *Curr. Opin. Immunol.* 35, 1–8. doi: 10.1016/j.coi.2015.05.001

Nixon, C. C., Mavigner, M., Sampey, G. C., Brooks, A. D., Spagnuolo, R. A., Irlbeck, D. M., et al. (2020). Systemic HIV and SIV latency reversal via non-canonical NF-κB signalling *in vivo*. *Nature.* 578, 160–165. doi: 10.1038/s41586-020-1951-3

Nolan, D. J., Rose, R., Rodriguez, P. H., Salemi, M., Singer, E. J., Lamers, S. L., et al. (2017). The spleen is an HIV-1 sanctuary during combined antiretroviral therapy. *AIDS Res. Hum. Retroviruses* 34, 123–125. doi: 10.1089/aid.2017.0254

Novis, C. L., Archin, N. M., Buzon, M. J., Verdin, E., Round, J. L., Lichterfeld, M., et al. (2013). Reactivation of latent HIV-1 in central memory CD4+T cells through TLR-1/2 stimulation. *Retrovirology* 10, 119. doi: 10.1186/1742-4690-10-119

O'Doherty, U., Swiggard, W. J., Jeyakumar, D., McGain, D., and Malim, M. H. (2002). A sensitive, quantitative assay for human immunodeficiency virus Type 1 integration. *J. Virol.* 76, 10942–10950. doi: 10.1128/JVI.76.21.10942-10950.2002

Ophinni, Y., Inoue, M., Kotaki, T., and Kameoka, M. (2018). CRISPR/Cas9 system targeting regulatory genes of HIV-1 inhibits viral replication in infected T-cell cultures. *Sci. Rep.* 8:7784. doi: 10.1038/s41598-018-26190-1

Osuna, C. E., Lim, S.-Y., Kublin, J. L., Apps, R., Chen, E., Mota, T. M., et al. (2018). Evidence that CD32a does not mark the HIV-1 latent reservoir. *Nature* 561, E20–E28. doi: 10.1038/s41586-018-0495-2

Pankrac, J., Klein, K., and Mann, J. F. S. (2017). Eradication of HIV-1 latent reservoirs through therapeutic vaccination. *AIDS Res. Ther.* 14:45. doi: 10.1186/s12981-017-0177-4

Pantaleo, G., and Levy, Y. (2016). Therapeutic vaccines and immunological intervention in HIV infection: a paradigm change. *Curr. Opin. HIV AIDS* 11, 576–584. doi: 10.1097/COH.0000000000000324

Pardons, M., Baxter, A. E., Massanella, M., Pagliuzza, A., Fromentin, R., Dufour, C., et al. (2019a). Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection. *PLoS Pathog.* 15:e1007619. doi: 10.1371/journal.ppat.1007619

Pardons, M., Fromentin, R., Pagliuzza, A., Routy, J.-P., and Chomont, N. (2019b). Latency-Reversing agents induce differential responses in distinct memory CD4 T cell subsets in individuals on antiretroviral therapy. *Cell Rep.* 29, 2783–2795.e5. doi: 10.1016/j.celrep.2019.10.101

Passaes, C. P. B., Bruel, T., Decalf, J., David, A., Angin, M., Monceaux, V., et al. (2017). Ultrasensitive HIV-1 p24 assay detects single infected cells and differences in reservoir induction by latency reversal agents. *J. Virol.* 91, e02296–e02216. doi: 10.1128/JVI.02296-16

Pasternak, A. O., and Berkhout, B. (2018). What do we measure when we measure cell-associated HIV RNA. *Retrovirology* 15:13. doi: 10.1186/s12977-018-0397-2

Pasternak, A. O., de Bruin, M., Jurriaans, S., Bakker, M., Berkhout, B., Prins, J. M., et al. (2012). Modest nonadherence to antiretroviral therapy promotes residual HIV-1 replication in the absence of virological rebound in plasma. *J. Infect. Dis.* 206, 1443–1452. doi: 10.1093/infdis/jis502

Pasternak, A. O., Jurriaans, S., Bakker, M., Prins, J. M., Berkhout, B., and Lukashov, V. V. (2009). Cellular levels of HIV unspliced RNA from patients on combination antiretroviral therapy with undetectable plasma viremia predict the therapy outcome. *PLoS ONE* 4:e8490. doi: 10.1371/journal.pone.0008490

Perelson, A. S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., et al. (1997). Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 387, 188–191. doi: 10.1038/387188a0

Pérez, L., Anderson, J., Chipman, J., Thorkelson, A., Chun, T.-W., Moir, S., et al. (2018). Conflicting evidence for HIV enrichment in CD32+ CD4 T cells. *Nature* 561, E9–E16. doi: 10.1038/s41586-018-0493-4

Perez, M., de Vinuesa, A. G., Sanchez-Duffhues, G., Marquez, N., Bellido, M. L., Munoz-Fernandez, M. A., et al. (2010). Bryostatin-1 synergizes with histone deacetylase inhibitors to reactivate HIV-1 from latency. *Curr. HIV Res.* 8, 418–429. doi: 10.2174/157016210793499312

Persaud, D., Gay, H., Ziemniak, C., Chen, Y. H., Piatak, M. Jr., Chun, T.-W., et al. (2013). Absence of detectable HIV-1 viremia after treatment cessation in an infant. *N. Engl. J. Med.* 369, 1828–1835. doi: 10.1056/NEJMoa1302976

Pierson, T., Hoffman, T. L., Blankson, J., Finzi, D., Chadwick, K., Margolick, J. B., et al. (2000). Characterization of chemokine receptor utilization of viruses in the latent reservoir for human immunodeficiency virus Type 1. *J. Virol.* 74, 7824–7833. doi: 10.1128/JVI.74.17.7824-7833.2000

Pierson, T. C., Kieffer, T. L., Ruff, C. T., Buck, C., Gange, S. J., and Siliciano, R. F. (2002). Intrinsic stability of episomal circles formed during human immunodeficiency virus type 1 replication. *J. Virol.* 76, 4138–4144. doi: 10.1128/JVI.76.8.4138-4144.2002

Plesa, G., Dai, J., Baytop, C., Riley, J. L., June, C. H., and O'Doherty, U. (2007). Addition of deoxynucleosides enhances human immunodeficiency virus type 1 integration and 2LTR formation in resting CD4+ T cells. *J. Virol.* 81, 13938–13942. doi: 10.1128/JVI.01745-07

Pollard, R. B., Rockstroh, J. K., Pantaleo, G., Asmuth, D. M., Peters, B., Lazzarin, A., et al. (2014). Safety and efficacy of the peptide-based therapeutic vaccine for HIV-1, Vacc-4×: a phase 2 randomised, double-blind, placebo-controlled trial. *Lancet Infect. Dis.* 14, 291–300. doi: 10.1016/S1473-3099(13)70343-8

Prins, J. M., Jurriaans, S., van Praag, R. M. E., Blaak, H., van Rij, R., Schellekens, P. T. A., et al. (1999). Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. *AIDS* 13, 2405–2410. doi: 10.1097/00002030-199912030-00012

Procopio, F. A., Fromentin, R., Kulpa, D. A., Brehm, J. H., Bebin, A. G., Strain, M. C., et al. (2015). A novel assay to measure the magnitude of the inducible viral reservoir in HIV-infected individuals. *EBiomedicine* 2, 874–883. doi: 10.1016/j.ebiom.2015.06.019

Puertas, M. C., Gómez-Mora, E., Santos, J. R., Moltó, J., Urrea, V., Morón-López, S., et al. (2018). Impact of intensification with raltegravir on HIV-1-infected

individuals receiving monotherapy with boosted PIs. *J. Antimicrob. Chemother.* 73, 1940–1948. doi: 10.1093/jac/dky106

Puray-Chavez, M., Tedbury, P. R., Huber, A. D., Ukah, O. B., Yapo, V., Liu, D., et al. (2017). Multiplex single-cell visualization of nucleic acids and protein during HIV infection. *Nat. Commun.* 8:1882. doi: 10.1038/s41467-017-01693-z

Rosenbloom, D. I. S., Bacchetti, P., Stone, M., Deng, X., Bosch, R. J., Richman, D. D., et al. (2019). Assessing intra-lab precision and inter-lab repeatability of outgrowth assays of HIV-1 latent reservoir size. *PLoS Comput. Biol.* 15:e1006849. doi: 10.1371/journal.pcbi.1006849

Rosenbloom, D. I. S., Elliott, O., Hill, A. L., Henrich, T. J., Siliciano, J. M., and Siliciano, R. F. (2015). Designing and interpreting limiting dilution assays: general principles and applications to the latent reservoir for human immunodeficiency virus-1. *Open Forum Infect. Dis.* 2:ofv123. doi: 10.1093/ofid/ofv123

Rosenbloom, D. I. S., Hill, A. L., Laskey, S. B., and Siliciano, R. F. (2017). Re-evaluating evolution in the HIV reservoir. *Nature* 551, E6–E9. doi: 10.1038/nature24634

Rouzioux, C., and Avettand-Fenoël, V. (2018). Total HIV DNA: a global marker of HIV persistence. *Retrovirology* 15:30. doi: 10.1186/s12977-018-0412-7

Rouzioux, C., Mélard, A., and Avéttand-Fénoël, V. (2014). "Quantification of total HIV1-DNA in peripheral blood mononuclear cells," in *Methods in Molecular Biology*, eds E. Vicenzi and G. Poli (Totowa, NJ: Humana Press), 261–270. doi: 10.1007/978-1-62703-670-2_21

Ruggiero, A., Malatinkova, E., Rutsaert, S., Paxton, W. A., Vandekerckhove, L., and de Spiegelaere, W. (2017). Utility of integrated HIV-1 DNA quantification in cure studies. *Future Virol.* 12, 215–225. doi: 10.2217/fvl-2016-0130

Rutsaert, S., Bosman, K., Trypsteen, W., Nijhuis, M., and Vandekerckhove, L. (2018a). Digital PCR as a tool to measure HIV persistence. *Retrovirology* 15:16. doi: 10.1186/s12977-018-0399-0

Rutsaert, S., De Spiegelaere, W., Van Hecke, C., De Scheerder, M.-A., Kiselinova, M., Vervisch, K., et al. (2018b). In-depth validation of total HIV-1 DNA assays for quantification of various HIV-1 subtypes. *Sci. Rep.* 8:17274. doi: 10.1038/s41598-018-35403-6

Sahu, G. K., Sango, K., Selliah, N., Ma, Q., Skowron, G., and Junghans, R. P. (2013). Anti-HIV designer T cells progressively eradicate a latently infected cell line by sequentially inducing HIV reactivation then killing the newly gp120-positive cells. *Virology* 446, 268–275. doi: 10.1016/j.virol.2013.08.002

Sanchez, G., Xu, X., Chermann, J. C., and Hirsch, I. (1997). Accumulation of defective viral genomes in peripheral blood mononuclear cells of human immunodeficiency virus type 1-infected individuals. *J. Virol.* 71, 2233–2240. doi: 10.1128/JVI.71.3.2233-2240.1997

Schmid, A., Gianella, S., von Wyl, V., Metzner, K. J., Scherrer, A. U., Niederöst, B., et al. (2010). Profound depletion of HIV-1 transcription in patients initiating antiretroviral therapy during acute infection. *PLoS ONE* 5:e13310. doi: 10.1371/journal.pone.0013310

Schooley, R. T., Spritzler, J., Wang, H., Lederman, M. M., Havlir, D., Kuritzkes, D. R., et al. (2010). AIDS clinical trials group 5197: a placebo-controlled trial of immunization of HIV-1-infected persons with a replication-deficient adenovirus Type 5 vaccine expressing the HIV-1 core protein. *J. Infect. Dis.* 202, 705–716. doi: 10.1086/655468

Sengupta, S., and Siliciano, R. F. (2018). Targeting the Latent Reservoir for HIV-1. *Immunity* 48, 872–895. doi: 10.1016/j.immuni.2018.04.030

Sgadari, C., Monini, P., Tripiciano, A., Picconi, O., Casabianca, A., Orlandi, C., et al. (2019). Continued decay of HIV proviral DNA upon vaccination with HIV-1 Tat of subjects on long-term ART: an 8-year follow-up study. *Front. Immunol.* 10:233. doi: 10.3389/fimmu.2019.00233

Shan, L., Deng, K., Shroff, N. S., Durand, C. M., Rabi, S. A., Yang, H.-C., et al. (2012). Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity* 36, 491–501. doi: 10.1016/j.immuni.2012.01.014

Shan, L., Rabi, S. A., Laird, G. M., Eisele, E. E., Zhang, H., Margolick, J. B., et al. (2013). A novel PCR assay for quantification of HIV-1 RNA. *J. Virol.* 87, 6521–6525. doi: 10.1128/JVI.00006-13

Siliciano, J. D., Kajdas, J., Finzi, D., Quinn, T. C., Chadwick, K., Margolick, J. B., et al. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4 + T cells. *Nat. Med.* 9, 727–728. doi: 10.1038/nm880

Siliciano, J. D., and Siliciano, R. F. (2005). Enhanced culture assay for detection and quantitation of latently infected, resting CD4+ T-cells carrying replication-competent virus in HIV-1-infected individuals. *Methods Mol. Biol.* 304, 3–15. doi: 10.1385/1-59259-907-9:003

Siliciano, R. F., and Greene, W. C. (2011). HIV latency. *Cold Spring Harb. Perspect. Med.* 1:a007096. doi: 10.1101/cshperspect.a007096

Simonetti, F. R., Sobolewski, M. D., Fyne, E., Shao, W., Spindler, J., Hattori, J., et al. (2016). Clonally expanded CD4+ T cells can produce infectious HIV-1 *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* 113, 1883–1888. doi: 10.1073/pnas.1522675113

Sloan, R. D., and Wainberg, M. A. (2011). The role of unintegrated DNA in HIV infection. *Retrovirology* 8:52. doi: 10.1186/1742-4690-8-52

Soriano-Sarabia, N., Bateson, R. E., Dahl, N. P., Crooks, A. M., Kuruc, J. D., Margolis, D. M., et al. (2014). Quantitation of replication-competent HIV-1 in populations of resting CD4+ T cells. *J. Virol.* 88, 14070–14077. doi: 10.1128/JVI.01900-14

Strain, M. C., Lada, S. M., Luong, T., Rought, S. E., Gianella, S., Terry, V. H., et al. (2013). Highly precise measurement of HIV DNA by droplet digital PCR. *PLoS ONE* 8:e55943. doi: 10.1371/journal.pone.0055943

Sun, H., Kim, D., Li, X., Kiselinova, M., Ouyang, Z., Vandekerckhove, L., et al. (2015). Th1/17 Polarization of CD4 T cells supports HIV-1 persistence during antiretroviral therapy. *J. Virol.* 89, 11284–11293. doi: 10.1128/JVI.01595-15

Sung, J. A., Patel, S., Clohosey, M. L., Roesch, L., Tripic, T., Kuruc, J. D., et al. (2018). HIV-specific, *ex vivo* expanded T cell therapy: feasibility, safety, and efficacy in ART-suppressed HIV-infected individuals. *Mol. Ther.* 26, 2496–2506. doi: 10.1016/j.ymthe.2018.08.015

Sunshine, S., Kirchner, R., Amr, S. S., Mansur, L., Shakhbatyan, R., Kim, M., et al. (2016). HIV integration site analysis of cellular models of HIV latency with a probe-enriched next-generation sequencing assay. *J. Virol.* 90, 4511–4519. doi: 10.1128/JVI.01617-15

Suzuki, K., Juelich, T., Lim, H., Ishida, T., Watanebe, T., Cooper, D. A., et al. (2008). Closed chromatin architecture is induced by an RNA duplex targeting the HIV-1 promoter region. *J. Biol. Chem.* 283, 23353–23363. doi: 10.1074/jbc.M709651200

Swiggard, W. J., Baytop, C., Yu, J. J., Dai, J., Li, C., Schretzenmair, R., et al. (2005). Human immunodeficiency virus type 1 can establish latent infection in resting CD4+ T Cells in the absence of activating stimuli. *J. Virol.* 79, 14179–14188. doi: 10.1128/JVI.79.22.14179-14188.2005

Swiggard, W. J., O'Doherty, U., McGain, D., Jeyakumar, D., and Malim, M. H. (2004). Long HIV Type 1 reverse transcripts can accumulate stably within resting CD4+ T cells while short ones are degraded. *AIDS Res. Hum. Retroviruses* 20, 285–295. doi: 10.1089/088922204322996527

Swingler, S., Mann, A. M., Zhou, J., Swingler, C., and Stevenson, M. (2007). Apoptotic killing of HIV-1-infected macrophages is subverted by the viral envelope glycoprotein. *PLoS Pathog.* 3:e134. doi: 10.1371/journal.ppat.0030134

Symons, J., Chopra, A., Malatinkova, E., de Spiegelaere, W., Leary, S., Cooper, D., et al. (2017). HIV integration sites in latently infected cell lines: evidence of ongoing replication. *Retrovirology* 14:2. doi: 10.1186/s12977-016-0325-2

Tagarro, A., Chan, M., Zangari, P., Ferns, B., Foster, C., De Rossi, A., et al. (2018). Early and highly suppressive antiretroviral therapy are main factors associated with low viral reservoir in european perinatally HIV-infected children. *J. Acquir. Immune Defic. Syndr.* 79, 269–276. doi: 10.1097/QAI.0000000000001789

Tapia, G., Højen, J. F., Ökvist, M., Olesen, R., and Leth, S., Nissen, S. K., et al. (2017). Sequential Vacc-4x and romidepsin during combination antiretroviral therapy (cART): immune responses to Vacc-4x regions on p24 and changes in HIV reservoirs. *J. Infect.* 75, 555–571. doi: 10.1016/j.jinf.2017.09.004

Tateishi, H., Monde, K., Anraku, K., Koga, R., Hayashi, Y., Ciftci, H. I., et al. (2017). A clue to unprecedented strategy to HIV eradication: "Lock-in and apoptosis." *Sci. Rep.* 7:8957. doi: 10.1038/s41598-017-09129-w

Tebas, P., Stein, D., Tang, W. W., Frank, I., Wang, S. Q., Lee, G., et al. (2014). Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. *N. Engl. J. Med.* 370, 901–910. doi: 10.1056/NEJMoa1300662

Thibault, S., Imbeault, M., Tardif, M. R., and Tremblay, M. J. (2009). TLR5 stimulation is sufficient to trigger reactivation of latent HIV-1 provirus in T lymphoid cells and activate virus gene expression in central memory CD4+ T cells. *Virology* 389, 20–25. doi: 10.1016/j.virol.2009.04.019

Thomas, J., Ruggiero, A., Procopio, F. A., Pantaleo, G., Paxton, W. A., and Pollakis, G. (2019). Comparative analysis and generation of a robust HIV-1 DNA quantification assay. *J. Virol. Methods* 263, 24–31. doi: 10.1016/j.jviromet.2018.10.010

Tran, T.-A., de Goër de Herve, M.-G., Hendel-Chavez, H., Dembele, B., Le Névot, E., Abbed, K., et al. (2008). Resting regulatory CD4 T cells: a site of HIV persistence in patients on long-term effective antiretroviral therapy. *PLoS ONE* 3:e3305. doi: 10.1371/journal.pone.0003305

Trypsteen, W., Vynck, M., de Neve, J., Bonczkowski, P., Kiselinova, M., Malatinkova, E., et al. (2015). Ddpcrquant: threshold determination for single channel droplet digital PCR experiments. *Anal. Bioanal. Chem.* 407, 5827–5834. doi: 10.1007/s00216-015-8773-4

Tsai, A., Irrinki, A., Kaur, J., Cihlar, T., Kukolj, G., Sloan, D. D., et al. (2017). Toll-like receptor 7 agonist GS-9620 induces HIV expression and HIV-specific immunity in cells from HIV-infected individuals on suppressive antiretroviral therapy. *J. Virol.* 91:e02166-16. doi: 10.1128/JVI.02166-16

Ueda, S., Ebina, H., Kanemura, Y., Misawa, N., and Koyanagi, Y. (2016). Anti-HIV-1 potency of the CRISPR/Cas9 system insufficient to fully inhibit viral replication. *Microbiol. Immunol.* 60, 483–496. doi: 10.1111/1348-0421.12395

Valverde-Villegas, J. M., Matte, M. C. C., Medeiros, R. M., de, and Chies, J. A. B. (2015). New insights about Treg and Th17 cells in HIV infection and disease progression. *J. Immunol. Res.* 2015:647916. doi: 10.1155/2015/647916

van der Sluis, R. M., Kumar, N. A., Pascoe, R. D., Zerbato, J. M., Evans, V. A., Dantanarayana, A. I., et al. (2020). Combination immune checkpoint blockade to reverse HIV latency. *J. Immunol.* 204, 1242–1254. doi: 10.4049/jimmunol.1901191

van der Sluis, R. M., van Montfort, T., Centlivre, M., Schopman, N. C. T., Cornelissen, M., Sanders, R. W., et al. (2013). Quantitation of HIV-1 DNA with a sensitive TaqMan assay that has broad subtype specificity. *J. Virol. Methods* 187, 94–102. doi: 10.1016/j.jviromet.2012.09.019

Van Zyl, G. U., Katusiime, M. G., Wiegand, A., McManus, W. R., Bale, M. J., Halvas, E. K., et al. (2017). No evidence of HIV replication in children on antiretroviral therapy. *J. Clin. Invest.* 127, 3827–3834. doi: 10.1172/JCI94582

Vandergeeten, C., Fromentin, R., Merlini, E., Lawani, M. B., DaFonseca, S., Bakeman, W., et al. (2014). Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *J. Virol.* 88, 12385–12396. doi: 10.1128/JVI.00609-14

Vatakis, D. N., Bristol, G., Wilkinson, T. A., Chow, S. A., and Zack, J. A. (2007). Immediate activation fails to rescue efficient human immunodeficiency virus replication in quiescent CD4+ T Cells. *J. Virol.* 81, 3574–3582. doi: 10.1128/JVI.02569-06

Vitone, F., Gibellini, D., Schiavone, P., and Re, M. C. (2005). Quantitative DNA proviral detection in HIV-1 patients treated with antiretroviral therapy. *J. Clin. Virol.* 33, 194–200. doi: 10.1016/j.jcv.2004.11.003

von Stockenstrom, S., Odevall, L., Lee, E., Sinclair, E., Bacchetti, P., Killian, M., et al. (2015). Longitudinal genetic characterization reveals that cell proliferation maintains a persistent HIV Type 1 DNA pool during effective HIV therapy. *J. Infect. Dis.* 212, 596–607. doi: 10.1093/infdis/jiv092

Wagner, T. A. (2018). Quarter century of anti-HIV CAR T cells. *Curr. HIV/AIDS Rep.* 15, 147–154. doi: 10.1007/s11904-018-0388-x

Wagner, T. A., McKernan, J. L., Tobin, N. H., Tapia, K. A., Mullins, J. I., and Frenkel, L. M. (2013). An increasing proportion of monotypic HIV-1 DNA sequences during antiretroviral treatment suggests proliferation of HIV-infected cells. *J. Virol.* 87, 1770–1778. doi: 10.1128/JVI.01985-12

Wagner, T. A., McLaughlin, S., Garg, K., Cheung, C. Y. K., Larsen, B. B., Styrchak, S., et al. (2014). Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* 345, 570–573. doi: 10.1126/science.1256304

Wang, G., Zhao, N., Berkhout, B., and Das, A. T. (2016). CRISPR-Cas9 can inhibit HIV-1 replication but NHEJ repair facilitates virus escape. *Mol. Ther.* 24, 522–526. doi: 10.1038/mt.2016.24

Wang, Q., Chen, S., Xiao, Q., Liu, Z., Liu, S., Hou, P., et al. (2017). Genome modification of CXCR4 by *Staphylococcus aureus* Cas9 renders cells resistance to HIV-1 infection. *Retrovirology* 14:51. doi: 10.1186/s12977-017-0375-0

Wang, Q., Liu, S., Liu, Z., Ke, Z., Li, C., Yu, X., et al. (2018). Genome scale screening identification of SaCas9/gRNAs for targeting HIV-1 provirus and suppression of HIV-1 infection. *Virus Res.* 250, 21–30. doi: 10.1016/j.virusres.2018.04.002

Wang, W., Ye, C., Liu, J., Zhang, D., Kimata, J. T., and Zhou, P. (2014). CCR5 gene disruption via lentiviral vectors expressing Cas9 and single guided RNA renders cells resistant to HIV-1 infection. *PLoS ONE* 9:e115987. doi: 10.1371/journal.pone.0115987

Wang, Z., Gurule, E. E., Brennan, T. P., Gerold, J. M., Kwon, K. J., Hosmane, N. N., et al. (2018). Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane. *Proc. Natl. Acad. Sci. U.S.A.* 115, E2575–E2584. doi: 10.1073/pnas.1720665115

Weinberger, A. D., and Weinberger, L. S. (2013). XStochastic fate selection in HIV-infected patients. *Cell* 155, 497–499. doi: 10.1016/j.cell.2013.09.039

Whitney, J. B., Hill, A. L., Sanisetty, S., Penaloza-Macmaster, P., Liu, J., Shetty, M., et al. (2014). Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 512, 74–77. doi: 10.1038/nature13594

Wilburn, K. M., Mwandumba, H. C., Jambo, K. C., Boliar, S., Solouki, S., Russell, D. G., et al. (2016). Heterogeneous loss of HIV transcription and proviral DNA from 8E5/LAV lymphoblastic leukemia cells revealed by RNA FISH: FLOW analyses. *Retrovirology* 13:55. doi: 10.1186/s12977-016-0289-2

Williams, J. P., Hurst, J., Stöhr, W., Robinson, N., Brown, H., Fisher, M., et al. (2014). HIV-1 DNA predicts disease progression and post-treatment virological control. *Elife* 3:e03821. doi: 10.7554/eLife.03821

Williams, S. A., Chen, L.-F., Kwon, H., Fenard, D., Bisgrove, D., Verdin, E., et al. (2004). Prostratin antagonizes HIV latency by activating NF-κB. *J. Biol. Chem.* 279, 42008–42017. doi: 10.1074/jbc.M402124200

Wonderlich, E. R., Subramanian, K., Cox, B., Wiegand, A., Lackman-Smith, C., Bale, M. J., et al. (2019). Effector memory differentiation increases detection of replication-competent HIV-l in resting CD4+ T cells from virally suppressed individuals. *PLoS Pathog.* 15:e1008074. doi: 10.1371/journal.ppat.1008074

Wong, J. K., Hezareh, M., Günthard, H. F., Havlir, D. V., Ignacio, C. C., Spina, C. A., et al. (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 278, 1291–1295. doi: 10.1126/science.278.5341.1291

Wong, M. E., Jaworowski, A., and Hearps, A. C. (2019). The HIV reservoir in monocytes and macrophages. *Front. Immunol.* 10:1435. doi: 10.3389/fimmu.2019.02517

Xiao, Q., Guo, D., and Chen, S. (2019). Application of CRISPR/Cas9-based gene editing in HIV-1/AIDS Therapy. *Front. Cell. Infect. Microbiol.* 9:69. doi: 10.3389/fcimb.2019.00069

Xing, S., Bullen, C. K., Shroff, N. S., Shan, L., Yang, H.-C., Manucci, J. L., et al. (2011). Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4+ T cell model without inducing global T cell activation. *J. Virol.* 85, 6060–6064. doi: 10.1128/JVI.02033-10

Xu, L., Yang, H., Gao, Y., Chen, Z., Xie, L., Liu, Y., et al. (2017). CRISPR/Cas9-mediated CCR5 ablation in human hematopoietic stem/progenitor cells confers HIV-1 resistance *in vivo*. *Mol. Ther.* 25, 1782–1789. doi: 10.1016/j.ymthe.2017.04.027

Yin, C., Zhang, T., Qu, X., Zhang, Y., Putatunda, R., Xiao, X., et al. (2017). *In vivo* excision of HIV-1 provirus by saCas9 and multiplex single-guide RNAs in animal models. *Mol. Ther.* 25, 1168–1186. doi: 10.1016/j.ymthe.2017.03.012

Yu, J. J., Wu, T. L., Liszewski, M. K., Dai, J., Swiggard, W. J., Baytop, C., et al. (2008). A more precise HIV integration assay designed to detect small differences finds lower levels of integrated DNA in HAART treated patients. *Virology* 379, 78–86. doi: 10.1016/j.virol.2008.05.030

Yucha, R. W., Hobbs, K. S., Hanhauser, E., Hogan, L. E., Nieves, W., Ozen, M. O., et al. (2017). High-throughput characterization of HIV-1 reservoir reactivation using a single-cell-in-droplet PCR assay. *EBiomedicine* 20, 217–229. doi: 10.1016/j.ebiom.2017.05.006

Yukl, S. A., Gianella, S., Sinclair, E., Epling, L., Li, Q., Duan, L., et al. (2010). Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. *J. Infect. Dis.* 202, 1553–1561. doi: 10.1086/656722

Yukl, S. A., Kaiser, P., Kim, P., Telwatte, S., Joshi, S. K., Vu, M., et al. (2018). HIV latency in isolated patient CD4+ T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing. *Sci. Transl. Med.* 10:eaap9927. doi: 10.1126/scitranslmed.aap9927

Zaikos, T. D., Painter, M. M., Sebastian Kettinger, N. T., Terry, V. H., and Collins, K. L. (2018). Class 1-selective histone deacetylase (HDAC) inhibitors enhance HIV latency reversal while preserving the activity of HDAC isoforms necessary for maximal HIV gene expression. *J. Virol.* 92:e02110–e02117. doi: 10.1128/JVI.02110-17

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past co-authorship with one of the authors GP.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 23 April 2020
doi: 10.3389/fcimb.2020.00176

Promise and Progress of an HIV-1 Cure by Adeno-Associated Virus Vector Delivery of Anti-HIV-1 Biologics

*Matthew R. Gardner**

Department of Immunology and Microbiology, The Scripps Research Institute, Jupiter, FL, United States

Despite the success of antiretroviral therapy (ART) at suppressing HIV-1 infection, a cure that eradicates all HIV-1-infected cells has been elusive. The latent viral reservoir remains intact in tissue compartments that are not readily targeted by the host immune response that could accelerate the rate of reservoir decline during ART. However, over the past decade, numerous broadly neutralizing antibodies (bNAbs) have been discovered and characterized. These bNAbs have also given rise to engineered antibody-like inhibitors that are just as or more potent than bNAbs themselves. The question remains whether bNAbs and HIV-1 inhibitors will be the effective "kill" to a shock-and-kill approach to eliminate the viral reservoir. Additional research over the past few years has sought to develop recombinant adeno-associated virus (rAAV) vectors to circumvent the need for continual administration of bNAbs and maintain persistent expression in a host. This review discusses the advancements made in using rAAV vectors for the delivery of HIV-1 bNAbs and inhibitors and the future of this technology in HIV-1 cure research. Numerous groups have demonstrated with great efficacy that rAAV vectors can successfully express protective concentrations of bNAbs and HIV-1 inhibitors. Yet, therapeutic concentrations, especially in non-human primate (NHP) models, are not routinely achieved. As new studies have been reported, more challenges have been identified for utilizing rAAV vectors, specifically how the host immune response limits the attainable concentrations of bNAbs and inhibitors. The next few years should provide improvements to rAAV vector delivery that will ultimately show whether they can be used for expressing bNAbs and HIV-1 inhibitors to eliminate the HIV-1 viral reservoir.

Keywords: adeno-associate virus, HIV-1, HIV-1 cure, broadly neutralizing antibodies (bNAbs), eCD4-Ig

OPEN ACCESS

Edited by:
Matthew Parsons,
Emory University, United States

Reviewed by:
Jeffrey R. Schneider,
Rush University, United States
Ann Jones Hessell,
Oregon Health and Science University, United States

***Correspondence:**
Matthew R. Gardner
mgardner@scripps.edu

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *05 February 2020*
Accepted: *02 April 2020*
Published: *23 April 2020*

Citation:
Gardner MR (2020) Promise and Progress of an HIV-1 Cure by Adeno-Associated Virus Vector Delivery of Anti-HIV-1 Biologics. Front. Cell. Infect. Microbiol. 10:176. doi: 10.3389/fcimb.2020.00176

INTRODUCTION

The 2019 UNAIDS Report and World Health Organization both estimate that there are ~38 million people living with HIV-1 infection (UNAIDS, 2019; WHO, 2019). Of those individuals, almost 25 million have access to antiretroviral therapy (ART) (UNAIDS, 2019). Various studies have shown the half-life of the HIV-1 reservoir can range somewhere between 44 months and 13 years and in some cohorts, no decay was observed at all (Siliciano et al., 2003; Chun et al., 2007; Besson et al., 2014; Bachmann et al., 2019). Thus, lifelong ART is required to maintain viral suppression and achieve the best health outcomes in the majority of individuals.

As more individuals living with HIV-1 receive daily ART, there is a push to investigate new means to cure the disease. Two definitions are routinely used in the field (Deeks et al., 2016). The first definition is a traditional cure, where the virus is eradicated or eliminated from the individual, including the latent viral reservoir. The second definition is a "functional cure" or remission, where an individual has their viremia suppressed to levels that limit transmission, and the individual does not progress to AIDS. Current ART falls in the second category, and thus, new therapies are needed in order to fully cure the disease.

Broadly neutralizing antibodies (bNAbs) and engineered HIV-1 inhibitors could serve as the new drugs that could help eliminate the latent reservoir and cure individuals with HIV-1. However, current uses of bNAbs in therapy require continual bNAb infusions along with reactivation of the latent viral reservoir, which could be considered a costly endeavor that is not practical for areas in developing nations with larger numbers of individuals living with HIV-1. Yet, bNAbs and antibody-like inhibitors utilize the Fc region that promotes effector functions via antibody interactions with immune cells (Lu et al., 2018), properties that may be useful for an HIV-1 cure. These effector functions include antibody-dependent complement-mediated lysis (ADCML) (Mujib et al., 2017), antibody-dependent cell-mediated cytotoxicity (ADCC) (Bruel et al., 2016; Von Bredow et al., 2016), and antibody-dependent cellular phagocytosis (ADCP) (Julg et al., 2017; Mayer et al., 2017). Through these mechanisms, bNAbs can identify and kill HIV-1-infected cells within the latent viral reservoir.

This review discusses the use of recombinant adeno-associated virus (rAAV) vectors to overcome the challenges associated with passive infusion of bNAbs and other HIV-1 inhibitors. The past decade has yielded numerous studies that evaluate the delivery of these biologics in different models as well as their protective and therapeutic efficacy. However, as more studies are reported, new challenges are identified that will need to be resolved for rAAV vectors to be employed in the clinic. Additionally, questions still remain as to whether bNAbs and other HIV-1 inhibitors can increase the rate of decay of the viral reservoir and provide the necessary means to cure individuals living with HIV-1. Despite these hurdles, rAAV delivery of bNAbs and HIV-1 inhibitors is a promising approach that warrants further investigation.

BROADLY NEUTRALIZING ANTIBODIES THAT TARGET THE HIV-1 ENVELOPE GLYCOPROTEIN

Due to the limitations of current antiretroviral therapies for reducing the viral reservoir and eradicating infected cells while viral loads are suppressed, there is a clear need for new drugs to treat those living with HIV-1. Antibodies are an obvious alternative due to their ability to not only bind and neutralize pathogens but because of their effector functions mediated by the Fc region. Prior to 2009, small molecule inhibitors were the only viable pathway for HIV-1 therapy because biologics, specifically antibodies, were not broad or potent enough to be used as therapeutics. Advances in single B cell sorting along with identification of HIV-1+ individuals with neutralizing sera from large patient cohorts allowed Walker et al. to usher in the second generation of bNAbs with their discovery of PG9 and PG16 (Walker et al., 2009). Since then, numerous bNAbs have been identified and characterized that potently neutralize hundreds of HIV-1 primary isolates in TZM-bl neutralization assays. These bNAbs are categorized based on the epitope of the HIV-1 envelope glycoprotein (Env) that they target. PG9 and PG16 are members of the class of bNAbs that target the V2 apex of Env. This class now includes PGT145 (Walker et al., 2011), PGDM1400 (Sok et al., 2014), and CAP256-VRC26.25 (Doria-Rose et al., 2016). One class that demonstrates extreme breadth is the CD4-binding site antibodies which includes VRC01 (Wu et al., 2010), VRC07-523LS (Rudicell et al., 2014), 3BNC117 (Scheid et al., 2011), N6 (Huang et al., 2016a), N49P7 (Sajadi et al., 2018), and 1-18 (Schommers et al., 2020). 10-1074 (Mouquet et al., 2012), PGT121, PGT122, and PGT128 (Walker et al., 2011) are examples bNAbs that target the N332 glycan near the base of V3 loop (V3g) of Env. 10E8 is a bNAb that targets the membrane proximal external region (MPER) of gp41 on Env (Huang et al., 2012) and has since been engineered for enhanced biophysical properties like improved solubility (10E8v4) (Kwon et al., 2016). More recent research identified newer epitopes on Env previously not known to be targets of antibody responses. One of these epitopes included the gp120/gp41 interface with examples including PGT151 (Falkowska et al., 2014), 35O22 (Huang et al., 2014), and 8ANC195 (Scharf et al., 2014). Kong et al. recently showed that the HIV-1 fusion peptide can also be targeted by bNAbs, specifically VRC34.01 (Kong et al., 2016). Lastly, two groups have shown that even the silent face of gp120 is a target for bNAbs with the identification of VRC-PG05 (Zhou et al., 2018) and SF12 (Schoofs et al., 2019). One epitope of note is the coreceptor binding site of Env. Early antibodies that target this site included 17b, E51, and 412d but are only effective when Env is in the CD4-bound state (Choe et al., 2003). More recently, antibodies targeting a peptide motif around the coreceptor-binding site have been described and include PGDM12 and PGDM21 (Sok et al., 2016). Although antibodies targeting the coreceptor-binding site are not as broad and potent as other classes of bNAbs, they are still useful reagents as the coreceptor-binding site is one of the most conserved regions on Env (Rizzuto et al., 1998).

With these bNAbs identified and characterized, the ideal method for employing them for HIV-1 therapy would be through a therapeutic vaccination. However, numerous limitations exist that render current vaccine approaches insufficient to elicit bNAbs. First, the bNAbs appear to arise over years of infection in a small subset of individuals living with HIV-1 (Wu et al., 2015). Second, bNAbs have long heavy chain CDR3 regions with high degrees of somatic hypermutation (reviewed in Burton and Hangartner, 2016), characteristics achievable due to chronic exposure to the virus rather than immunization. Third, properties of the virus have made immunogen design challenging. These include the low number of Env trimers on the surface of the virion (Zhu et al., 2003) as well as a protective glycan shield on Env (Wei et al., 2003). Engineering immunogens

that circumvent these properties is ongoing, but currently there is no viable HIV-1 vaccine that induces bNAbs nearly 40 years after the virus was first identified. Should an effective HIV-1 therapeutic vaccine that elicits bNAbs be realized, data from clinical trials indicate that more than one active bNAb will be necessary to maintain viral suppression.

ENGINEERED HIV-1 INHIBITORS

To address the need for targeting multiple epitopes on Env, a few groups have undertaken the engineering of bispecific antibodies and fusion proteins. Bispecific antibodies employ two techniques required for their generation. First, multiple mutations in IgG CH2 and CH3 regions of the Fc domain are introduced for a knobs-in-holes approach (Ridgway et al., 1996). These mutations link the two different IgG Fc domains at a success rate of nearly 95%. The second technique has been termed "CrossMAb" technology, where one of the two Fabs of the engineered bispecific antibody has swapped the CH1 and CL region (Schaefer et al., 2011). This technique reduces the mixing of heavy chains and light chains of the two antibodies during production of the bispecific antibody. The first demonstration of a bispecific antibody targeted the CD4-binding site and apex regions (Asokan et al., 2015). A later study used the IgG3 Fc with an open hinge that targeted the CD4-binding site and N332 V3 glycan (Bournazos et al., 2016). Both studies showed that bispecific antibodies had increased breadth compared to their individual components. One design applied to bispecific antibodies to avoid the need for a CrossMab technique is to use the full Fab of one antibody and a single-chain variable fragment (scFv) of the second antibody. A recent study by Davis-Gardner et al. applying this approach demonstrated that a bispecific antibody using the Fab from V3g bNAbs with the scFv form of CAP256-VRC26.25 had increased breadth compared to the two parent antibodies. This inhibitor maintained nearly the same potency and achieved 100% neutralization against the isolates assayed (Davis-Gardner et al., 2020). Interestingly, the most potent of bispecific antibodies comes from a design that features an HIV-1 bNAb on one arm with an anti-receptor antibody as the other arm. Huang et al. showed that their lead candidate, a bispecific antibody that targeted CD4 with one arm and used a modified 10E8 (10E8v2.0) Fab as the second arm, had 100% breadth with a mean 50% inhibitory concentration of 0.002 μg/mL against a panel of 118 HIV-1 isolates (Huang et al., 2016b).

A few groups have taken the bispecific antibody approach a step further and engineered trispecific antibodies. These antibody constructs utilize tandem scFvs fused together on one arm with a heavy and light chain from a third antibody as the other arm. Two groups showed that trispecific antibodies targeting the MPER, CD4-binding site, and either the apex or V3g had extreme coverage with great neutralization potency (Xu et al., 2017; Steinhardt et al., 2018). A third trispecific antibody recently described targeted CCR5 with an scFv and targeted the MPER and apex with the other arms of the antibody (Khan et al., 2018). Taken together, bispecific and trispecific antibodies represent single HIV-1 inhibitors with increased coverage while at least maintaining the original potency of their individual components.

Another direction of engineered inhibitors includes those that target the CD4– and coreceptor-binding sites of Env simultaneously. The first one described used a linker to fuse two domains of soluble CD4 to the scFv of 17b (Lagenaur et al., 2010). Later work has seen these kinds of inhibitors use an IgG1 Fc domain that improves the inhibitor's half-life and adds the effector functions of an antibody. One of these inhibitors came from Dimiter Dimitrov and colleagues, who used phage display to identify a stable version of CD4 domain 1 (Chen et al., 2014). This CD4 domain 1 was then fused to the N-terminus of CH1 and C-terminus of CH3 of an IgG1. Fused to the N-terminus of the CL domain is the scFv of m36.4, an antibody that targets the coreceptor-binding site. This inhibitor, termed 4Dm2m, had 100% breadth and exceptional potency against over 60 isolates it was tested against.

Our lab has taken a similar approach to developing the antibody-like HIV-1 entry inhibitor eCD4-Ig (Gardner et al., 2015). This inhibitor fuses a short, coreceptor-mimetic peptide to the C-terminus of CD4-Ig. When tested against more than 270 isolates, including HIV-2 and SIV, eCD4-Ig potently neutralizes 100% of these isolates with >99% maximum inhibition. The addition of the coreceptor-mimetic peptide also limits CD4-enhancement on CD4-negative/CCR5-positive cells. Recently we have shown that three mutations in CD4 domain 1 increase its potency by an average of 9-fold (Fetzer et al., 2018). Furthermore, *in vitro* studies showed that continuous passaging of virus in the presence of eCD4-Ig only yielded partial resistance to the inhibitor (Fellinger et al., 2019). These resistance mutations also conferred a high fitness cost to the virus in entry assays. Taken together, the broadest HIV-1 inhibitors are these fusion inhibitors that target the CD4– and coreceptor-binding sites because they target the two most conserved regions on Env.

ADENO-ASSOCIATED VIRUS VECTORS FOR DELIVERY OF HIV-1 INHIBITORS

An ideal deployment of anti-HIV-1 biologics for therapy would be through constant expression to avoid lapses in maintaining therapeutic concentrations. One way to achieve this would be through the use of rAAV vectors. AAV is a small DNA virus that is widely used in numerous gene therapy applications. It was discovered as a contaminant during adenovirus preparations in 1965 (Atchison et al., 1965). AAV belongs to the *Parvoviradae* family under the genus *Dependoparvovirus*. An icosadhedral protein capsid encases its DNA genome. The wild-type AAV genome encodes three genes—*rep*, *cap*, and *aap* (Wang et al., 2019). The *rep* gene is responsible for the production of Rep78, Rep68, Rep52, and Rep40. These genes are necessary for replication. The cap gene encodes three subunit proteins needed for capsid assembly—VP1, VP2, and VP3. While the AAV1 capsid was the first AAV capsid identified and the AAV2 capsid has been highly characterized *in vitro*, numerous capsids have been identified and characterized for their different transduction efficiencies and tissue tropism. The third gene encoded by

AAV is the *aap* gene, located in an alternate reading frame within the *cap* gene, encoding the assembly activating protein (AAP) which promotes viral assembly (Sonntag et al., 2010). As its classification implies, wild-type AAV requires a secondary infection, usually adenovirus or herpesvirus, in order to replicate. However, it is currently believed that a productive AAV infection does not cause human disease.

As a gene therapy vector, rAAV vectors are engineered to remove all parts of the AAV genome except for the two inverted terminal repeats (ITRs). The ITRs are especially important as they facilitate circularization and concatemerization in the nucleus of transduced cells (Duan et al., 1998). This process produces DNA episomes that are stable for long-term expression of the delivered transgene. The transgene encoded by rAAV is usually within the size constraints of the wild-type AAV genome (4.7 kb). However, transgenes up to 5.0 kb appear to be accommodated for vector delivery. Larger payloads can be encoded in two different rAAV vectors using splicing techniques that link the two separate portions of the delivered gene (Duan et al., 2000; Nakai et al., 2000; Sun et al., 2000).

The 2010's marked a productive decade for rAAV gene therapy. In 2012, the European Medicines Agency approved Glybera, an rAAV1-based gene therapy, which treats lipoprotein lipase deficiency. In a landmark decision, the United States FDA approved Luxturna in 2017 to treat inherited retinal disease. Luxturna is an rAAV2-based gene therapy delivering the natural form of RPE65. In 2019, Zolgensma became the second FDA-approved AAV gene therapy to treat spinal muscular atrophy in children under 2 years of age. Zolgensma is administered systemically using the AAV9 capsid to deliver the natural form of the *survival motor neuron 1* gene. A vast amount of gene therapy work has also examined treating hemophilia with rAAV vectors. Early clinical trials using rAAV2 vectors encoding Factor IX to treat hemophilia patients showed that up to 10 years after intramuscular inoculation, patients were still producing therapeutic amounts of Factor IX (Buchlis et al., 2012). This characteristic is one of the main reasons why rAAV vectors are viable for HIV-1 cure research. If long-term expression of bNAbs is critical for design of new therapies, rAAV vectors could translate into the "one-shot cure" desired by field.

While rAAV gene therapies were making headlines over the past decade, their use in HIV-1 research took off as well. All reported rAAV studies pertaining to HIV-1 research are highlighted in **Table 1**. Early studies were pioneered by the lab of Phil Johnson who described that rAAV2 vectors encoding the CD4-binding site antibody b12 could express the antibody for 24 weeks after intramuscular inoculation (Lewis et al., 2002). Mice given the highest dose of rAAV vectors had a range of b12 expression from 2 to 7 μg/mL and serum samples from the mice retained neutralization activity when assessed by *in vitro* neutralization assays. Johnson et al. took their research to the next step using rAAV1 vectors to deliver anti-SIV immunoadhesins

TABLE 1 | List of preclinical and clinical rAAV studies using HIV-1 bNAbs and inhibitors.

References	Purpose of study	AAV capsid	bNAbs	Virus	# with ADA out of total
Preclinical mouse studies					
Lewis et al. (2002)	Expression	2	b12	N/A	N/A
Balazs et al. (2012)	Protection	8	VRC01, b12, 2G12, 4E10, 2F5	NL4-3	N/A
Balazs et al. (2014)	Protection	8	VRC01, b12, VRC07	JR-CSF, REJO.c	N/A
Horwitz et al. (2013)	Therapy	8	10-1074, 3BNC117	YU2	N/A
Badamchi-Zadeh et al. (2018)	Therapy	1	PGT121	JR-CSF	4/4
Van Den Berg et al. (2019)	Expression	8	CAP256-VRC26.25	N/A	7/7
Preclinical NHP studies					
Johnson et al. (2009)	Protection	1	4L6, 5L7, N4 immunoadhesins	SIVmac316	3/9
Gardner et al. (2015)	Protection	1	eCD4-Ig	SHIV-AD8EO	2/4
Saunders et al. (2015)	Protection	8	VRC07	SHIV-BaIP4	4/4
Fuchs et al. (2015)	Protection	1	4L6, 5L7	SIVmac239	9/12
Martinez-Navio et al. (2016)	Expression, ADA response	1	4L6, 5L7, 3BNC117, 10E8, 10-1074, 1NC9, 8ANC195	N/A	24/24
Welles et al. (2018)	Protection	8	ITS01, ITS06.02	SIVsmE660	13/60
Gardner et al. (2019b)	Protection	1	3BNC117, NIH45-46, 10-1074, PGT121	SHIV-AD8EO	24/24
Martinez-Navio et al. (2019)	Therapy	1	3BNC117, 10-1074, N6, PGT128, PGT145, 35O22	SHIV-AD8EO	10/12
Gardner et al. (2019a)	Protection	1	eCD4-Ig	SIVmac239	4/4
Fuchs et al. (2019)	Expression	8, 1	4L6	N/A	12/12
Clincal studies					
Priddy et al. (2019)	Expression	1	PG9		10/16
Clinical trial NTC03374202	Expression	8	VRC07		2/7

N/A, not available.

and CD4-Ig to rhesus macaques (Johnson et al., 2009). In this study, four of six macaques that were expressing the anti-SIV immunoadhesins had concentrations >100 μg/mL and were protected from intravenous SIVmac316 challenges. The two animals that became infected were shown to have anti-drug antibody (ADA) responses targeting the immunoadhesin, which presumably limited the efficacy of inhibitor. Thus, this study highlights two major results. First, AAV-delivered SIV inhibitors could protect from SIV challenges, implying the potential for delivering HIV-1 bNAbs and inhibitors. Second, the host immune response is a barrier to successful utilization of rAAV vectors for therapy.

In David Baltimore's lab, Alex Balazs and colleagues were the first to show that rAAV vectors delivering HIV-1 bNAbs could protect humanized mice from HIV-1 challenges. In Balazs et al., the authors engineered the rAAV transgene cassette to produce full-length antibodies using an F2A peptide to separate and generate both the heavy and light chains of VRC01, as well as early generations of HIV-1 neutralizing antibodies, b12, 4E10, 2F5, and 2G12 (Balazs et al., 2012). Results were extremely encouraging not only because of the observed protection from intravenous HIV-1 challenges, but also because serum concentrations of VRC01, b12, and 2G12 were >100 μg/mL. The first study using rAAV2-delivered b12 employed two different promoters to drive expression of the heavy and light chains, in contrast to this study using the F2A peptide to produce both chains and rAAV8 vectors. The F2A peptide was previously shown to drive high levels of antibody expression in mice (Fang et al., 2005), possibly indicating this design to be superior to two promoters. Balazs et al. followed up this initial study with a second study showing that VRC07W could be measured at concentrations >100 μg/mL following rAAV8 intramuscular inoculation in humanized mice (Balazs et al., 2014). These levels mediated complete protection from vaginal challenges of the transmitted-founder isolate REJO.c. These results highlighted that an rAAV-delivered bNAb could mediate protection from challenges that mimicked most HIV-1 transmission events. Overall, these two studies provided a stable foundation for AAV gene therapy in HIV-1 research. Since then, other groups have explored rAAV delivery of different bNAbs in mice, including PGT121 and CAP256.VRC26.25 (Badamchi-Zadeh et al., 2018; Van Den Berg et al., 2019).

As demonstrated in Johnson et al., the non-human primate (NHP) model presents a more difficult challenge when evaluating rAAV-delivery of HIV-1 bNAbs and inhibitors. The host immune response generated by rhesus macaques has made evaluating rAAV vectors in NHPs very difficult. The humanized mouse model has numerous benefits compared to the NHP model, one of which is that these studies utilize HIV-1 isolates. However, humanized mice do not have a fully functioning immune response like humans and NHPs do. There is also a limited amount of time for studying HIV-1 infection in mice, which limits the ability to evaluate new therapies. In contrast, the physiology of macaques more closely resembles that of humans. However, HIV-1 does not replicate in macaques. Thus, the NHP model uses SIV isolates or SIV/HIV-1 chimeras (SHIVs) for both prophylaxis and therapy studies. SIV has shown to closely mimic the disease progression to AIDS that has been observed in HIV-1 infected individuals, which makes it a good model in macaques. Yet, HIV-1 bNAbs do not bind to SIV Env so SHIVs are widely used to evaluate bNAb efficacy in NHPs. With the pros and cons acknowledged for both NHP and humanized mouse studies, there is a need to demonstrate that bNAbs are effective in NHPs to be convinced they can work in humans.

An early study by Fuchs et al. set out to evaluate the protective efficacy of the SIV antibodies 4L6 and 5L7 in rhesus macaques (Fuchs et al., 2015). Using a similar transgene cassette design as Balazs et al. with the F2A peptide to generate both the heavy and light chains, the authors used rAAV1 vectors encoding either 4L6 or 5L7 to inoculate the quadriceps muscles of rhesus macaques. Peak concentration of 4L6 ranged from 50 to 150 μg/mL while 5L7 concentrations ranged from 20 to 70 μg/mL in five of six macaques. However, the last macaque in the 5L7 group had peak and stable concentrations >200 μg/mL, a concentration that has now been achieved for over 6 years (Martinez-Navio et al., 2020). Unfortunately, antibody concentrations in most macaques began to decline due to the emergence of an ADA response against the delivered antibody. Although low-dose SIVmac239 challenges did not yield significant protection, it is of note that the macaque expressing >200 μg/mL of 5L7 was protected from all six challenges, including a high-dose intravenous challenge that has been shown to infect all historical controls. This single animal provides some encouragement that when rAAV delivery of antibody is efficient, protective efficacy will most likely be observed. However, what concentration needs to be observed in order to mediate protection is still not completely elucidated.

As Fuchs et al. described in their study, ADA was a clear hurdle to successful expression of antibodies from rAAV vectors. A similar result was observed by Saunders et al. in 2015 using rAAV8 vectors to deliver VRC07 (Saunders et al., 2015). Because VRC07 is a human-derived bNAb, the authors initially engineered VRC07 to be more "simian-like" in hopes of avoiding an immune response against the bNAb in rhesus macaques. Despite their best efforts, four of four macaques that received rAAV8 vectors encoding the simian version of VRC07 had concentrations that peaked between 2.5 and 7.7 μg/mL and then declined to concentrations below the limits of detection by 9 weeks following inoculation. The crash in VRC07 concentrations correlated with the emergence of an ADA response in all four macaques. The authors then treated a second group of six macaques with cyclosporine A (CsA) to limit the immune response of the rAAV8 inoculated animals. CsA treatment appeared to be beneficial as the mean peak concentration of VRC07 in these animals rose to 38.12 μg/mL. It was noted that one macaque generated an ADA response against VRC07 while being treated with CsA and two others generated ADA after CsA treatment was discontinued. All three of these macaques had concentrations of VRC07 decline to undetectable concentrations. However, four of six macaques were protected from a single challenge of SHIV-BalP4.

Both these NHP studies highlight the emergence of an ADA response against the expressed antibodies in rhesus macaques. Martinez-Navio and colleagues took a deeper look into their ADA responses from both their rhesus macaque study of 4L6

and 5L7 as well as rAAV1 delivery of five "rhesusized" HIV-1 bNAbs (Martinez-Navio et al., 2016). As noted in Fuchs et al., 9 of the 12 macaques that received rAAV1 vectors delivering 4L6 or 5L7 developed ADA responses. This study showed that the ADA response targeted the variable regions of the expressed antibodies. This was also observed in eight of eight macaques that received a cocktail of "rhesusized" bNAbs (bNAbs that were engineered to have the human variable regions with the rhesus macaque IgG1 constant and light chain constant regions). The two cocktails were rAAV1 vectors encoding 3BNC117, 1NC9, and 8ANC195 or 3BNC117, 10-1074, and 10E8. The authors showed that the degree of ADA responses against an individual bNAb correlated with its divergence from its closest germline precursor. The magnitude of somatic hypermutation may need to be evaluated for determining which bNAbs should be used in rAAV studies.

We have recently published a study that confirms the limitations caused by ADA responses (Gardner et al., 2019b). In our study, we took a different approach, giving four groups of three macaques a combination of rAAV1 vectors encoding either 10-1074 and 3BNC117 or NIH45-46 and PGT121. We also evaluated the role of IgG isotype as one group of each combination had a rhesus macaque IgG1 Fc domain and the other group had a rhesus macaque IgG2 Fc domain. Like Martinez-Navio et al., we observed all 12 macaques generate ADA responses against both expressed bNAbs they were treated with. In some cases, ADA responses did not completely correlate with clearance of the bNAb. In these four macaques, we observed protection from two SHIV-AD8 challenges, which indeed correlated with higher concentrations of 10-1074 or PGT121. In another NHP study, Welles et al. evaluated the delivery of anti-SIV antibodies, ITS01 and ITS06.02, using rAAV8 vectors (Welles et al., 2018). In this study, ITS01 was observed to express at higher concentrations than ITS06.02, and in only 20% of the animals that received rAAV8 vectors did macaques generate ADA responses against the expressed antibody. Stable expression was usually observed around 10 μg/mL and these concentrations were sufficient for protection from SIVsmE660 challenges. Although they have a lower level of somatic hypermutation compared to HIV-1 bNAbs, these anti-SIV antibodies were naturally produced rhesus macaque antibodies. In contrast, 4L6 and 5L7 were derived from phage display. Taken together, these studies highlight concentrations that may be protective against HIV-1 transmission but also indicate that the high amounts of somatic hypermutation of HIV-1 bNAbs may limit their protective efficacy after delivery with rAAV vectors.

In addition to studying rAAV delivered HIV-1 bNAbs, our lab has also evaluated the protective efficacy of rAAV1 expressed eCD4-Ig in rhesus macaques (Gardner et al., 2015, 2019a). In these studies, we have used the rhesus macaque CD4 domains 1 and 2 sequences with an I39N mutation that increases CD4's affinity for HIV-1 and SIV gp120 (Humes et al., 2012). We also engineered eCD4-Ig to have a rhesus macaque IgG2 Fc domain with a coreceptor-mimetic peptide fused to the C-terminus. In two different studies, two groups of four macaques each have received rAAV1 vectors encoding eCD4-Ig as well as a second vector encoding the rhesus macaque TPST2 enzyme to ensure the coreceptor-mimetic peptide gets efficiently sulfated. In our first study, macaques expressed eCD4-Ig for over 1 year with concentrations ranging from 17 to 77 μg/mL (Gardner et al., 2015). These concentrations of eCD4-Ig protected rhesus macaques from six infectious SHIV-AD8 challenges, the last challenge being 4-times higher than the dose needed to infect the last macaque in the control group. In the second study, eCD4-Ig concentrations ranged from 3 to 18 μg/mL (Gardner et al., 2019a). Although lower than the first study, these concentrations protected the treated macaques from two SIVmac239 challenges needed to infect all eight macaques in the control group. As we increased the dose of the challenges, all macaques eventually became infected with the last one getting infected at a dose 32-times greater than the dose needed to infect the macaques in the control group. Despite viral loads not being controlled after infection, we did observe eCD4-Ig-mediated pressure on the viral swarms. Three of the four macaques had noted mutations in the CD4-binding site of SIVmac239 Env, while the fourth macaque had three mutations in gp41. *In vitro* assays showed that these mutations only mediated resistance against eCD4-Ig and not complete escape, which had a clear effect on viral fitness. When looking at both studies, six of eight macaques did generate ADA responses against eCD4-Ig, however the magnitude of the responses was lower than that we observed with rAAV1-expressed HIV-1 bNAbs. In all six of these macaques, the ADA did decline over time to background levels, correlating with stable eCD4-Ig expression in these eight macaques. The lower levels of ADA against eCD4-Ig, combined with observations described in Martinez-Navio et al. and Welles et al., suggest that antibodies and inhibitors that have protein sequences closer to naturally occurring host proteins may result in long-term expression after rAAV inoculation.

The ADA issue present in NHP studies was also observed in a recent phase 1 clinical trial evaluating the delivery of PG9 from rAAV1 vectors (Priddy et al., 2019). In Priddy et al., the authors describe results from their study where participants received rAAV1 doses ranging from 4×10^{12} to 1.2×10^{14} vector genomes (vgs) inoculated intramuscularly. Importantly, no serious adverse events were noted in the study. However, PG9 concentrations were not measurable by ELISA, although the authors did note that a few participants had serum neutralizing activity against the HIV-1 NL4.3 isolate. Despite not measuring PG9 concentrations by ELISA, 10 of 16 participants did have a measurable ADA response against PG9, suggesting a low amount of PG9 was being expressed. A second clinical trial evaluating rAAV8 delivery of VRC07 (https://clinicaltrials.gov/ct2/show/NCT03374202) is currently on-going. Preliminary results were presented at the 2020 Conference on Retroviruses and Opportunistic Infections (CROI, Boston, 2020). All seven volunteers had measurable amounts of VRC07 in the plasma and two volunteers in the high-dose group (2.5×10^{12} vector genomes/kg) had plasma concentrations of VRC01 ranging from 1.1 to 1.2 μg/mL for more than 6 months. One encouraging note was that only two of seven volunteers had measurable ADA responses against VRC07. In the meantime, future pre-clinical studies to improve upon these two clinical trials will help determine the variables that need to be changed to yield better clinical trial results. These variables

include choosing the right capsid, engineering a high-expressing AAV transgene cassette, and possibly choosing a bNAb with less somatic hypermutation than PG9 and VRC07.

rAAV-DELIVERED BNABS FOR THE CURE

With numerous successes in demonstrating protective efficacy of rAAV-delivered bNAbs and HIV-1 inhibitors (i.e., eCD4-Ig), the question now remains whether rAAV vectors can be used for treating and curing an HIV-1 infection. Mouse studies thus far have shown what appears to be the highest concentrations of bNAbs expressed from rAAV vectors (>100 μg/mL for some bNAbs). Would these levels be sufficient for maintaining viral suppression? The NHP studies have yet to show that these concentrations can be routinely achieved in rhesus macaques. Would low concentrations of bNAbs or HIV-1 inhibitors have any impact on viral loads, let alone maintain viral suppression, or would it facilitate viral escape and render the bNAbs useless? These questions are critical for rAAV vector-mediated bNAb therapy to replace current ART.

Despite some of the current limitations observed in animal models and the PG9 clinical trial, a few studies have been encouraging. In a study published by Horwitz et al. (2013), the authors evaluated whether rAAV2-delivered 10-1074 could maintain viral suppression after ART lift (Horwitz et al., 2013). For this study, humanized mice were infected with the HIV-1 isolate YU2 and given ART to suppress infection. Five days after ART began, 10-1074 was administered via passive infusion. ART was halted after 16 days post infusion and the mice were then treated with rAAV2 vectors encoding 10-1074 12 days later. After 67 days, only one of the seven mice treated with rAAV2 vectors encoding 10-1074 had viral rebound, which was noted to have escape mutations against 10-1074. High concentrations of 10-1074 were observed, usually >100 μg/mL, which most likely played a role in viral suppression. One important note the authors made was the use of an "antibody bridge" to wash out the ART after lift and before inoculating the rAAV vectors. The need for a bridge was due to an observed decrease in antibody expression in mice on ART when inoculated with rAAV vectors. This finding may have an impact should rAAV vectors advance to clinic for treating HIV-1 infected individuals as it suggests some ART components may limit AAV transduction. The authors did address that although the results are encouraging, there are major differences between humanized mice and humans. These include the lack of viral diversity before treatment began and is highlighted by the results showing one antibody is sufficient for suppressing viremia, humanized mice are not immunologically competent, and study designs with humanized mice limit the amount of time to monitor efficacy of treatment.

While NHP studies facilitate longer-term evaluation of therapies in a host with a functional immune system, they are not capable of studying actual HIV-1 infection. SHIV isolates are routinely used for evaluating therapeutic efficacy of HIV-1 inhibitors, however, there are instances where macaques can spontaneously resolve SHIV infection without any intervention. With these limitations in mind, the Desrosiers lab published an interesting study last year looking at the therapeutic efficacy of rAAV-delivered bNAbs to suppress an on-going SHIV-AD8 infection (Martinez-Navio et al., 2019). In their first study group, four macaques received rAAV1 vectors encoding 10-1074, 3BNC117, and 10E8 at 86 weeks post infection. Unfortunately, one macaque spontaneously controlled infection immediately before rAAV1 treatment. In the remaining three macaques, one macaque (rh2438, nicknamed the "Miami monkey") suppressed viral loads to below the limits of detection (<15 viral RNA copies/mL) for 3 years after rAAV inoculation. This macaque had high concentrations of both 10-1074 (>100 μg/mL) and 3BNC117 (>50 μg/mL) during that time frame. What makes the Miami monkey result remarkable was that these two antibodies presumably mediated viral suppression before viral escape against both antibodies could be generated. The authors also showed a drop in both viral RNA and DNA measured in PBMCs from the Miami monkey post rAAV inoculation. In the second group from this study, six macaques were treated with a combination 10-1074 and 3BNC117 or N6, 35O22, PGT128, and PGT145. All 12 animals were given rAAV8 inoculations at 36 weeks post SHIV-AD8 infection and then boosted with an rAAV1 inoculation at 60 weeks post SHIV-AD8 infection. Although none of the 12 macaques repeated the results observed in the Miami monkey, two macaques in the group that received four bNAbs demonstrated suppression, with numerous time points where viremia was below the limits of detection. In both these animals, measurable amounts of PGT128 were shown while one of the macaques also had a measurable amount of N6. Despite only reporting one Miami monkey and two additional macaques with suppressed viremia in this study, it is worth noting that the Berlin patient inspired further efforts into cure research. Thus, these results are the first steps toward demonstrating that rAAV vectors delivering bNAbs and HIV-1 inhibitors could be an alternative to ART in future years.

Our lab's current studies have been evaluating the therapeutic efficacy of rAAV-delivered eCD4-Ig in SHIV-AD8-infected rhesus macaques. In a pilot study, six macaques were treated with ART 12 weeks after infection. Two macaques received rAAV8 vectors encoding eCD4-Ig and TPST2 at 104 weeks post infection. ART was lifted 4 weeks after rAAV8 inoculation. The other four macaques received rAAV8 vectors at 56 weeks post infection and rAAV1 vectors at 70 weeks post infection. Again, ART was lifted 4 weeks after rAAV1 inoculations. In all six macaques we have observed measurable eCD4-Ig concentrations for over 2 years, ranging from 4 to 15 μg/mL. While viral rebound was observed in each macaque, we have observed subsequent viral suppression for the past year in each animal, usually with viral loads measuring <100 viral RNA copies/mL. All animals have had viral loads below the limits of detection (<15 viral RNA copies/mL) at multiple time points, and we have yet to observe viral escape from eCD4-Ig. These results are encouraging; however, they highlight the need for further vector improvement to increase eCD4-Ig concentrations. Presumably, higher eCD4-Ig concentrations could delay or suppress viral rebound after ART cessation and hopefully maintain viremia below the limits of detection in all animals over a long period of time. Additionally, because eCD4-Ig is functional against HIV-1,

HIV-2, and SIV, future studies evaluating the therapeutic efficacy of rAAV-delivered eCD4-Ig against more stringent SIV isolates, like SIVmac239, would be more convincing that this therapy would translate when used in human clinical trials. What is evident in all these studies is that the next few years may be critical in advancing rAAV vectors into the clinic for preventing and treating HIV-1 infection.

LEARNING FROM PASSIVE INFUSION EFFICACY STUDIES

Preclinical Studies in Humanized Mice and NHPs

Despite the limited published studies analyzing AAV-delivered HIV-1 inhibitors as a means for an HIV-1 cure, the past decade has produced several passive infusion studies that we can use as insight for the therapeutic efficacy of these inhibitors. The 2010s added to the solid groundwork from numerous studies in the late 1990s and 2000s evaluating passive infusion of first generation bNAbs (like b12 and 2G12) for SHIV protection (Mascola et al., 1999, 2000; Shibata et al., 1999; Baba et al., 2000; Parren et al., 2001; Hessell et al., 2007, 2009). The studies from the past 10 years have shown which second generation bNAbs (like VRC01, 3BNC117, 10-1074, and PGT121) have favorable properties for pharmacokinetics and are effective at suppressing an on-going infection. The first of these studies showed that individual bNAbs had little to no therapeutic efficacy against a clade B infection (YU2) in humanized mice, but a cocktail of five bNAbs yielded complete suppression of viremia, albeit, for a short period of time (Klein et al., 2012). Two studies were published a year later, where in both studies, cocktails of bNAbs were passively infused into rhesus macaques infected with SHIVs. In one study, macaques chronically infected with SHIV-SF162P3 were infused with a cocktail of three bNAbs (3BNC117, b12, and PGT121) or two bNAbs (3BNC117 and PGT121) (Barouch et al., 2013). Viremia decreased to below limits of detection in 12 of 14 animals. In the second study, rhesus macaques infected with SHIV-AD8EO were passively administered 3BNC117 and 10-1074 (Shingai et al., 2013). In 10 of 11 macaques, plasma viremia was decreased to levels below the limit of detection during treatment. However, as bNAb concentrations declined, viremia rebounded in all macaques, with resistance mutations to the bNAbs identified in the Env sequences post viral rebound. Additionally, the combination of 3BNC117 and 10-1074 in macaques acutely infected with SHIV-AD8EO could maintain viral suppression for a 56–177 days in which rebound correlated with antibody half-life. In a similar study by Hessel et al., the authors evaluated the therapeutic efficacy of PGT121 and VRC07-523 in infant rhesus macaques 24 h after being challenged with SHIV-SF162P3 (Hessell et al., 2016). As in the previous study, all animals that received the bNAb therapy were free of viremia in both the plasma and tissue measurements 6 months after treatment. A later study showed that in infant macaques, treatment with the same combination of bNAbs at 30 h post SHIV-SF162P3 challenge, but not at 48 h, was sufficient for keeping the animals from becoming infected (Shapiro et al., 2020). These studies show that bNAb therapy could be used for treating early infections if the treatment is started within a very early window of exposure.

Additional studies in non-human primates (NHPs) described that mutations in the CH3 region of the IgG1 Fc, M428L/N434S (LS), could improve the half-life and protective efficacy of bNAbs *in vivo* (Ko et al., 2014). For example, the LS mutations improved the median protective effect of 3BNC117 from 13 to 17 weeks and of 10-1074, from 12.5 to 27 weeks (Gautam et al., 2018). Of note, one macaque was protected from repeated low-dose intrarectal challenges for 37 weeks after the infusion of 10-1074 with the LS mutations. Due to these results, it is not surprising that the LS mutations have been included in evaluating the safety of bNAbs in clinical trials. LS versions VRC01 and VRC07-523 have been evaluated in the clinic and have reported half-lives of 71 and 38 days, respectively (Gaudinski et al., 2018, 2019). Having measurable quantities of bNAbs in the blood for longer periods of time would presumably lead to less frequent dosing, and ultimately, lower the cost of production.

Clinical Studies

As more bNAbs enter phase 1 evaluation in healthy adults, a few bNAbs have already been assessed for their therapeutic efficacy in individuals living with HIV-1. In the first report from Caskey et al., 10 of 11 participants that received a 10 or 30 mg/kg dose saw plasma viremia decrease that ranged from 0.8 to 2.5 log10 viral RNA copies/mL (Caskey et al., 2015). The participant that did not respond to 3BNC117 treatment had a viral swarm resistant to 3BNC117. Although viremia rebounded in all trial participants, only some had viral swarms with changes in Env that were associated with 3BNC117 resistance. Follow-up studies based on this trial showed that 3BNC117 (and other bNAbs) could accelerate the killing of HIV-1-infected cells in humanized mice as well as improve the antibody responses against HIV-1 in participants treated with 3BNC117 (Lu et al., 2016; Schoofs et al., 2016).

Soon after, Lynch et al. reported their clinical findings for VRC01 treatment (Lynch et al., 2015). In their study, six of eight participants had a reduction of plasma viremia ranging from 1.1 to 1.8 log10 viral RNA copies/mL. Similar to that observed for 3BNC117, the two participants that did not respond to VRC01 treatment had pre-existing resistance to VRC01. Additionally, this study also showed that VRC01 treatment had no effect on cell-associated viral loads in participants treated while still on antiretroviral drug therapy. Should bNAb immunotherapy be useful for an HIV-1 cure, there will need to be evidence that bNAbs can reduce the viral reservoir (i.e., demonstrating reduction in cell-associated viral DNA measurements). A recent study showed similar results with participants acutely infected with HIV-1 (Crowell et al., 2019). These participants treated with VRC01 also had a delay to viral rebound compared to a placebo group. A third bNAb tested for immunotherapy was 10-1074 (Caskey et al., 2017). In 13 viremic individuals, 11 participants saw an average of 1.5 log10 reduction in plasma viremia after 10-1074 infusion. Overall, these three studies highlight two key points. First, bNAb immunotherapy can be effective at treating on-going infection. Second, because of the emergence of viremia

after treatment which can be associated with escape, future bNAb therapies will require a cocktail of bNAbs to be most effective. To this point, a recent clinical trial evaluated the use of 3BNC117 and 10-1074 combination immunotherapy (Bar-On et al., 2018). In this study, participants that responded to treatment had an average reduction of >2 log10 viral RNA copies/mL. A few participants also had suppressed viremia for nearly 3 months after treatment and most viral isolates sequenced after therapy did not escape from the bNAb combination. These data show the promise of bNAb cocktails for therapy although future analysis will be needed to determine which bNAbs are used in combination therapy.

While these first few clinical trials showed that bNAb immunotherapy could suppress an ongoing infection, the question remains whether bNAbs have a role in eradicating virus from infected individuals. One approach has been to administer bNAb infusions while participants are on ART and then undergo an analytical treatment interruption (ATI) to monitor viral rebound. The underlying hypothesis tested in these clinical trials is that a delay in viral rebound should correlate with a decrease in viral reservoir mediated by bNAb immunotherapy. Two initial trials evaluating VRC01 in this situation indeed showed that participants treated with VRC01 while still on ART had delayed viral rebound during the ATI when compared to historical controls (Bar et al., 2016). Sequencing of the *env* gene indicated VRC01-selective pressure in the rebounding virus with most participants developing VRC01-resistant virus during rebound.

Another study evaluated 3BNC117 immunotherapy in participants that had viral loads suppressed by ART (Scheid et al., 2016). In this trial, participants that received infusions of 3BNC117 and then were taken off ART, had viral rebound ranging from 5 to 19 weeks after their final treatment. Like the VRC01 trial, these times to rebound were significantly longer when compared to historical controls. Although these results were encouraging, there was no direct analysis to definitively show that 3BNC117 immunotherapy decreased the latent reservoir. However, it was shown that rebound virus during ATI in participants treated with 3BNC117 was not the predominant species observed in the latent reservoir prior to ATI (Cohen et al., 2018). Additionally, although all participants were screened to be sensitive to 3BNC117 immunotherapy, resistance mutations were identified in the latent reservoir before treatment was initiated. Thus, this study is another example for the need of bNAb combinations even when participants are pre-screened for sensitivity to one bNAb. Mendozza et al. have reported successful results when using the combination bNAb immunotherapy using 3BNC117 and 10-1074 (Mendoza et al., 2018). Participants that received this therapy had suppressed viremia for a median of 21 weeks after ATI was initiated. Viral rebound was observed in all but two participants, and this usually correlated with the clearance of 3BNC117, meaning the participants were effectively on monotherapy at the time of rebound.

Overall, these results are very encouraging in that, at the very least, bNAb combinations can suppress viremia without ART for relatively long periods of time and could be a future alternative to current ART. However, for passive infusion of bNAbs to work as an HIV-1 therapy, individuals will continuously need to receive these infusions, multiple times a year and thus, a requirement for constant production of bNAbs would be needed. Considering the average American male and female are roughly 90 and 77 kg, respectively, more than two grams of bNAbs would be needed per treatment. The underlying question is whether this type of treatment is feasible on a global scale.

CURRENT LIMITATIONS WHEN USING RAAV VECTORS

In order to pursue clinical trials evaluating the therapeutic efficacy of bNAbs delivered by rAAV vectors, three areas of research will be critical to success. The first is identifying which bNAbs or HIV-1 inhibitors should be used in a cocktail of antibodies. No single bNAb described to date can neutralize 100% of HIV-1 isolates tested. Thus, it is a forgone conclusion that multiple bNAbs will be necessary to treat an ongoing HIV-1 infection. The clinical trials have especially shown this in that individual bNAb therapy results in viral rebound in both ART-treated participants and participants undergoing ATI. However, the clinical data that shows long-term suppression after ATI when combining 3BNC117 and 10-1074 underscores the value of bNAb combinations for therapy (Mendoza et al., 2018). Mathematical modeling has been used to identify the best bNAb combinations for specific areas with HIV-1 infected populations (Wagh et al., 2016, 2018). Combinations of bNAbs and bispecific antibodies may be even more effective than bNAbs alone. However, bispecific antibodies provide a challenge for rAAV vectors in that both arms of the antibody cannot fit into a single rAAV transgene cassette. eCD4-Ig may be a useful reagent to combine with bNAbs as current studies show that it neutralizes all HIV-1 and HIV-2 isolates tested. Additionally, viral resistance mutations against eCD4-Ig come with a fitness cost to the virus. Binding studies have shown that eCD4-Ig may pair well with V3g bNAbs (Davis-Gardner et al., 2017). eCD4-Ig also has the benefit at enhancing the ADCC activity of non-neutralizing antibodies that are elicited during an HIV-1 infection (Davis-Gardner et al., 2017). What still needs to be shown is that high concentrations of eCD4-Ig can be expressed routinely from rAAV vectors, and that eCD4-Ig is safe and well-tolerated after passive infusion in phase I clinical trials. As shown in **Figure 1**, the process for identifying the correct cocktail will likely be dependent upon the viral swarm present in the individual living with HIV-1. Viral swarms will need to be characterized for each individual to determine an active cocktail of bNAbs and inhibitors to use on that swarm. Is this necessary? Most likely. Is it practical for the millions of people living with HIV-1? Possibly not as it would require that manufacturing and production of numerous rAAV vectors encoding many different bNAbs or inhibitors.

A second area of research that requires more analysis is the ability for bNAbs, bispecific antibodies, and HIV-1 inhibitors to reduce the latent viral reservoir. Both clinical and pre-clinical bNAb therapy studies have yet to definitively show an increase in rate of reduction of the latent viral reservoir. The latent reservoir is a tough target as it remains hidden

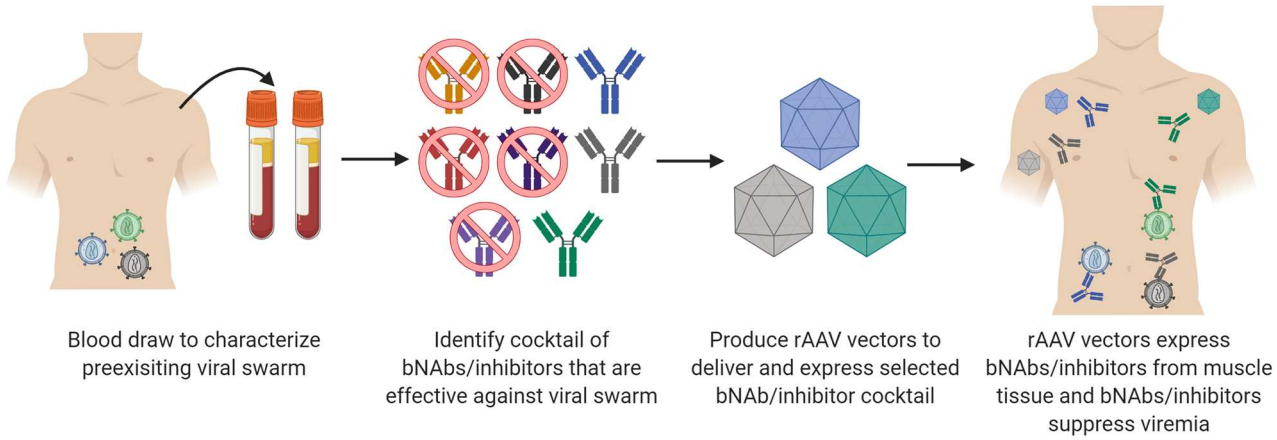


FIGURE 1 | Process for selecting a bNAb/inhibitor cocktail for delivery by rAAV vectors. Similar to ART, it is highly unlikely that a single bNAb or inhibitor cocktail will be effective for every individual living with HIV-1. Therefore, cocktails will most likely be tailored to the viral swarm present for each individual or endemic region. Initial screening will need to be conducted on the preexisting viral swarm to identify which bNAbs or inhibitors have activity against that swarm. rAAV vectors that encode each of the individual components of the cocktail will need to be produced. Delivery of the rAAV vectors will then yield a cocktail of inhibitors made by individual that target the HIV-1 swarm and suppress infection.

in numerous tissue compartments like lymph nodes and gut-associated lymphoid tissues. Can bNAbs and HIV-1 inhibitors access these tissue compartments to kill infected cells and would they reach these tissue compartments when expressed from AAV vectors? Will we need latency reversal agents (LRAs) to stimulate virus production to generate infected cells for bNAbs to target? Are the current LRAs good enough to be combined with bNAb immunotherapy to reduce the viral reservoir? A few studies have investigated these questions. Advancements in visualizing exactly where these antibodies go once delivered into a host include fluorescent imaging of tagged-bNAbs (Schneider et al., 2017) and using PET scans in animal models. Additionally, there is a clinical trial using radiolabeled VRC01 that is in the recruiting phase to use PET-MR imaging that will give insight into bNAb distribution in humans (https://clinicaltrials.gov/ct2/show/NCT03729752). These studies will then lead to downstream questions about whether bNAb and HIV-1 inhibitors expressed from AAV vectors get distributed to the same areas of the host. Pertaining to stimulating viral production in infected cells, the TLR7 agonist GS-9620 induced viremia in SIVmac251-infected rhesus macaques that had viremia suppressed by ART (Lim et al., 2018). In another study, macaques infected with SHIV-SF162P3 and with viremia suppressed on ART were treated with GS-9620 and PGT121 immunotherapy (Borducchi et al., 2018). This group of treated animals had a delay in viral rebound and lower set point viremia in 6 of the 11 macaques that had rebound after ART lift. Of note, in this study GS-9620 did not induce transient viremia. Thus, other reagents may be more effective to reverse latency and induce viral replication. SMAC-mimetic inhibitors that stimulate the non-canonical NF-kB pathway have shown promise as future LRAs to evaluate with bNAbs in NHP studies (Pache et al., 2015; Nixon et al., 2020). Additionally, an IL-15 superagonist (N-803) coupled with CD8+ T cell depletion could provide another avenue to explore (Mcbrien et al., 2020). Combining these LRAs with rAAV-delivered bNAbs or HIV-1 inhibitors could provide valuable insights for HIV-1 cure research. Dosing LRAs in the presence of persistent expression of bNAbs or HIV-1 inhibitors could provide the field with the data necessary for determining whether the shock-and-kill approach reduces the viral reservoir.

Potential outcomes of rAAV-delivered bNAbs combined with and without LRAs are highlighted in **Figure 2**. First, should individuals living with HIV-1 not treated with ART receive subtherapeutic concentrations of bNAbs, viremia will remain stable and viral escape mutations are likely to occur (**Figure 2A**). However, high concentrations of bNAbs, like that observed in the Miami monkey, may be able to suppress infection and result in a functional cure. Second, expression after rAAV inoculation that is again below the therapeutic threshold in individuals with suppressed viremia from ART will likely result in viral rebound and escape during ATI (**Figure 2B**). Yet, should high concentrations of bNAbs be observed, there is a possibility of no viral rebound during ATI, such as that observed in the clinical trial that used 3BNC117 and 10-1074 immunotherapy. Lastly, in the best-case scenario where a functional cure is observed using rAAV-delivered bNAbs, LRA administration may help stimulate viral replication that could ultimately reduce the viral reservoir (**Figure 2C**). The only way to cure individuals living with HIV-1 is through this scenario where viral suppression is maintained and the viral reservoir is reduced by bNAbs.

A third area of research that needs to be resolved for rAAV vectors to work is the immunogenicity issues. As highlighted in the NHP studies and confirmed in the PG9 clinical trial, ADA responses against the expressed bNAb may limit measurable concentrations and functionality of the bNAb. Interestingly, ADA against eCD4-Ig appears to decline over time. Is it possible to engineer bNAbs to have such a quality or do we need to identify the best bNAbs with least amount of somatic hypermutation to

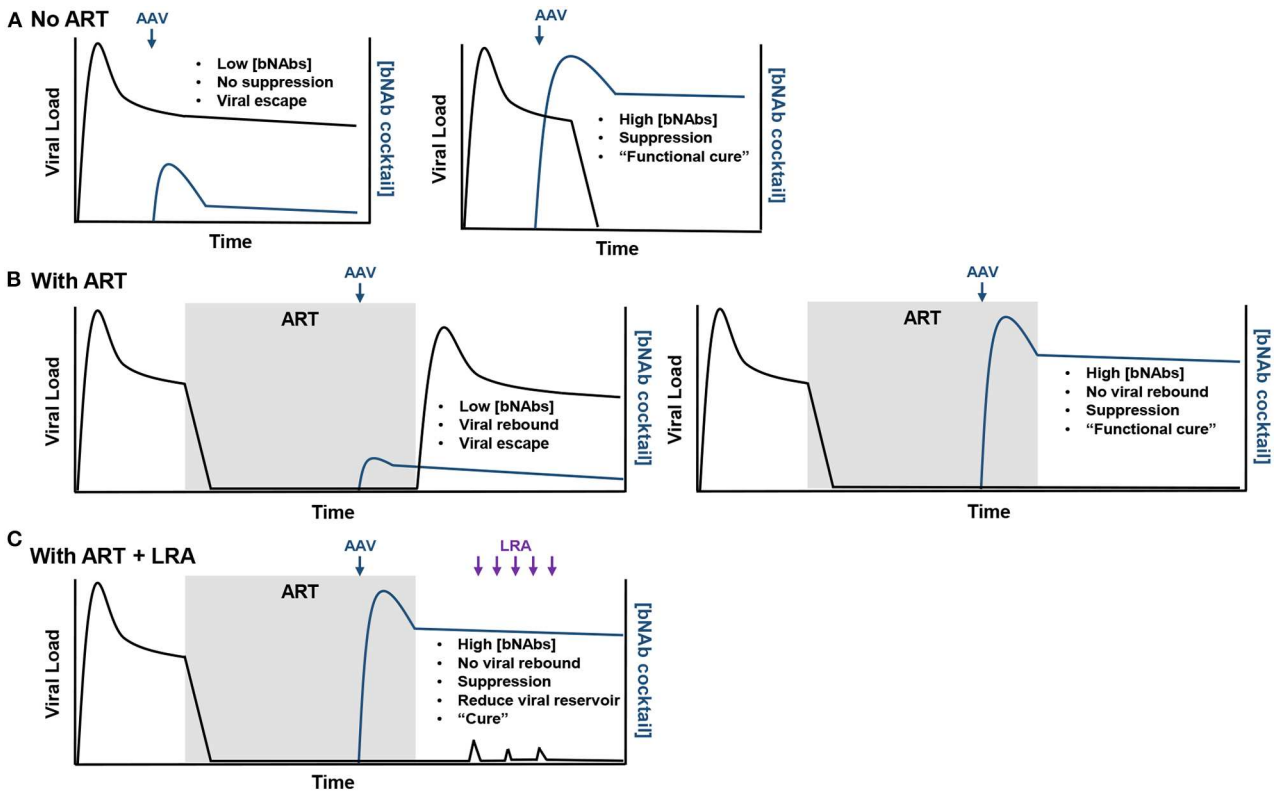


FIGURE 2 | Possible outcomes from rAAV-mediated bNAb therapy. **(A)** In the scenario where rAAV-delivered bNAbs (blue arrow) are used to treat chronic infection, if the bNAb concentrations do not reach therapeutic levels, no viral suppression will be observed and viral escape from the bNAbs will be likely (left). However, therapeutic concentrations may suppress viremia and provide a functional cure for the individual (right). **(B)** In individuals treated with ART, low concentrations of rAAV-expressed bNAbs will likely result in viral rebound (left). High concentrations of bNAbs will maintain viral suppression after ART lift and provide a functional cure (right). **(C)** Should therapeutic concentrations maintain viral suppression after ART lift, LRAs (purple arrows) may be administered to stimulate replication (viral load blips) in latently infected cells and provide a target for bNAbs. If this approach is successful, a measurable decrease in the viral reservoir should be observed and eventually lead the individual to being cured of the HIV-1 infection.

use for rAAV delivery? Would these be the bNAbs that work best in combination for treating HIV-1 infection? Additionally, the rAAV capsid presents its own immunogenicity issues. Delivery of rAAV vectors will likely cause T cell and antibody responses against the capsid, limiting the number of times a capsid can be used to just one dose (Ertl and High, 2016). T cell responses also play a role in removing transduced cells, thus lowering the total concentration of expressed transgene. Mouse studies have shown that different AAV capsids do have different immunogenicity properties. For example, the AAV1 capsid appears to be more immunogenic than the AAV8 capsid as it stimulates greater T cell responses (Lu and Song, 2009). Generation of regulatory T cells (Tregs) has also been correlated with lower immune responses against rAAV delivered transgenes (Cao et al., 2007). Inducing tolerance to rAAV transgene is thus an interesting approach to promote persistence of transgene expression. One way to promote tolerance has been to target the liver with rAAV vectors to produce more Tregs. In a recent study, intravenous administration of rAAV8 vectors encoding 4L6 followed by an intramuscular rAAV1 vector inoculation 14 weeks later, resulted in robust 4L6 concentrations in three of three rhesus macaques (Fuchs et al., 2019). Thus, the liver expression of rAAV transgenes may provide a means of tolerance. However, liver transduction may be complicated by another immunogenicity factor. Pre-existing neutralizing antibodies against specific AAV capsids limit the ability to use specific capsids for transduction. Studies evaluating the seropositive status of humans against AAV capsids have shown that a high prevalence of the human population already has neutralizing antibodies against at least one AAV capsid (Halbert et al., 2006; Calcedo et al., 2009; Boutin et al., 2010; Hüser et al., 2017). It has also been shown that AAV neutralizing antibodies do not have as much of an effect when inoculations are administered intramuscularly (Greig et al., 2016), which makes skeletal muscle tissue an easier target compared to the liver. Another approach could be through the use of synthetic capsids (Büning and Srivastava, 2019; Pekrun et al., 2019). Both rational design and directed evolution studies are being employed to generate synthetic capsids that improve AAV transduction to specific tissues and avoid the pre-existing neutralizing antibody responses. Another issue is that the AAV genome itself is immunogenic as it is a target of the TLR9 pathway (Zhu et al., 2009). Genome engineering studies, such as CpG depletion (Faust et al., 2013), have been effective at lowering the TLR9 response but studies in NHPs are warranted. Overall,

overcoming the numerous host immune responses against AAV will be critical for rAAV vectors to be utilized in the clinic, not only for delivering HIV-1 bNAbs and inhibitors, but for other gene therapies as well.

Lastly, the safety of AAV gene therapy vectors will need to be addressed. Ideally, systems would be in place that can regulate transgene expression in case adverse events are observed after infusion. A few studies have delved into regulating transgene expression in AAV systems. Some studies investigating transgene regulation can induce expression using small molecules (Yen et al., 2004; Mou et al., 2018; Zhong et al., 2020). However, in a situation where AAV vectors are used for delivery anti-HIV-1 biologics for a cure, ideally the vector would constitutively express the inhibitor and contain elements for a kill-switch. A functional kill-switch for AAV vectors has yet to be realized, but it can be imagined that Cre/LoxP or CRISPR/Cas systems might solve the issue in the coming years. Overcoming the safety concerns as well as the other hurdles that were noted would improve a gene therapy system that already has a great foundation. The promise that AAV gene therapy provides for treating numerous diseases supports future studies to solve these issues.

SUMMARY

A true cure that eliminates the HIV-1 reservoir is a formidable goal, yet one that is worth pursuing. As noted in this review, bNAbs and HIV-1 inhibitors may play a role as part of an HIV-1 cure, but questions persist whether these drugs can ultimately impact the rate of decay of the reservoir. The Miami Monkey provides the field with evidence that rAAV vectors could be part of a "one-shot cure" in that these vectors could provide long-term expression of bNAbs and inhibitors and at therapeutic concentrations, suppress an HIV-1 infection in place of daily ART. Thus, a pathway to curing HIV-1 using bNAbs or inhibitors is a functional cure without adherence to ART. Further studies are warranted combining rAAV-delivered bNAbs and HIV-1 inhibitors with LRAs to determine whether the shock-and-kill approach is viable with these reagents. Overall, the future research in the HIV-1 cure field remains promising as we enter a new decade with an arsenal of new and effective tools at our disposable.

AUTHOR CONTRIBUTIONS

MG composed the manuscript and figures.

FUNDING

MG is currently supported by the National Institutes of Health (NIH) Grant K99AI138860.

ACKNOWLEDGMENTS

The author would like to thank Dr. Meredith Davis-Gardner for her valuable discussion, insight, and editing of the manuscript.

REFERENCES

Asokan, M., Rudicell, R. S., Louder, M., Mckee, K., O'dell, S., Stewart-Jones, G., et al. (2015). Bispecific antibodies targeting different epitopes on the HIV-1 envelope exhibit broad and potent neutralization. *J. Virol.* 89, 12501–12512. doi: 10.1128/JVI.02097-15

Atchison, R. W., Casto, B. C., and Hammon, W. M. (1965). Adenovirus-associated defective virus particles. *Science* 149, 754–755. doi: 10.1126/science.149.3685.754

Baba, T. W., Liska, V., Hofmann-Lehmann, R., Vlasak, J., Xu, W., Ayehunie, S., et al. (2000). Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian–human immunodeficiency virus infection. *Nat. Med.* 6, 200–206. doi: 10.1038/72309

Bachmann, N., Von Siebenthal, C., Vongrad, V., Turk, T., Neumann, K., Beerenwinkel, N., et al. (2019). Determinants of HIV-1 reservoir size and long-term dynamics during suppressive ART. *Nat. Commun.* 10:3193. doi: 10.1038/s41467-019-10884-9

Badamchi-Zadeh, A., Tartaglia, L. J., Abbink, P., Bricault, C. A., Liu, P.-T., Boyd, M., et al. (2018). Therapeutic efficacy of vectored PGT121 gene delivery in HIV-1-infected humanized mice. *J. Virol.* 92, e01925–e01917. doi: 10.1128/JVI.01925-17

Balazs, A. B., Chen, J., Hong, C. M., Rao, D. S., Yang, L., and Baltimore, D. (2012). Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature* 481, 81–84. doi: 10.1038/nature10660

Balazs, A. B., Ouyang, Y., Hong, C. M., Chen, J., Nguyen, S. M., Rao, D. S., et al. (2014). Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. *Nat. Med.* 20, 296–300. doi: 10.1038/nm.3471

Bar, K. J., Sneller, M. C., Harrison, L. J., Justement, J. S., Overton, E. T., Petrone, M. E., et al. (2016). Effect of HIV antibody VRC01 on viral rebound after treatment interruption. *N. Engl. J. Med.* 375, 2037–2050. doi: 10.1056/NEJMoa1608243

Bar-On, Y., Gruell, H., Schoofs, T., Pai, J. A., Nogueira, L., Butler, A. L., et al. (2018). Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. *Nat. Med.* 24, 1701–1707. doi: 10.1038/s41591-018-0186-4

Barouch, D. H., Whitney, J. B., Moldt, B., Klein, F., Oliveira, T. Y., Liu, J., et al. (2013). Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* 503, 224–228. doi: 10.1038/nature12744

Besson, G. J., Lalama, C. M., Bosch, R. J., Gandhi, R. T., Bedison, M. A., Aga, E., et al. (2014). HIV-1 DNA Decay dynamics in blood during more than a decade of suppressive antiretroviral therapy. *Clin. Infect. Dis.* 59, 1312–1321. doi: 10.1093/cid/ciu585

Borducchi, E. N., Liu, J., Nkolola, J. P., Cadena, A. M., Yu, W.-H., Fischinger, S., et al. (2018). Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* 563, 360–364. doi: 10.1038/s41586-018-0600-6

Bournazos, S., Gazumyan, A., Seaman, M. S., Nussenzweig, M. C., and Ravetch, J. V. (2016). Bispecific anti-HIV-1 antibodies with enhanced breadth and potency. *Cell* 165, 1609–1620. doi: 10.1016/j.cell.2016.04.050

Boutin, S., Monteilhet, V., Veron, P., Leborgne, C., Benveniste, O., Montus, M. F., et al. (2010). Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. *Hum. Gene Ther.* 21, 704–712. doi: 10.1089/hum.2009.182

Bruel, T., Guivel-Benhassine, F., Amraoui, S., Malbec, M., Richard, L., Bourdic, K., et al. (2016). Elimination of HIV-1-infected cells by broadly neutralizing antibodies. *Nat. Commun.* 7:10844. doi: 10.1038/ncomms10844

Buchlis, G., Podsakoff, G. M., Radu, A., Hawk, S. M., Flake, A. W., Mingozzi, F., et al. (2012). Factor IX expression in skeletal muscle of a severe hemophilia B patient 10 years after AAV-mediated gene transfer. *Blood* 119, 3038–3041. doi: 10.1182/blood-2011-09-382317

Büning, H., and Srivastava, A. (2019). Capsid modifications for targeting and improving the efficacy of AAV vectors. *Mol. Ther. Methods Clin. Dev.* 12, 248–265. doi: 10.1016/j.omtm.2019.01.008

Burton, D. R., and Hangartner, L. (2016). Broadly neutralizing antibodies to HIV and their role in vaccine design. *Annu. Rev. Immunol.* 34, 635–659. doi: 10.1146/annurev-immunol-041015-055515

Calcedo, R., Vandenberghe, L. H., Gao, G., Lin, J., and Wilson, J. M. (2009). Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. *J. Infect. Dis.* 199, 381–390. doi: 10.1086/595830

Cao, O., Dobrzynski, E., Wang, L., Nayak, S., Mingle, B., Terhorst, C., et al. (2007). Induction and role of regulatory CD4+CD25+ T cells in tolerance to the transgene product following hepatic *in vivo* gene transfer. *Blood* 110, 1132–1140. doi: 10.1182/blood-2007-02-073304

Caskey, M., Klein, F., Lorenzi, J. C. C., Seaman, M. S., West, A. P. Jr., Buckley, N., et al. (2015). Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature* 522, 487–491. doi: 10.1038/nature14411

Caskey, M., Schoofs, T., Gruell, H., Settler, A., Karagounis, T., Kreider, E. F., et al. (2017). Antibody 10-1074 suppresses viremia in HIV-1-infected individuals. *Nat. Med.* 23, 185–191. doi: 10.1038/nm.4268

Chen, W., Feng, Y., Prabakaran, P., Ying, T., Wang, Y., Sun, J., et al. (2014). Exceptionally potent and broadly cross-reactive, bispecific multivalent HIV-1 inhibitors based on single human CD4 and antibody domains. *J. Virol.* 88, 1125–1139. doi: 10.1128/JVI.02566-13

Choe, H., Li, W., Wright, P. L., Vasilieva, N., Venturi, M., Huang, C.-C., et al. (2003). Tyrosine sulfation of human antibodies contributes to recognition of the CCR5 binding region of HIV-1 gp120. *Cell* 114, 161–170. doi: 10.1016/s0092-8674(03)00508-7

Chun, T.-W., Justement, J. S., Moir, S., Hallahan, C. W., Maenza, J., Mullins, J. I., et al. (2007). Decay of the HIV reservoir in patients receiving antiretroviral therapy for extended periods: implications for eradication of virus. *J. Infect. Dis.* 195, 1762–1764. doi: 10.1086/518250

Cohen, Y. Z., Lorenzi, J. C. C., Krassnig, L., Barton, J. P., Burke, L., Pai, J., et al. (2018). Relationship between latent and rebound viruses in a clinical trial of anti-HIV-1 antibody 3BNC117. *J. Exp. Med.* 215, 2311–2324. doi: 10.1084/jem.20180936

Crowell, T. A., Colby, D. J., Pinyakorn, S., Sacdalan, C., Pagliuzza, A., Intasan, J., et al. (2019). Safety and efficacy of VRC01 broadly neutralising antibodies in adults with acutely treated HIV (RV397): a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet HIV* 6, e297–e306. doi: 10.1016/S2352-3018(19)30053-0

Davis-Gardner, M. E., Alfant, B., Weber, J. A., Gardner, M. R., and Farzan, M. (2020). A bispecific antibody that simultaneously recognizes the V2- and V3-glycan epitopes of the HIV-1 envelope glycoprotein is broader and more potent than its parental antibodies. *MBio* 11:e03080–19. doi: 10.1128/mBio.03080-19

Davis-Gardner, M. E., Gardner, M. R., Alfant, B., and Farzan, M. (2017). eCD4-Ig promotes ADCC activity of sera from HIV-1-infected patients. *PLoS Pathog.* 13:e1006786. doi: 10.1371/journal.ppat.1006786

Deeks, S. G., Lewin, S. R., Ross, A. L., Ananworanich, J., Benkirane, M., Cannon, P., et al. (2016). International AIDS society global scientific strategy: towards an HIV cure 2016. *Nat. Med.* 22, 839–850. doi: 10.1038/nm.4108

Doria-Rose, N. A., Bhiman, J. N., Roark, R. S., Schramm, C. A., Gorman, J., Chuang, G.-Y., et al. (2016). New member of the V1V2-directed CAP256-VRC26 lineage that shows increased breadth and exceptional potency. *J. Virol.* 90, 76–91. doi: 10.1128/JVI.01791-15

Duan, D., Sharma, P., Yang, J., Yue, Y., Dudus, L., Zhang, Y., et al. (1998). Circular intermediates of recombinant adeno-associated virus have defined structural characteristics responsible for long-term episomal persistence in muscle tissue. *J. Virol.* 72, 8568–8577. doi: 10.1128/JVI.72.11.8568-8577.1998

Duan, D., Yue, Y., Yan, Z., and Engelhardt, J. F. (2000). A new dual-vector approach to enhance recombinant adeno-associated virus-mediated gene expression through intermolecular cis activation. *Nat. Med.* 6, 595–598. doi: 10.1038/75080

Ertl, H. C. J., and High, K. A. (2016). Impact of AAV capsid-specific T-cell responses on design and outcome of clinical gene transfer trials with recombinant adeno-associated viral vectors: an evolving controversy. *Hum. Gene Ther.* 28, 328–337. doi: 10.1089/hum.2016.172

Falkowska, E., Le, K. M., Ramos, A., Doores, K. J., Lee, J. H., Blattner, C., et al. (2014). Broadly neutralizing HIV antibodies define a glycan-dependent epitope on the prefusion conformation of gp41 on cleaved envelope trimers. *Immunity* 40, 657–668. doi: 10.1016/j.immuni.2014.04.009

Fang, J., Qian, J.-J., Yi, S., Harding, T. C., Tu, G. H., Vanroey, M., et al. (2005). Stable antibody expression at therapeutic levels using the 2A peptide. *Nat. Biotechnol.* 23, 584–590. doi: 10.1038/nbt1087

Faust, S. M., Bell, P., Cutler, B. J., Ashley, S. N., Zhu, Y., Rabinowitz, J. E., et al. (2013). CpG-depleted adeno-associated virus vectors evade immune detection. *J. Clin. Invest.* 123, 2994–3001. doi: 10.1172/JCI68205

Fellinger, C. H., Gardner, M. R., Weber, J. A., Alfant, B., Zhou, A. S., and Farzan, M. (2019). eCD4-Ig limits HIV-1 escape more effectively than CD4-Ig or a broadly neutralizing antibody. *J. Virol.* 93, e00443–e00419. doi: 10.1128/JVI.00443-19

Fetzer, I., Gardner, M. R., Davis-Gardner, M. E., Prasad, N. R., Alfant, B., Weber, J. A., et al. (2018). eCD4-Ig variants that more potently neutralize HIV-1. *J. Virol.* 92, e02011–02017. doi: 10.1128/JVI.02011-17

Fuchs, S. P., Martinez-Navio, J. M., Piatak, M. Jr., Lifson, J. D., Gao, G., and Desrosiers, R. C. (2015). AAV-delivered antibody mediates significant protective effects against SIVmac239 challenge in the absence of neutralizing activity. *PLoS Pathog.* 11:e1005090. doi: 10.1371/journal.ppat.1005090

Fuchs, S. P., Martinez-Navio, J. M., Rakasz, E. G., Gao, G., and Desrosiers, R. C. (2019). Liver-directed but not muscle-directed AAV-antibody gene transfer limits humoral immune responses in rhesus monkeys. *Mol. Ther. Methods Clin. Dev.* 16, 94–102. doi: 10.1016/j.omtm.2019.11.010

Gardner, M. R., Fellinger, C. H., Kattenhorn, L. M., Davis-Gardner, M. E., Weber, J. A., Alfant, B., et al. (2019a). AAV-delivered eCD4-Ig protects rhesus macaques from high-dose SIVmac239 challenges. *Sci. Trans. Med.* 11:eaau5409. doi: 10.1126/scitranslmed.aau5409

Gardner, M. R., Fetzer, I., Kattenhorn, L. M., Davis-Gardner, M. E., Zhou, A. S., Alfant, B., et al. (2019b). Anti-drug antibody responses impair prophylaxis mediated by AAV-delivered HIV-1 broadly neutralizing antibodies. *Mol. Ther.* 27, 650–660. doi: 10.1016/j.ymthe.2019.01.004

Gardner, M. R., Kattenhorn, L. M., Kondur, H. R., Von Schaewen, M., Dorfman, T., Chiang, J. J., et al. (2015). AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges. *Nature* 519, 87–91. doi: 10.1038/nature14264

Gaudinski, M. R., Coates, E. E., Houser, K. V., Chen, G. L., Yamshchikov, G., Saunders, J. G., et al. (2018). Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: a phase 1 open-label clinical trial in healthy adults. *PLoS Med.* 15:e1002493. doi: 10.1371/journal.pmed.1002493

Gaudinski, M. R., Houser, K. V., Doria-Rose, N. A., Chen, G. L., Rothwell, R. S. S., Berkowitz, N., et al. (2019). Safety and pharmacokinetics of broadly neutralising human monoclonal antibody VRC07-523LS in healthy adults: a phase 1 dose-escalation clinical trial. *Lancet HIV* 6, e667–e679. doi: 10.1016/S2352-3018(19)30181-X

Gautam, R., Nishimura, Y., Gaughan, N., Gazumyan, A., Schoofs, T., Buckler-White, A., et al. (2018). A single injection of crystallizable fragment domain-modified antibodies elicits durable protection from SHIV infection. *Nat. Med.* 24, 610–616. doi: 10.1038/s41591-018-0001-2

Greig, J., Calcedo, R., Grant, R., Peng, H., Medina-Jaszek, C., Ahonkhai, O., et al. (2016). Intramuscular administration of AAV overcomes pre-existing neutralizing antibodies in rhesus macaques. *Vaccine* 34, 6323–6329. doi: 10.1016/j.vaccine.2016.10.053

Halbert, C. L., Miller, A. D., Mcnamara, S., Emerson, J., Gibson, R. L., Ramsey, B., et al. (2006). Prevalence of neutralizing antibodies against adeno-associated virus (AAV) types 2, 5, and 6 in cystic fibrosis and normal populations: implications for gene therapy using AAV vectors. *Hum. Gene Ther.* 17, 440–447. doi: 10.1089/hum.2006.17.440

Hessell, A. J., Hangartner, L., Hunter, M., Havenith, C. E. G., Beurskens, F. J., Bakker, J. M., et al. (2007). Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* 449, 101–104. doi: 10.1038/nature06106

Hessell, A. J., Jaworski, J. P., Epson, E., Matsuda, K., Pandey, S., Kahl, C., et al. (2016). Early short-term treatment with neutralizing human monoclonal antibodies halts SHIV infection in infant macaques. *Nat. Med.* 22, 362–368. doi: 10.1038/nm.4063

Hessell, A. J., Rakasz, E. G., Poignard, P., Hangartner, L., Landucci, G., Forthal, D. N., et al. (2009). Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. *PLoS Pathog.* 5:e1000433. doi: 10.1371/journal.ppat.1000433

Horwitz, J. A., Halper-Stromberg, A., Mouquet, H., Gitlin, A. D., Tretiakova, A., Eisenreich, T. R., et al. (2013). HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. *Proc. Natl. Acad. Sci. U.S.A.* 110, 16538–16543. doi: 10.1073/pnas.1315295110

Huang, J., Kang, B. H., Ishida, E., Zhou, T., Griesman, T., Sheng, Z., et al. (2016a). Identification of a CD4-binding-site antibody to HIV that evolved near-pan neutralization breadth. *Immunity* 45, 1108–1121. doi: 10.1016/j.immuni.2016.10.027

Huang, J., Kang, B. H., Pancera, M., Lee, J. H., Tong, T., Feng, Y., et al. (2014). Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-gp120 interface. *Nature* 515, 138–142. doi: 10.1038/nature13601

Huang, J., Ofek, G., Laub, L., Louder, M. K., Doria-Rose, N. A., Longo, N. S., et al. (2012). Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature* 491, 406–412. doi: 10.1038/nature11544

Huang, Y., Yu, J., Lanzi, A., Yao, X., Andrews, C. D., Tsai, L., et al. (2016b). Engineered bispecific antibodies with exquisite HIV-1-neutralizing activity. *Cell* 165, 1621–1631. doi: 10.1016/j.cell.2016.05.024

Humes, D., Emery, S., Laws, E., and Overbaugh, J. (2012). A species-specific amino acid difference in the macaque CD4 receptor restricts replication by global circulating HIV-1 variants representing viruses from recent infection. *J. Virol.* 86, 12472–12483. doi: 10.1128/JVI.02176-12

Hüser, D., Khalid, D., Lutter, T., Hammer, E.-M., Weger, S., Heßler, M., et al. (2017). High prevalence of infectious adeno-associated virus (AAV) in human peripheral blood mononuclear cells indicative of T lymphocytes as sites of AAV persistence. *J. Virol.* 91, e02137–e02116. doi: 10.1128/JVI.02137-16

Johnson, P. R., Schnepp, B. C., Zhang, J., Connell, M. J., Greene, S. M., Yuste, E., et al. (2009). Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nat. Med.* 15, 901–906. doi: 10.1038/nm.1967

Julg, B., Sok, D., Schmidt, S. D., Abbink, P., Newman, R. M., Broge, T., et al. (2017). Protective efficacy of broadly neutralizing antibodies with incomplete neutralization activity against simian-human immunodeficiency virus in rhesus monkeys. *J. Virol.* 91, e01187–e01117. doi: 10.1128/JVI.01187-17

Khan, S. N., Sok, D., Tran, K., Movsesyan, A., Dubrovskaya, V., Burton, D. R., et al. (2018). Targeting the HIV-1 spike and coreceptor with Bi- and trispecific antibodies for single-component broad inhibition of entry. *J. Virol.* 92, e00384–e00318. doi: 10.1128/JVI.00384-18

Klein, F., Halper-Stromberg, A., Horwitz, J. A., Gruell, H., Scheid, J. F., Bournazos, S., et al. (2012). HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* 492, 118–122. doi: 10.1038/nature11604

Ko, S.-Y., Pegu, A., Rudicell, R. S., Yang, Z.-Y., Joyce, M. G., Chen, X., et al. (2014). Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* 514, 642–645. doi: 10.1038/nature13612

Kong, R., Xu, K., Zhou, T., Acharya, P., Lemmin, T., Liu, K., et al. (2016). Fusion peptide of HIV-1 as a site of vulnerability to neutralizing antibody. *Science* 352, 828–833. doi: 10.1126/science.aae0474

Kwon, Y. D., Georgiev, I. S., Ofek, G., Zhang, B., Asokan, M., Bailer, R. T., et al. (2016). Optimization of the solubility of HIV-1-neutralizing antibody 10E8 through somatic variation and structure-based design. *J. Virol.* 90, 5899–5914. doi: 10.1128/JVI.03246-15

Lagenaur, L. A., Villarroel, V. A., Bundoc, V., Dey, B., and Berger, E. A. (2010). sCD4-17b bifunctional protein: extremely broad and potent neutralization of HIV-1 Env pseudotyped viruses from genetically diverse primary isolates. *Retrovirology* 7:11. doi: 10.1186/1742-4690-7-11

Lewis, A. D., Chen, R., Montefiori, D. C., Johnson, P. R., and Clark, K. R. (2002). Generation of neutralizing activity against human immunodeficiency virus type 1 in serum by antibody gene transfer. *J. Virol.* 76, 8769–8775. doi: 10.1128/jvi.76.17.8769-8775.2002

Lim, S.-Y., Osuna, C. E., Hraber, P. T., Hesselgesser, J., Gerold, J. M., Barnes, T. L., et al. (2018). TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci. Trans. Med.* 10:eaao4521. doi: 10.1126/scitranslmed.aao4521

Lu, C.-L., Murakowski, D. K., Bournazos, S., Schoofs, T., Sarkar, D., Halper-Stromberg, A., et al. (2016). Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 *in vivo*. *Science* 352, 1001–1004. doi: 10.1126/science.aaf1279

Lu, L. L., Suscovich, T. J., Fortune, S. M., and Alter, G. (2018). Beyond binding: antibody effector functions in infectious diseases. *Nat. Rev. Immunol.* 18, 46–61. doi: 10.1038/nri.2017.106

Lu, Y., and Song, S. (2009). Distinct immune responses to transgene products from rAAV1 and rAAV8 vectors. *Proc. Natl. Acad. Sci. U.S.A.* 106, 17158–17162. doi: 10.1073/pnas.0909520106

Lynch, R. M., Boritz, E., Coates, E. E., Dezure, A., Madden, P., Costner, P., et al. (2015). Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. *Sci. Trans. Med.* 7:319ra206. doi: 10.1126/scitranslmed.aad5752

Martinez-Navio, J. M., Fuchs, S. P., Mendes, D. E., Rakasz, E. G., Gao, G., Lifson, J. D., et al. (2020). Long-term delivery of an Anti-SIV monoclonal antibody with AAV. *Front. Immunol.* 11:449. doi: 10.3389/fimmu.2020.00449

Martinez-Navio, J. M., Fuchs, S. P., Pantry, S. N., Lauer, W. A., Duggan, N. N., Keele, B. F., et al. (2019). Adeno-associated virus delivery of anti-HIV monoclonal antibodies can drive long-term virologic suppression. *Immunity* 50, 567–575.e565. doi: 10.1016/j.immuni.2019.02.005

Martinez-Navio, J. M., Fuchs, S. P., Pedreño-López, S., Rakasz, E. G., Gao, G., and Desrosiers, R. C. (2016). Host anti-antibody responses following adeno-associated virus-mediated delivery of antibodies against HIV and SIV in rhesus monkeys. *Mol. Ther.* 24, 76–86. doi: 10.1038/mt.2015.191

Mascola, J. R., Lewis, M. G., Stiegler, G., Harris, D., Vancott, T. C., Hayes, D., et al. (1999). Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J. Virol.* 73, 4009–4018. doi: 10.1128/JVI.73.5.4009-4018.1999

Mascola, J. R., Stiegler, G., Vancott, T. C., Katinger, H., Carpenter, C. B., Hanson, C. E., et al. (2000). Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat. Med.* 6, 207–210. doi: 10.1038/72318

Mayer, K. H., Seaton, K. E., Huang, Y., Grunenberg, N., Isaacs, A., Allen, M., et al. (2017). Safety, pharmacokinetics, and immunological activities of multiple intravenous or subcutaneous doses of an anti-HIV monoclonal antibody, VRC01, administered to HIV-uninfected adults: results of a phase 1 randomized trial. *PLoS Med.* 14:e1002435. doi: 10.1371/journal.pmed.1002435

Mcbrien, J. B., Mavigner, M., Franchitti, L., Smith, S. A., White, E., Tharp, G. K., et al. (2020). Robust and persistent reactivation of SIV and HIV by N-803 and depletion of CD8+ cells. *Nature* 578, 154–159. doi: 10.1038/s41586-020-1946-0

Mendoza, P., Gruell, H., Nogueira, L., Pai, J. A., Butler, A. L., Millard, K., et al. (2018). Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature* 561, 479–484. doi: 10.1038/s41586-018-0531-2

Mou, H., Zhong, G., Gardner, M. R., Wang, H., Wang, Y.-W., Cheng, D., et al. (2018). Conditional regulation of gene expression by ligand-induced occlusion of a microRNA target sequence. *Mol. Ther.* 26, 1277–1286. doi: 10.1016/j.ymthe.2018.02.021

Mouquet, H., Scharf, L., Euler, Z., Liu, Y., Eden, C., Scheid, J. F., et al. (2012). Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc. Natl. Acad. Sci. U.S.A.* 109, E3268–E3277. doi: 10.1073/pnas.1217207109

Mujib, S., Liu, J., Rahman, A. K. M. N.-U., Schwartz, J. A., Bonner, P., Yue, F. Y., et al. (2017). Comprehensive cross-clade characterization of antibody-mediated recognition, complement-mediated lysis, and cell-mediated cytotoxicity of HIV-1 envelope-specific antibodies toward eradication of the HIV-1 reservoir. *J. Virol.* 91, e00634–e00617. doi: 10.1128/JVI.00634-17

Nakai, H., Storm, T. A., and Kay, M. A. (2000). Increasing the size of rAAV-mediated expression cassettes *in vivo* by intermolecular joining of two complementary vectors. *Nat. Biotechnol.* 18, 527–532. doi: 10.1038/75390

Nixon, C. C., Mavigner, M., Sampey, G. C., Brooks, A. D., Spagnuolo, R. A., Irlbeck, D. M., et al. (2020). Systemic HIV and SIV latency reversal via non-canonical NF-κB signalling *in vivo*. *Nature* 578, 160–165. doi: 10.1038/s41586-020-1951-3

Pache, L., Dutra, M. S., Spivak, A. M., Marlett, J. M., Murry, J. P., Hwang, Y., et al. (2015). BIRC2/cIAP1 Is a negative regulator of HIV-1 transcription and can be targeted by smac mimetics to promote reversal of viral latency. *Cell Host Microbe* 18, 345–353. doi: 10.1016/j.chom.2015.08.009

Parren, P. W., Marx, P. A., Hessell, A. J., Luckay, A., Harouse, J., Cheng-Mayer, C., et al. (2001). Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization *in vitro*. *J. Virol.* 75, 8340–8347. doi: 10.1128/jvi.75.17.8340-8347.2001

Pekrun, K., De Alencastro, G., Luo, Q.-J., Liu, J., Kim, Y., Nygaard, S., et al. (2019). Using a barcoded AAV capsid library to select for clinically relevant gene therapy vectors. *JCI Insight* 4:e131610. doi: 10.1172/jci.insight.131610

Priddy, F. H., Lewis, D. J. M., Gelderblom, H. C., Hassanin, H., Streatfield, C., Labranche, C., et al. (2019). Adeno-associated virus vectored immunoprophylaxis to prevent HIV in healthy adults: a phase 1 randomised controlled trial. *Lancet HIV* 6, e230–e239. doi: 10.1016/S2352-3018(19)30003-7

Ridgway, J. B. B., Presta, L. G., and Carter, P. (1996). 'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization. *Protein Eng. Des. Sel.* 9, 617–621. doi: 10.1093/protein/9.7.617

Rizzuto, C. D., Wyatt, R., Hernández-Ramos, N., Sun, Y., Kwong, P. D., Hendrickson, W. A., et al. (1998). A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* 280, 1949–1953. doi: 10.1126/science.280.5371.1949

Rudicell, R. S., Kwon, Y. D., Ko, S.-Y., Pegu, A., Louder, M. K., Georgiev, I. S., et al. (2014). Enhanced potency of a broadly neutralizing HIV-1 antibody *in vitro* improves protection against lentiviral infection *in vivo*. *J. Virol.* 88, 12669–12682. doi: 10.1128/JVI.02213-14

Sajadi, M. M., Dashti, A., Rikhtegaran Tehrani, Z., Tolbert, W. D., Seaman, M. S., Ouyang, X., et al. (2018). Identification of near-pan-neutralizing antibodies against HIV-1 by deconvolution of plasma humoral responses. *Cell* 173, 1783–1795.e1714. doi: 10.1016/j.cell.2018.03.061

Saunders, K. O., Wang, L., Joyce, M. G., Yang, Z.-Y., Balazs, A. B., Cheng, C., et al. (2015). Broadly neutralizing human immunodeficiency virus type 1 antibody gene transfer protects nonhuman primates from mucosal simian-human immunodeficiency virus infection. *J. Virol.* 89, 8334–8345. doi: 10.1128/JVI.00908-15

Schaefer, W., Regula, J. T., Bähner, M., Schanzer, J., Croasdale, R., Dürr, H., et al. (2011). Immunoglobulin domain crossover as a generic approach for the production of bispecific IgG antibodies. *Proc. Natl. Acad. Sci. U.S.A.* 108, 11187–11192. doi: 10.1073/pnas.1019002108

Scharf, L., Scheid, J. F., Lee, J. H., West, A. P. Jr., Chen, C., Gao, H., et al. (2014). Antibody 8ANC195 reveals a site of broad vulnerability on the HIV-1 envelope spike. *Cell Rep.* 7, 785–795. doi: 10.1016/j.celrep.2014.04.001

Scheid, J. F., Horwitz, J. A., Bar-On, Y., Kreider, E. F., Lu, C.-L., Lorenzi, J. C. C., et al. (2016). HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 535, 556–560. doi: 10.1038/nature18929

Scheid, J. F., Mouquet, H., Ueberheide, B., Diskin, R., Klein, F., Oliveira, T. Y. K., et al. (2011). Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* 333, 1633–1637. doi: 10.1126/science.1207227

Schneider, J. R., Carias, A. M., Bastian, A. R., Cianci, G. C., Kiser, P. F., Veazey, R. S., et al. (2017). Long-term direct visualization of passively transferred fluorophore-conjugated antibodies. *J. Immunol. Methods* 450, 66–72. doi: 10.1016/j.jim.2017.07.009

Schommers, P., Gruell, H., Abernathy, M. E., Tran, M.-K., Dingens, A. S., Gristick, H. B., et al. (2020). Restriction of HIV-1 escape by a highly broad and potent neutralizing antibody. *Cell* 180, 471–489.e22. doi: 10.1016/j.cell.2020.01.010

Schoofs, T., Barnes, C. O., Suh-Toma, N., Golijanin, J., Schommers, P., Gruell, H., et al. (2019). Broad and potent neutralizing antibodies recognize the silent face of the HIV envelope. *Immunity* 50, 1513–1529.e1519. doi: 10.1016/j.immuni.2019.04.014

Schoofs, T., Klein, F., Braunschweig, M., Kreider, E. F., Feldmann, A., Nogueira, L., et al. (2016). HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science* 352, 997–1001. doi: 10.1126/science.aaf0972

Shapiro, M. B., Cheever, T., Malherbe, D. C., Pandey, S., Reed, J., Yang, E. S., et al. (2020). Single-dose bNAb cocktail or abbreviated ART post-exposure regimens achieve tight SHIV control without adaptive immunity. *Nat. Commun.* 11:70. doi: 10.1038/s41467-019-13972-y

Shibata, R., Igarashi, T., Haigwood, N., Buckler-White, A., Ogert, R., Ross, W., et al. (1999). Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV−1/SIV chimeric virus infections of macaque monkeys. *Nat. Med.* 5, 204–210. doi: 10.1038/5568

Shingai, M., Nishimura, Y., Klein, F., Mouquet, H., Donau, O. K., Plishka, R., et al. (2013). Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* 503, 277–280. doi: 10.1038/nature12746

Siliciano, J. D., Kajdas, J., Finzi, D., Quinn, T. C., Chadwick, K., Margolick, J. B., et al. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat. Med.* 9, 727–728. doi: 10.1038/nm880

Sok, D., Pauthner, M., Briney, B., Lee, J. H., Saye-Francisco, K. L., Hsueh, J., et al. (2016). A prominent site of antibody vulnerability on HIV envelope incorporates a motif associated with CCR5 binding and its camouflaging glycans. *Immunity* 45, 31–45. doi: 10.1016/j.immuni.2016.06.026

Sok, D., Van Gils, M. J., Pauthner, M., Julien, J.-P., Saye-Francisco, K. L., Hsueh, J., et al. (2014). Recombinant HIV envelope trimer selects for quaternary-dependent antibodies targeting the trimer apex. *Proc. Natl. Acad. Sci. U.S.A.* 111, 17624–17629. doi: 10.1073/pnas.1415789111

Sonntag, F., Schmidt, K., and Kleinschmidt, J. A. (2010). A viral assembly factor promotes AAV2 capsid formation in the nucleolus. *Proc. Natl. Acad. Sci. U.S.A.* 107, 10220–10225. doi: 10.1073/pnas.1001673107

Steinhardt, J. J., Guenaga, J., Turner, H. L., Mckee, K., Louder, M. K., O'dell, S., et al. (2018). Rational design of a trispecific antibody targeting the HIV-1 Env with elevated anti-viral activity. *Nat. Commun.* 9:877. doi: 10.1038/s41467-018-03335-4

Sun, L., Li, J., and Xiao, X. (2000). Overcoming adeno-associated virus vector size limitation through viral DNA heterodimerization. *Nat. Med.* 6, 599–602. doi: 10.1038/75087

UNAIDS (2019). *Global HIV and AIDS Statistics - 2019 Fact Sheet*. Available online at: https://www.unaids.org/en/resources/fact-sheet (accessed February 1, 2020).

Van Den Berg, F. T., Makoah, N. A., Ali, S. A., Scott, T. A., Mapengo, R. E., Mutsvunguma, L. Z., et al. (2019). AAV-mediated expression of broadly neutralizing and vaccine-like antibodies targeting the HIV-1 envelope V2 region. *Mol. Ther. Methods Clin. Dev.* 14, 100–112. doi: 10.1016/j.omtm.2019.06.002

Von Bredow, B., Arias, J. F., Heyer, L. N., Moldt, B., Le, K., Robinson, J. E., et al. (2016). Comparison of antibody-dependent cell-mediated cytotoxicity and virus neutralization by HIV-1 Env-specific monoclonal antibodies. *J. Virol.* 90, 6127–6139. doi: 10.1128/JVI.00347-16

Wagh, K., Bhattacharya, T., Williamson, C., Robles, A., Bayne, M., Garrity, J., et al. (2016). Optimal combinations of broadly neutralizing antibodies for prevention and treatment of HIV-1 clade C infection. *PLoS Pathog.* 12:e1005520. doi: 10.1371/journal.ppat.1005520

Wagh, K., Seaman, M. S., Zingg, M., Fitzsimons, T., Barouch, D. H., Burton, D. R., et al. (2018). Potential of conventional & bispecific broadly neutralizing antibodies for prevention of HIV-1 subtype A, C & D infections. *PLoS Pathog.* 14:e1006860. doi: 10.1371/journal.ppat.1006860

Walker, L. M., Huber, M., Doores, K. J., Falkowska, E., Pejchal, R., Julien, J.-P., et al. (2011). Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* 477, 466–470. doi: 10.1038/nature10373

Walker, L. M., Phogat, S. K., Chan-Hui, P.-Y., Wagner, D., Phung, P., Goss, J. L., et al. (2009). Broad and potent neutralizing antibodies from an african donor reveal a new HIV-1 vaccine target. *Science* 326, 285–289. doi: 10.1126/science.1178746

Wang, D., Tai, P. W. L., and Gao, G. (2019). Adeno-associated virus vector as a platform for gene therapy delivery. *Nat. Rev. Drug Discov.* 18, 358–378. doi: 10.1038/s41573-019-0012-9

Wei, X., Decker, J. M., Wang, S., Hui, H., Kappes, J. C., Wu, X., et al. (2003). Antibody neutralization and escape by HIV-1. *Nature* 422, 307–312. doi: 10.1038/nature01470

Welles, H. C., Jennewein, M. F., Mason, R. D., Narpala, S., Wang, L., Cheng, C., et al. (2018). Vectored delivery of anti-SIV envelope targeting mAb via AAV8 protects rhesus macaques from repeated limiting dose intrarectal swarm SIVsmE660 challenge. *PLoS Pathog.* 14:e1007395. doi: 10.1371/journal.ppat.1007395

WHO (2019). *HIV/AIDS Key Facts*. Available online at: https://www.who.int/news-room/fact-sheets/detail/hiv-aids (accessed February 1, 2020).

Wu, X., Yang, Z.-Y., Li, Y., Hogerkorp, C.-M., Schief, W. R., Seaman, M. S., et al. (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* 329, 856–861. doi: 10.1126/science.1187659

Wu, X., Zhang, Z., Schramm, C. A., Joyce, M. G., Kwon, Y. D., Zhou, T., et al. (2015). Maturation and diversity of the VRC01-antibody lineage over 15 years of chronic HIV-1 infection. *Cell* 161, 470–485. doi: 10.1016/j.cell.2015.03.004

Xu, L., Pegu, A., Rao, E., Doria-Rose, N., Beninga, J., Mckee, K., et al. (2017). Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques. *Science* 358, 85–90. doi: 10.1126/science.aan8630

Yen, L., Svendsen, J., Lee, J.-S., Gray, J. T., Magnier, M., Baba, T., et al. (2004). Exogenous control of mammalian gene expression through modulation of RNA self-cleavage. *Nature* 431, 471–476. doi: 10.1038/nature02844

Zhong, G., Wang, H., He, W., Li, Y., Mou, H., Tickner, Z. J., et al. (2020). A reversible RNA on-switch that controls gene expression of AAV-delivered therapeutics *in vivo*. *Nat. Biotechnol.* 38, 169–175. doi: 10.1038/s41587-019-0357-y

Zhou, T., Zheng, A., Baxa, U., Chuang, G.-Y., Georgiev, I. S., Kong, R., et al. (2018). A neutralizing antibody recognizing primarily N-linked glycan targets the silent face of the HIV envelope. *Immunity* 48, 500–513.e506. doi: 10.1016/j.immuni.2018.02.013

Zhu, J., Huang, X., and Yang, Y. (2009). The TLR9-MyD88 pathway is critical for adaptive immune responses to adeno-associated virus gene therapy vectors in mice. *J. Clin. Invest.* 119, 2388–2398. doi: 10.1172/JCI37607

Zhu, P., Chertova, E., Bess, J. Jr., Lifson, J. D., Arthur, L. O., Liu, J., et al. (2003). Electron tomography analysis of envelope glycoprotein trimers on HIV and simian immunodeficiency virus virions. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15812–15817. doi: 10.1073/pnas.2634931100

Conflict of Interest: MG is a co-founder and shareholder of Emmune, Inc., a start-up company dedicated to the development of eCD4-Ig and AAV-delivered eCD4-Ig for clinical trials.

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 05 May 2020
doi: 10.3389/fcimb.2020.00175

TIGIT Blockade: A Multipronged Approach to Target the HIV Reservoir

*Kayla A. Holder and Michael D. Grant**

Immunology and Infectious Diseases Program, Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL, Canada

During chronic human immunodeficiency virus type 1 (HIV-1) infection, upregulation of inhibitory molecules contributes to effector cell dysfunction and exhaustion. This, in combination with the ability of HIV-1 to reside dormant in cellular reservoirs and escape immune recognition, makes the pathway to HIV-1 cure particularly challenging. An idealized strategy to achieve HIV-1 cure proposes combined viral and immune activation by "shock"ing HIV-1 out of latency and into an immunologically visible state to be recognized and "kill"ed by immune effector cells. Here we outline the potential for blockade of the inhibitory immune checkpoint T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) to overcome natural killer (NK) cell and T cell inhibition associated with HIV-1 infection and invigorate antiviral effector cell responses against HIV-1 reactivated from the latent cellular reservoir.

Keywords: HIV-1, TIGIT, PVR, checkpoint inhibitor, T cell, NK cell

OPEN ACCESS

Edited by:
Andrés Finzi,
Université de Montréal, Canada

Reviewed by:
Nicolas Chomont,
Université de Montréal, Canada
Matthew Parsons,
Emory University, United States
Catherine A. Blish,
Stanford University, United States

***Correspondence:**
Michael D. Grant
mgrant@mun.ca

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *05 February 2020*
Accepted: *02 April 2020*
Published: *05 May 2020*

Citation:
Holder KA and Grant MD (2020) TIGIT Blockade: A Multipronged Approach to Target the HIV Reservoir. Front. Cell. Infect. Microbiol. 10:175. doi: 10.3389/fcimb.2020.00175

INTRODUCTION

Combination antiretroviral therapy (cART) reduces human immunodeficiency virus type 1 (HIV-1) replication to levels where the amount of viral ribonucleic acid (RNA) in the bloodstream falls below current limits of detection. In most cases, maintenance of undetectable viral loads requires strict adherence to therapy (Chun et al., 1995, 1997; Finzi et al., 1999). Despite their efficacy, complete eradication of HIV-1 is unattainable with current cART regimes. During early infection, HIV-1 establishes proviral reservoirs, concealing itself within various cell types in different anatomical niches (Wong and Yukl, 2016; Baxter et al., 2018). In this largely dormant state, the HIV-1 reservoir is invisible to the immune system and insensitive to cART (Finzi et al., 1997). As a consequence of this widespread thorough concealment, if cART is interrupted, HIV-1 reactivates and produces replication-competent viruses capable of nascent infection (Wong et al., 1997; Finzi et al., 1999). Organs and tissues such as the gut and lymph nodes are key sites enriched for cells harboring HIV-1 provirus (Wong and Yukl, 2016). Although various types of cells including macrophages, monocytes and astrocytes can serve as HIV-1 reservoirs, the predominant cell type containing HIV-1 provirus are $CD4^+$ T cells and, thus, they are the predominant source of viral replication with withdrawal of cART (Finzi et al., 1999; Wong et al., 2019). Seeding itself in long-lived memory $CD4^+$ T cells during acute and ongoing infection allows HIV-1 to persist indefinitely, despite consistent and effective cART suppression.

In the absence of cART, activation of the resting $CD4^+$ T cells harboring HIV-1 provirus drives HIV-1 out from latency, replenishes the reservoir and promotes disease progression. Cure of the "Berlin patient" in 2008 and the "London patient" in 2019 with HIV-1-resistant bone marrow transplants provides proof of concept that HIV-1 can be eradicated in those already living with the virus (Hutter et al., 2009; Gupta et al., 2019). Although application of this approach is not feasible

for the vast majority of people living with HIV-1 (PLWH), other elimination strategies are under investigation. These can include "block and lock" or gene editing, both of which aim to fix latent proviral HIV in a permanent inactive state with either drug therapy or *in situ* HIV genome editing. Conversely, a "kick/shock and kill" approach focuses on purging the latent HIV-1 reservoir by forced HIV activation from reservoir cells, thereby exposing it to the immune system and/or cART (Deeks, 2012; Shan et al., 2012; Qu et al., 2013; Ahlenstiel et al., 2015; Mousseau et al., 2015; Zhu et al., 2015; Karpinski et al., 2016; Margolis et al., 2016). To completely cure HIV-1 infection by this latter approach, two currently unattainable objectives must be met. Firstly, viral reactivation needs to occur in all latently infected cells bearing replication competent viral genomes. Secondly, those cells in which HIV-1 reactivates must be eliminated efficiently enough to prevent spread to uninfected cells. The second goal requires enhanced antiviral immune function, likely combined with novel pharmacologic strategies. Direct reservoir cytolysis by T cell and specific antibody-dependent NK cell mechanisms is a key element of this goal. Incomplete purging of the latent HIV-1 reservoir, although not an absolute cure, may be sufficient to reduce or even remove dependence upon cART for suppression of HIV replication and yield a functional cure for HIV-1 infection. In light of the role that the immune system will play, similarities between cancer and chronic viral infection imply that administration of checkpoint inhibitors can benefit immune-based HIV-1 cure and treatment strategies.

Like cancer, chronic viral infection often progresses to a stage where effector cell functions fundamental for its control are severely impaired (Wherry and Kurachi, 2015; Bi and Tian, 2017). Following activation, T cells upregulate inhibitory receptors such as CTLA-4 and PD-1 to limit T cell responses and prevent immune pathology arising from unregulated responses (Wherry and Kurachi, 2015). In settings of chronic infection with persistent microbial replication, T cell function is dysregulated by sustained high expression of these inhibitory checkpoint receptors (Attanasio and Wherry, 2016; Wykes and Lewin, 2018). Checkpoint inhibitors targeting different inhibitory receptors on immune cells or their corresponding ligands are transforming cancer therapy and many are relevant to immunotherapy for HIV-1 infection. We focused this review on the T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) immune checkpoint receptor as expression of TIGIT, its competitors, and its ligands are broadly dysregulated on multiple cell types in HIV-1 infection. Furthermore, recent studies indicate that TIGIT negatively regulates both T cell and NK cell antiviral effector functions. We will discuss findings that suggest that this regulatory axis is an especially exploitable immune checkpoint in HIV-1 reservoir elimination strategies engaging antiviral effector cells.

Differential TIGIT Expression on Immune Cells

Most NK cells and multiple T cell subsets, including memory T cells, regulatory T cells and follicular helper T cells (T_{FH}), express TIGIT (Boles et al., 2009; Stanietsky et al., 2009; Yu et al., 2009; Levin et al., 2011; Wang et al., 2015; Wu et al., 2016). After interaction with either of its ligands, poliovirus receptor (PVR or CD155 or Necl-5), or PVRL2 (CD112 or nectin-2), TIGIT inhibits activation of T cell or NK cell effector functions (Stanietsky et al., 2009; Yu et al., 2009; Stengel et al., 2012). TIGIT belongs to a larger family of nectin and nectin-like receptors that all recognize the same group of ligands (Chan et al., 2012; Pauken and Wherry, 2014). Like TIGIT, TACTILE (CD96), and PVR-related Ig domain (PVRIG or CD112R) bind PVR, and PVRL2, respectively, whereas DNAM-1 (CD226) is a costimulatory counter receptor that competes with both TIGIT and TACTILE for PVR engagement and with PVRIG for PVRL2 binding (**Figure 1**) (Anderson et al., 2016; Zhu et al., 2016; Dougall et al., 2017; Xu et al., 2017; Sanchez-Correa et al., 2019). The inhibitory receptor PVRIG is expressed on activated T cells and NK cells (**Figure 1**), however, there is a lack of conclusive evidence in human NK cell studies as to whether TACTILE negatively or positively regulates activation (Fuchs et al., 2004; Georgiev et al., 2018; Whelan et al., 2019). Although PVR is a common ligand for TIGIT, TACTILE, and DNAM-1, the binding affinities vastly differ, with TIGIT having a greater affinity for PVR than either DNAM-1 or TACTILE (**Figure 1**) (Yu et al., 2009). This domination TIGIT has over DNAM-1 for ligand binding favors effector cell inhibition over effector cell costimulation, thereby dampening immune responses. Another means by which TIGIT controls T cell or NK cell activation is by interfering with DNAM-1 homodimerization by forming a heterodimer with DNAM-1 in *cis* (**Figure 1**) (Johnston et al., 2014). The intracellular TIGIT/DNAM-1 complex prevents effective intercellular DNAM-1/ligand interactions and reduces effector cell costimulation. This family of paired receptors and ligands constitute a regulatory signaling pathway resembling that of CD28 and CTLA-4 with antagonistic effects conveyed through differential receptor binding of the same ligand (Martinet and Smyth, 2015).

One hallmark of chronic HIV-1 infection is disruption of normal lymphocyte functions, leading to signs and symptoms of immune exhaustion. This exhaustion profile is illustrated by increased expression of multiple inhibitory immune checkpoint molecules including PD-1, CTLA-4, TIM-3, and LAG-3 on $CD8^+$ T cells and in some instances, on NK cells (Wherry et al., 2007; Anderson et al., 2016). In contrast to these well-characterized exhaustion markers, TIGIT is found to varying extents on NK cells and naïve $CD8^+$ T cells and is further upregulated after activation (Yu et al., 2009). There is convincing evidence of a central role for TIGIT in control of $CD8^+$ T cell maturation and exhaustion (Johnston et al., 2014). However, considering its parallel regulation of NK cell functions, targeting TIGIT with checkpoint inhibitors may have even greater implications for bolstering antiviral immunity than targeting PD-1 or CTLA-4. Of all lymphocyte subsets, NK cells have the highest fraction of cells constitutively expressing TIGIT receptors (Wang et al., 2015). Between 20 and 90% of resting NK cells express TIGIT and levels are increased by acute and chronic viral infections or cancers (Bi et al., 2014; Johnston et al., 2014; Wang et al., 2015; Zhang et al., 2018).

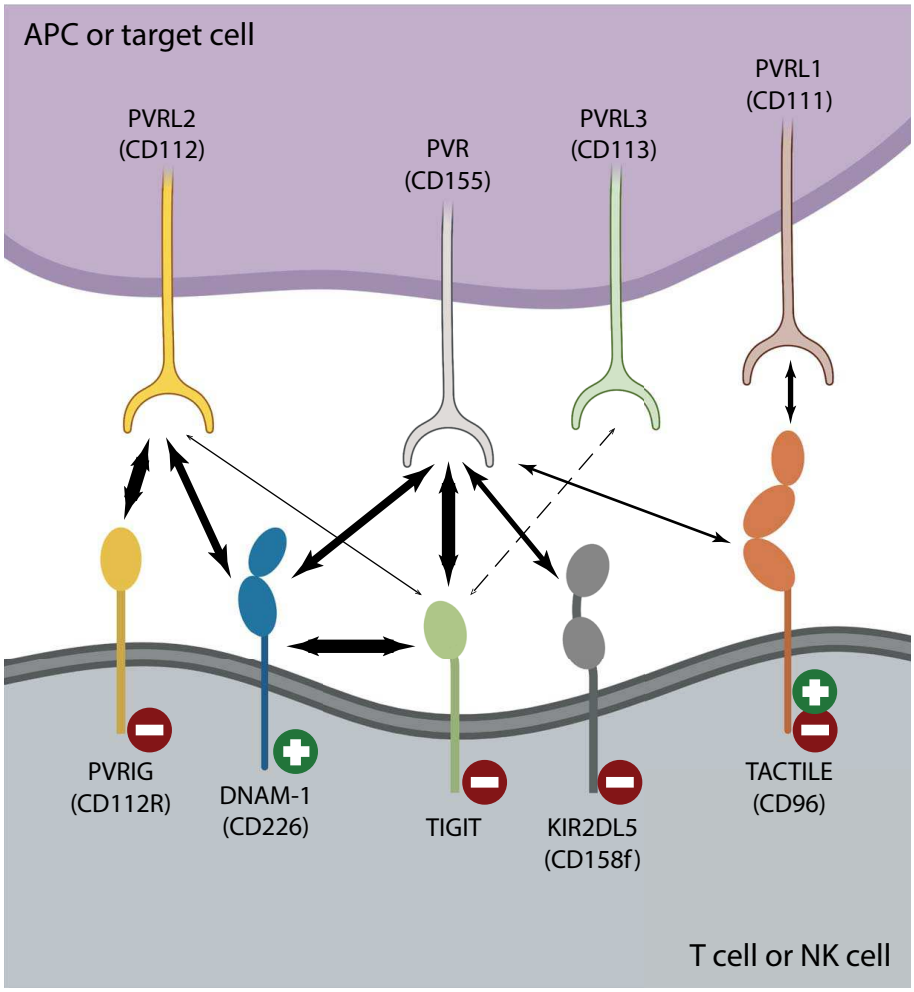


FIGURE 1 | The TIGIT/DNAM-1 immune checkpoint axis. Interactions between inhibiting (⊖) and activating (⊕) T cell or NK cell receptors belonging to the nectin or nectin-like family of receptors and their corresponding family of ligands are depicted. Strong interactions such as those between TIGIT and PVR or DNAM-1 in *cis* or PVRIG and PVRL2 are illustrated with heavy arrows. There is no clear consensus regarding whether TIGIT binds PVRL3 (dotted arrow) and it is unclear whether TIGIT/PVRL2 interactions are physiologically relevant *in vivo* (Stanietsky et al., 2009; Yu et al., 2009; Whelan et al., 2019). DNAM-1 interacts with both PVR and PVRL2 to counter inhibition, yet does so with lower affinity than either TIGIT or PVRIG. TACTILE preferentially interacts with PVRL1 over PVR (Holmes et al., 2019). The affinity of KIR2DL5 for PVR binding is currently unknown, as is whether any other nectin or nectin-like ligand or receptor can serve as its binding partner.

Targeting TIGIT is an especially attractive approach to incorporate into HIV-1 cure strategies as it impacts multiple functions of multiple types of effector cells. Its widespread expression on NK cells and $CD8^+$ T cells enhances the likelihood of TIGIT blockade having a meaningful impact in the setting of chronic infection. In this setting, $CD8^+$ T cells acquire expression of inhibitory receptors, including TIGIT, all contributing to maintenance of an immune exhausted state. Utilizing therapeutic monoclonal antibodies (mAb) to release the brakes on exhausted $CD8^+$ T cells and on NK cells expressing high amounts of TIGIT can counter inhibition to favor restoration of productive antiviral effector functions.

TIGIT Regulates Effector Cells in HIV-1 Infection

Expression of TIGIT is broadly dysregulated on both $CD8^+$ T cells and NK cells in HIV-1 infection. An increased fraction of $CD8^+$ T cells expressing TIGIT arises despite early initiation of effective cART (Chew et al., 2016; Tauriainen et al., 2017). The high potential impact of targeting TIGIT as a therapeutic strategy to invigorate effector cell responses against HIV-1 is emphasized by TIGIT expression on more than half of $CD8^+$ T cells and almost all HIV-1-specific $CD8^+$ T cells in PLWH (Chew et al., 2016; Tauriainen et al., 2017). Cells expressing TIGIT proliferated less and mounted weaker antiviral cytokine responses compared with their $TIGIT^{neg}$ $CD8^+$ T

cell counterparts, indicating a prominent role for TIGIT as a negative regulator of HIV-1-specific $CD8^+$ T cell immunity (Chew et al., 2016). Additionally, $TIGIT^{pos}$ $CD8^+$ T cells from PLWH have increased PD-1 co-expression, which correlates with HIV-1 disease progression (Chew et al., 2016). ADDIN EN.CITE (Cella et al., 2010; Tauriainen et al., 2017; Yin et al., 2018) Interrupting TIGIT signaling using therapeutic mAb blockade rescues $CD8^+$ T cell antiviral activity. If signaling through either TIGIT or PD-1 receptors is prevented by mAb, $CD8^+$ T cell interferon (IFN)-γ responses and cytotoxicity increase (Johnston et al., 2014; Chew et al., 2016). However, IL-2 production and T cell proliferation is reestablished only when blockade of both receptors is imposed (**Figure 2A**) (Johnston et al., 2014; Chew et al., 2016). In parallel with increased TIGIT on $CD8^+$ T cells, its costimulatory counterpart, DNAM-1, is often downregulated, further contributing to T cell exhaustion (Cella et al., 2010; Tauriainen et al., 2017). This "one-two punch" increases inhibitory intercellular TIGIT/PVR interactions and *cis* TIGIT/DNAM-1 heterodimers further restrict the potential for productive costimulation mediated by DNAM-1/PVR interactions (**Figure 1**).

Similar to the relationship seen with $CD8^+$ T cells, higher levels of TIGIT on NK cells correlate with HIV-1 disease progression (Yin et al., 2018). Although TIGIT blockade can rescue NK cell function against cancer, further evidence illustrating the potential benefits of targeting the TIGIT axis in the context of HIV-1 infection is needed (Zhang et al., 2018). While TIGIT expression is increased on NK cells from treatment naïve PLWH, cART may return TIGIT expression to similar levels as that of healthy controls (Yin et al., 2018; Vendrame et al., 2020). In untreated PLWH, NK cells expressing higher amounts of TIGIT were less likely to degranulate and produce IFN-γ in response to cytokine stimuli than those that did not express TIGIT. In this case, baseline NK cell function was rescued by mAb against TIGIT (Yin et al., 2018). In another study in which NK cells were activated for 3 days with IL-2, blockade of TIGIT provided no benefits to NK cells responding against *in vitro* HIV-1 infected autologous primary $CD4^+$ T cells (Vendrame et al., 2020). In the setting of active HIV-1 infection, TIGIT expression is increased on subsets of NK cells coexpressing DNAM-1 (Yin et al., 2018; Vendrame et al., 2020). Combining viral reactivation strategies with effector cell reinvigoration by preventing TIGIT interactions with either its ligand or DNAM-1 should promote cytolysis of infected cells (**Figure 2B**). More evidence is needed to delineate the cytotoxic potential of these cells. Expression of TIGIT on $CD8^+$ T cells and NK cells suggests that TIGIT-specific mAb therapy could synergistically unleash both types of antiviral effector cells to more robustly target active HIV-1 infection.

A Ligand for TIGIT Is Enriched on HIV-1 Reservoir Cells

Although expression levels of many inhibitory checkpoint molecules increase on multiple types of effector cells during HIV-1 infection, inhibition relies on the interactions between these receptors, and their cognate ligands. The predominant ligand for TIGIT and DNAM-1 is PVR, which is expressed on monocytes, dendritic cells, T cells and other cell types including tumor cells and HIV-1-infected cells (Mendelsohn et al., 1989; Pende et al., 2006; Chauvin et al., 2015; Chew et al., 2016). Originally identified in 1989 as a receptor for poliovirus, PVR belongs to a larger family of molecules that facilitate cell adhesion and migration, while over-expression of PVR in transformed cells promotes proliferation (Mendelsohn et al., 1989; Takai et al., 2008). Stimulated T cells have increased total PVR protein and cell surface expression levels, with preferential PVR expression on proliferating T cells in the S or G_2/M cell cycle phase (Ardolino et al., 2011). Increased cellular PVR expression occurs after the DNA damage response (DDR) pathway is induced (Ardolino et al., 2011). Although activated primary $CD4^+$ T cells express PVR, whether or not HIV-1 influences PVR expression on circulating primary $CD4^+$ T cells remains controversial (Davis et al., 2017).

During infection, expression of HIV-1-encoded Vpr helps promote cell cycle arrest in G_2 via the DDR pathway (Andersen et al., 2008). Through this same Vpr-dependent mechanism, PVR was reported to be upregulated on the surface of HIV-1-infected Jurkat T cells, yet expression of Nef and/or Vpu reduced surface-expressed PVR on both Jurkat and primary $CD4^+$ T cells (Matusali et al., 2012; Vassena et al., 2013; Bolduan et al., 2014). Another study reported no role for HIV-1-specific modulation of PVR expression on primary $CD4^+$ T cells (Davis et al., 2017). These studies used various *in vitro* systems with $CD4^+$ T cell lines or *ex vivo* $CD4^+$ T cells from healthy controls infected with different laboratory passaged HIV-1 strains. In all cases, PVR expression was assessed on all infected T cells, yet *in vitro*-infected $CD4^+$ T cells can be subsequently distinctly grouped into either $CD4^{pos}$ or $CD4^{neg}$ cells (Tremblay-McLean et al., 2017). In so doing, Tremblay-McLean *et al.* found that surface PVR expression is reduced on infected $CD4^{neg}$ T cells compared with infected $CD4^{pos}$ T cells (Tremblay-McLean et al., 2017). This could indicate that if HIV-1 does regulate PVR expression *in vivo*, productively infected or reservoir T_{FH} cells that maintain their expression of CD4 may have a different PVR expression profile than their $CD4^{neg}$ T cell counterparts.

Investigation of *ex vivo* PVR expression on $CD4^+$ T cells from PLWH has been limited. Very low levels of PVR expression on circulating $CD4^+$ T cells combined with the relative inaccessibility of lymph node sections from PLWH make informed assessment of PVR expression problematic (Yin et al., 2018; Vendrame et al., 2020). Upregulation of PVR can occur on $CD4^+$ T cells in HIV-1 infection, especially on lymph node T_{FH} $CD4^+$ T cells, which are the major site of HIV-1 reservoir concentration (Perreau et al., 2013; Banga et al., 2016; Tauriainen et al., 2017). Further, within the lymph nodes from PLWH, PVR is expressed on both germinal center $CD3^+$ cells and interdigitating follicular DCs (Cella et al., 2010). This compact compartment comprised of cells expressing PVR in proximity to $CD4^+$ T cells enriched in HIV-1 provirus could exploit higher localized TIGIT expression on $CD8^+$ T cells and NK cells to limit effector cell functions as they transit through lymph nodes. As NK cell and $CD8^+$ T cell expression of TIGIT increases with acute HIV-1 infection, introducing mAb therapy to overcome the higher affinity TIGIT/PVR inhibitory interaction in favor of DNAM-1/PVR-mediated activation is a rational strategy to address lingering HIV-1 infection (Yin et al., 2018). In this event, PVR expressed on reservoir $CD4^+$ T cells would render them

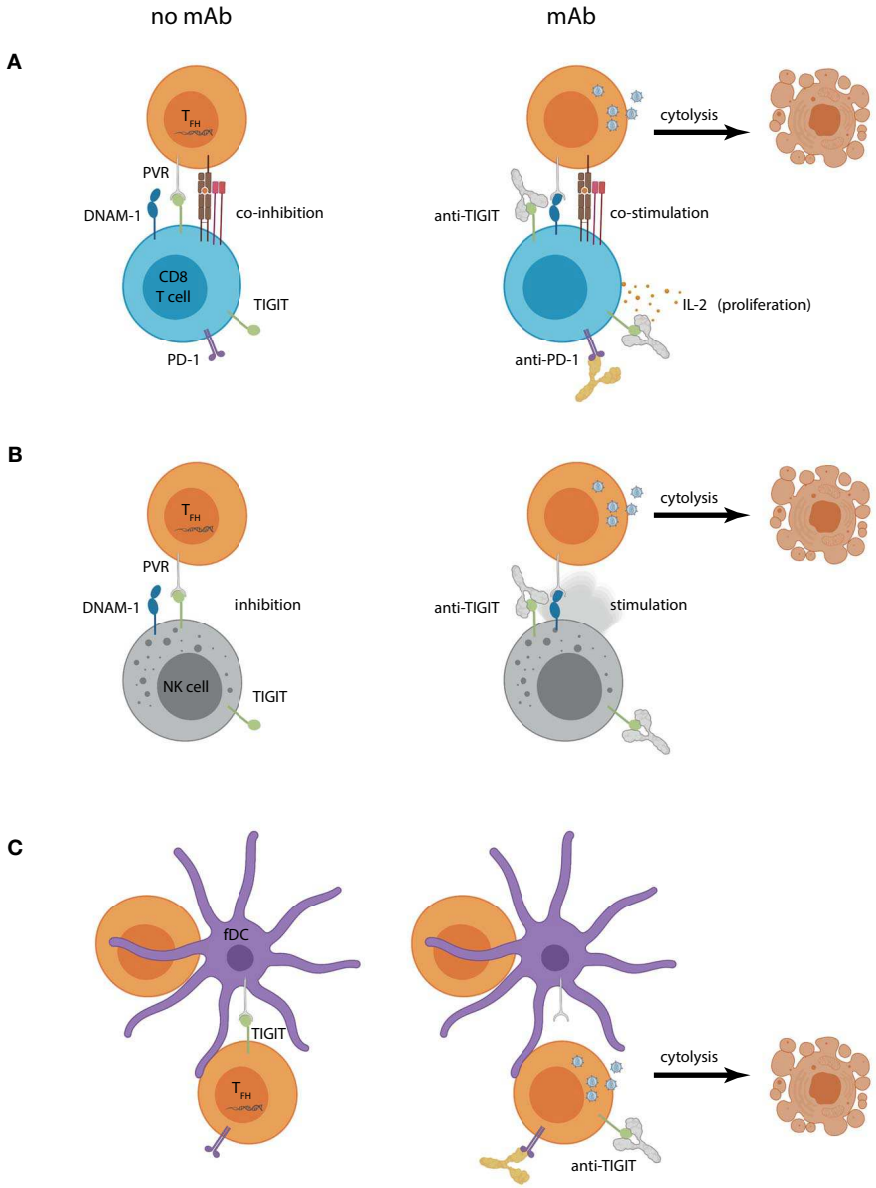


FIGURE 2 | Hypothetical outcomes of using TIGIT blockade within a "shock and kill" approach for HIV-1 curative therapy. Increased PVR expression on lymph node $CD4^+$ T_{FH} cells can contribute to **(A)** $CD8^+$ T cell or **(B)** NK cell dysregulation by engaging TIGIT and (co)inhibiting effector functions (left panel). Combined $CD4^+$ T cell reservoir activation and TIGIT mAb (right panel) could create a scenario where previously latent HIV-1 actively replicates, introducing targets for HIV-1-specific $CD8^+$ T cell or NK cell recognition. Reservoir cytolysis is promoted in this scenario by preventing inhibitory TIGIT interactions and allowing DNAM-1 (co)stimulation. Combination TIGIT and PD-1 mAbs would also allow $CD8^+$ T cell proliferation and IL-2 production. **(C)** Follicular DCs (fDCs) express PVR and interact closely with reservoir $CD4^+$ T cells. TIGIT is also expressed on lymph node $CD4^+$ T cells and may contribute to their suppression (left panel). Humanized anti-TIGIT mAb could aid in "shock"ing latent cells into productive infection by preventing $CD4^+$ T_{FH} cell TIGIT interactions with PVR expressed on fDCs leading to virus-induced or effector cell mediated cytolysis.

more susceptible targets for DNAM-1-expressing $CD8^+$ T cells and NK cells (**Figures 2A,B**).

In 2019, killer cell immunoglobulin-like receptor (KIR)2DL5, an inhibitory receptor expressed on NK cells and $CD8^+$ T cells, was identified as a binding partner for PVR, adding another facet to this already complex regulatory pathway (Estefania et al., 2007; Husain et al., 2019). The genes encoding KIR2DL5 (*KIR2DL5A* and *KIR2DL5*) are highly polymorphic (Vilches et al., 2000a,b). Less than 10% of $CD56^{dim}$ NK cells and a very small fraction of the $CD8^+$ T cells of carriers express the most common allele, *2DL5A*001*, which is detectable by mAb UP-R1 (Estefania et al., 2007; Cisneros et al., 2012). An accurate measure of KIR2DL5

prevalence in the wider population is currently unavailable as it is unknown whether this is the only allele expressed or whether polymorphisms arising in other alleles alter epitopes recognized by UP-R1 (Cisneros et al., 2012, 2016). While multiple factors suggest that inhibiting TIGIT/PVR interactions is a suitable strategy to invigorate effector cell responses against HIV-1, PLWH expressing KIR2DL5 may be less likely to benefit from this approach. Studies are needed to determine the antiviral effector potential of NK cells expressing KIR2DL5 and whether they co-express other nectin or nectin-like receptors.

TIGIT Is Expressed on HIV-1 Reservoir Cells

A significant hurdle to achieving HIV-1 cure is the lack of HIV-1 antigen expression on reservoir $CD4^+$ T cells, which leaves no appropriate means to target them immunologically. Without specific cell surface markers or HIV-1 antigen expression/peptide presentation to identify HIV-1-infected cells, no level of competent effector cell function can eradicate HIV. Selectively targeting latently infected cells that comprise the HIV-1 reservoir is a subsidiary approach to HIV-1 cure. Together with $CD8^+$ T cells and NK cells, $CD4^+$ T cells, including T_{FH} cells residing deep within lymph node tissues, express TIGIT (Yu et al., 2009; Wu et al., 2016). The $CD4^+$ T cell fraction expressing TIGIT is enriched for integrated HIV-1 DNA and the frequency of $TIGIT^{pos}$ cells that also co-express PD-1 and LAG-3 correlates with the size of the HIV-1 reservoir (Fromentin et al., 2016). Expression of TIGIT on $CD4^+$ T cells, alone or in combination with other immune checkpoint receptors identifies a subset of $CD4^+$ T cells more likely to harbor latent HIV-1.

Although TIGIT expression can help identify HIV-1 reservoirs, these cells need to be activated or shocked into productive infection to express HIV-1 antigens or associated stress proteins enabling recognition by antiviral effector cells. Maintenance of stable HIV-1 reservoirs involves persistent inhibition through interactions between checkpoint inhibitors, such as PD-1 or TIGIT, and their ligands (Wykes and Lewin, 2018). Consistent with the latency reversal noted with anti-PD-1 mAb, introducing anti-TIGIT mAb to unleash negative regulation can help shock TIGIT-expressing $CD4^+$ T cells into activation and shift latent HIV-1 into active production (**Figure 2C**) (Chew et al., 2016; Fromentin et al., 2016, 2019; Evans et al., 2018; Guihot et al., 2018; Wykes and Lewin, 2018). Targeting TIGIT as part of a cure strategy for HIV-1 could concurrently help force HIV-1 out of hiding, while rescuing $CD8^+$ T cell and NK cell antiviral functions to bridge effector cell functions with recognition of HIV-1-infected cells–a multipronged "shock and kill" approach.

CONCLUSION

We have discussed findings that suggest TIGIT inhibition of $CD8^+$ T cell and NK cell surveillance against HIV-1-infected $CD4^+$ T cells and monocytes, compounded by dysregulation of PVR and DNAM-1 expression, constitutes an exploitable immune checkpoint in HIV-1 reservoir elimination strategies engaging antiviral effector cells. The reasons that TIGIT could be an especially attractive target are several fold. Most importantly, TIGIT is expressed on most NK cells and almost all HIV-specific $CD8^+$ T cells in PLWH (Wang et al., 2015; Tauriainen et al., 2017; Yin et al., 2018). While this may favor targeting TIGIT over other inhibitory receptors such as PD-1 or CTLA-4, there is a case for using combinations of checkpoint inhibitors. For example, blocking TIGIT increased degranulation, IFN-γ production and proliferation of antiviral effectors, however, blockade of both TIGIT and PD-1 rescued IL-2 production, an important correlate of immune stability in PLWH (Johnston et al., 2014; Chew et al., 2016). In some settings, TIGIT blockade increases NK cell natural degranulation and antiviral cytokine release and likewise enhances cytokine release by $CD8^+$ and $CD4^+$ T cells and degranulation of HIV-specific $CD8^+$ T cells (Chew et al., 2016; Fromentin et al., 2016; Tauriainen et al., 2017; Yin et al., 2018). Secondly, there is evidence that HIV-1 infection of $CD4^+$ T cells induces upregulation of PVR expression and that the latent HIV reservoir is to some extent concentrated in $CD4^+$ T cells expressing PVR and/or TIGIT (Cella et al., 2010; Tauriainen et al., 2017; Yin et al., 2018). Thus, at least a fraction of the $CD4^+$ T cells activated for nascent HIV-1 replication is pre-armed to inhibit antiviral effector cell function through PVR engagement of TIGIT. Previous studies indicating that endogenous HIV-specific $CD8^+$ T cell responses of PLWH are insufficient to address nascent HIV-1 reactivation underscore the necessity to enhance antiviral effector functions in concert with HIV-1 reactivation (Shan et al., 2012).

The breadth of its effects on T cells and NK cells as well as specificity for cells in which the HIV-1 reservoir is concentrated combine to highlight the potential of TIGIT blockade in immunotherapeutic HIV-1 cure strategies. Several humanized anti-TIGIT mAb (AB154 and Etigilimab) have already entered clinical trials in cancer therapy, alone and in combination with anti-PD-1. These early stage studies indicate a favorable safety profile with effective TIGIT blockade. Experience in the cancer setting should help inform strategies for TIGIT blockade in PLWH, including whether better outcomes can be achieved when used in combination with other checkpoint inhibitors. For cure strategies that involve widespread reactivation of HIV replication and purging of the exposed infected cells, it will be critical to determine which effector cells or functions can most rapidly be brought to bear against nascent HIV-1 replication.

AUTHOR CONTRIBUTIONS

KH and MG jointly wrote and edited the manuscript.

FUNDING

Research in the area addressed by this review was supported by a research operating grant from the Canadian Institutes of Health Research (CIHR) PJT 361426 awarded to MG. KH was supported by a CIHR CGS-D fellowship.

ACKNOWLEDGMENTS

Figures were created using BioRender and Adobe Illustrator.

REFERENCES

Ahlenstiel, C., Mendez, C., Lim, S. T., Marks, K., Turville, S., Cooper, D. A., et al. (2015). Novel RNA duplex locks HIV-1 in a latent state via chromatin-mediated transcriptional silencing. *Mol. Ther. Nucleic Acids* 4:e261. doi: 10.1038/mtna.2015.31

Andersen, J. L., Le Rouzic, E., and Planelles, V. (2008). HIV-1 Vpr: mechanisms of G2 arrest and apoptosis. *Exp. Mol. Pathol.* 85, 2–10. doi: 10.1016/j.yexmp.2008.03.015

Anderson, A. C., Joller, N., and Kuchroo, V. K. (2016). Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity* 44, 989–1004. doi: 10.1016/j.immuni.2016.05.001

Ardolino, M., Zingoni, A., Cerboni, C., Cecere, F., Soriani, A., Iannitto, M. L., et al. (2011). DNAM-1 ligand expression on Ag-stimulated T lymphocytes is mediated by ROS-dependent activation of DNA-damage response: relevance for NK-T cell interaction. *Blood* 117, 4778–4786. doi: 10.1182/blood-2010-08-300954

Attanasio, J., and Wherry, E. J. (2016). Costimulatory and coinhibitory receptor pathways in infectious disease. *Immunity* 44, 1052–1068. doi: 10.1016/j.immuni.2016.04.022

Banga, R., Procopio, F. A., Noto, A., Pollakis, G., Cavassini, M., Ohmiti, K., et al. (2016). PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat. Med.* 22, 754–761. doi: 10.1038/nm.4113

Baxter, A. E., O'Doherty, U., and Kaufmann, D. E. (2018). Beyond the replication-competent HIV reservoir: transcription and translation-competent reservoirs. *Retrovirology* 15:18. doi: 10.1186/s12977-018-0392-7

Bi, J., and Tian, Z. (2017). NK cell exhaustion. *Front. Immunol.* 8:760. doi: 10.3389/fimmu.2017.00760

Bi, J., Zhang, Q., Liang, D., Xiong, L., Wei, H., Sun, R., et al. (2014). T-cell Ig and ITIM domain regulates natural killer cell activation in murine acute viral hepatitis. *Hepatology* 59, 1715–1725. doi: 10.1002/hep.26968

Bolduan, S., Reif, T., Schindler, M., and Schubert, U. (2014). HIV-1 Vpu mediated downregulation of CD155 requires alanine residues 10, 14 and 18 of the transmembrane domain. *Virology* 464–465, 375–384. doi: 10.1016/j.virol.2014.07.034

Boles, K. S., Vermi, W., Facchetti, F., Fuchs, A., Wilson, T. J., Diacovo, T. G., et al. (2009). A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur. J. Immunol.* 39, 695–703. doi: 10.1002/eji.200839116

Cella, M., Presti, R., Vermi, W., Lavender, K., Turnbull, E., Ochsenbauer-Jambor, C., et al. (2010). Colonna and N. C. f. H. Immunology, A. V. Loss of DNAM-1 contributes to CD8+ T-cell exhaustion in chronic HIV-1 infection. *Eur. J. Immunol.* 40, 949–954. doi: 10.1002/eji.200940234

Chan, C. J., Andrews, D. M., and Smyth, M. J. (2012). Receptors that interact with nectin and nectin-like proteins in the immunosurveillance and immunotherapy of cancer. *Curr. Opin. Immunol.* 24, 246–251. doi: 10.1016/j.coi.2012.01.009

Chauvin, J. M., Pagliano, O., Fourcade, J., Sun, Z., Wang, H., Sander, C., et al. (2015). TIGIT and PD-1 impair tumor antigen-specific CD8(+) T cells in melanoma patients. *J. Clin. Invest.* 125, 2046–2058. doi: 10.1172/JCI80445

Chew, G. M., Fujita, T., Webb, G. M., Burwitz, B. J., Wu, H. L., Reed, J. S., et al. (2016). TIGIT marks exhausted T cells, correlates with disease progression, and serves as a target for immune restoration in HIV and SIV infection. *PLoS Pathog.* 12:e1005349. doi: 10.1371/journal.ppat.1005349

Chun, T. W., Carruth, L., Finzi, D., Shen, X., DiGiuseppe, J. A., Taylor, H., et al. (1997). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387, 183–188. doi: 10.1038/387183a0

Chun, T. W., Finzi, D., Margolick, J., Chadwick, K., Schwartz, D., and Siliciano, R. F. (1995). *In vivo* fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat. Med.* 1, 1284–1290. doi: 10.1038/nm1295-1284

Cisneros, E., Estefania, E., and Vilches, C. (2016). Allelic polymorphism determines surface expression or intracellular retention of the human NK cell receptor KIR2DL5A (CD158f). *Front. Immunol.* 7:698. doi: 10.3389/fimmu.2016.00698

Cisneros, E., Moraru, M., N., Gomez-Lozano, M., Lopez-Botet, and Vilches, C. (2012). KIR2DL5: an orphan inhibitory receptor displaying complex patterns of polymorphism and expression. *Front. Immunol.* 3:289. doi: 10.3389/fimmu.2012.00289

Davis, Z. B., Sowrirajan, B., Cogswell, A., Ward, J. P., Planelles, V., and Barker, E. (2017). CD155 on HIV-infected cells is not modulated by HIV-1 Vpu and Nef but synergizes with NKG2D ligands to trigger NK cell lysis of autologous primary HIV-infected cells. *AIDS Res. Hum. Retroviruses* 33, 93–100. doi: 10.1089/AID.2015.0375

Deeks, S. G. (2012). HIV: Shock and kill. *Nature* 487, 439–440. doi: 10.1038/487439a

Dougall, W. C., Kurtulus, S., Smyth, M. J., and Anderson, A. C. (2017). TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy. *Immunol. Rev.* 276, 112–120. doi: 10.1111/imr.12518

Estefania, E., Flores, R., Gomez-Lozano, N., Aguilar, H., Lopez-Botet, M., and Vilches, C. (2007). Human KIR2DL5 is an inhibitory receptor expressed on the surface of NK and T lymphocyte subsets. *J. Immunol.* 178, 4402–4410. doi: 10.4049/jimmunol.178.7.4402

Evans, V. A., van der Sluis, R. M., Solomon, A., Dantanarayana, A., McNeil, C., Garsia, R., et al. (2018). Programmed cell death-1 contributes to the establishment and maintenance of HIV-1 latency. *AIDS* 32, 1491–1497. doi: 10.1097/QAD.0000000000001849

Finzi, D., Blankson, J., Siliciano, J. D., Margolick, J. B., Chadwick, K., Pierson, T., et al. (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.* 5, 512–517. doi: 10.1038/8394

Finzi, D., Hermankova, M., Pierson, T., Carruth, L. M., Buck, C., Chaisson, R. E., et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295–1300. doi: 10.1126/science.278.5341.1295

Fromentin, R., Bakeman, W., Lawani, M. B., Khoury, G., Hartogensis, W., DaFonseca, S., et al. (2016). CD4+ T cells expressing PD-1, TIGIT and LAG-3 contribute to HIV persistence during ART. *PLoS Pathog.* 12:e1005761. doi: 10.1371/journal.ppat.1005761

Fromentin, R., DaFonseca, S., Costiniuk, C. T., El-Far, M., Procopio, F. A., Hecht, F. M., et al. (2019). PD-1 blockade potentiates HIV latency reversal *ex vivo* in CD4(+) T cells from ART-suppressed individuals. *Nat. Commun.* 10:814. doi: 10.1038/s41467-019-08798-7

Fuchs, A., Cella, M., Giurisato, E., Shaw, A. S., and Colonna, M. (2004). Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). *J. Immunol.* 172, 3994–3998. doi: 10.4049/jimmunol.172.7.3994

Georgiev, H., Ravens, I., Papadogianni, G., and Bernhardt, G. (2018). Coming of age: CD96 emerges as modulator of immune responses. *Front. Immunol.* 9:1072. doi: 10.3389/fimmu.2018.01072

Guihot, A., Marcelin, A. G., Massiani, M. A., Samri, A., Soulie, C., Autran, B., et al. (2018). Drastic decrease of the HIV reservoir in a patient treated with nivolumab for lung cancer. *Ann. Oncol.* 29, 517–518. doi: 10.1093/annonc/mdx696

Gupta, R. K., Abdul-Jawad, S., McCoy, L. E., Mok, H. P., Peppa, D., Salgado, M., et al. (2019). HIV-1 remission following CCR5Delta32/Delta32 haematopoietic stem-cell transplantation. *Nature* 568, 244–248. doi: 10.1038/s41586-019-1027-4

Holmes, V. M., Maluquer de Motes, C., Richards, P. T., Roldan, J., Bhargava, A. K., Orange, J. S., et al. (2019). Interaction between nectin-1 and the human natural killer cell receptor CD96. *PLoS ONE* 14:e0212443. doi: 10.1371/journal.pone.0212443

Husain, B., Ramani, S. R., Chiang, E., Lehoux, I., Paduchuri, S., Arena, T. A., et al. (2019). A platform for extracellular interactome discovery identifies novel functional binding partners for the immune receptors

B7-H3/CD276 and PVR/CD155. *Mol. Cell Proteomics* 18, 2310–2323. doi: 10.1074/mcp.TIR119.001433

Hutter, G., Nowak, D., Mossner, M., Ganepola, S., Mussig, A., Allers, K., et al. (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N. Engl. J. Med*. 360, 692–698. doi: 10.1056/NEJMoa0802905

Johnston, R. J., Comps-Agrar, L., Hackney, J., Yu, X., Huseni, M., Yang, Y., et al. (2014). The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell* 26, 923–937. doi: 10.1016/j.ccell.2014.10.018

Karpinski, J., Hauber, I., Chemnitz, J., Schafer, C., Paszkowski-Rogacz, M., Chakraborty, D., et al. (2016). Directed evolution of a recombinase that excises the provirus of most HIV-1 primary isolates with high specificity. *Nat. Biotechnol*. 34, 401–409. doi: 10.1038/nbt.3467

Levin, S. D., Taft, D. W., Brandt, C. S., Bucher, C., Howard, E. D., Chadwick, E. M., et al. (2011). Vstm3 is a member of the CD28 family and an important modulator of T-cell function. *Eur. J. Immunol*. 41, 902–15. doi: 10.1002/eji.201041136

Margolis, D. M., Garcia, J. V., Hazuda, D. J., and Haynes, B. F. (2016). Latency reversal and viral clearance to cure HIV-1. *Science* 353:aaf6517. doi: 10.1126/science.aaf6517

Martinet, L., and Smyth, M. J. (2015). Balancing natural killer cell activation through paired receptors. *Nat. Rev. Immunol*. 15, 243–254. doi: 10.1038/nri3799

Matusali, G., Potesta, M., Santoni, A., Cerboni, C., and Doria, M. (2012). The human immunodeficiency virus type 1 Nef and Vpu proteins downregulate the natural killer cell-activating ligand PVR. *J. Virol*. 86, 4496–4504. doi: 10.1128/JVI.05788-11

Mendelsohn, C. L., Wimmer, E., and Racaniello, V. R. (1989). Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* 56, 855–865. doi: 10.1016/0092-867490690-9

Mousseau, G., Kessing, C. F., Fromentin, R., Trautmann, L., Chomont, N., and Valente, S. T. (2015). The tat inhibitor didehydro-cortistatin a prevents HIV-1 reactivation from latency. *mBio* 6:e00465. doi: 10.1128/mBio.00465-15

Pauken, K. E., and Wherry, E. J. (2014). TIGIT and CD226: tipping the balance between costimulatory and coinhibitory molecules to augment the cancer immunotherapy toolkit. *Cancer Cell* 26, 785–787. doi: 10.1016/j.ccell.2014.11.016

Pende, D., Castriconi, R., Romagnani, P., Spaggiari, G. M., Marcenaro, S., Dondero, A., et al. (2006). Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: relevance for natural killer-dendritic cell interaction. *Blood* 107, 2030–2036. doi: 10.1182/blood-2005-07-2696

Perreau, M., Savoye, A. L., De Crignis, E., Corpataux, J. M., Cubas, R., Haddad, E. K., et al. (2013). Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J. Exp. Med*. 210, 143–156. doi: 10.1084/jem.20121932

Qu, X., Wang, P., Ding, D., Li, L., Wang, H., Ma, L., et al. (2013). Zinc-finger-nucleases mediate specific and efficient excision of HIV-1 proviral DNA from infected and latently infected human T cells. *Nucleic Acids Res*. 41, 7771–7782. doi: 10.1093/nar/gkt571

Sanchez-Correa, B., Valhondo, I., Hassouneh, F., Lopez-Sejas, N., Pera, A., Bergua, J. M., et al. (2019). DNAM-1 and the TIGIT/PVRIG/TACTILE axis: novel immune checkpoints for Natural Killer cell-based cancer immunotherapy. *Cancers* 11:877. doi: 10.3390/cancers11060877

Shan, L., Deng, K., Shroff, N. S., Durand, C. M., Rabi, S. A., Yang, H. C., et al. (2012). Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity* 36, 491–501. doi: 10.1016/j.immuni.2012.01.014

Stanietsky, N., Simic, H., Arapovic, J., Toporik, A., Levy, O., Novik, A., et al. (2009). The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc. Natl. Acad. Sci. U. S. A*. 106, 17858–17863. doi: 10.1073/pnas.0903474106

Stengel, K. F., Harden-Bowles, K., Yu, X., Rouge, L., Yin, J., Comps-Agrar, L., et al. (2012). Structure of TIGIT immunoreceptor bound to poliovirus receptor reveals a cell-cell adhesion and signaling mechanism that requires cis-trans receptor clustering. *Proc. Natl. Acad. Sci. U. S. A*. 109, 5399–5404. doi: 10.1073/pnas.1120606109

Takai, Y., Miyoshi, J., Ikeda, W., and Ogita, H. (2008). Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. *Nat. Rev. Mol. Cell Biol*. 9, 603–615. doi: 10.1038/nrm2457

Tauriainen, J., Scharf, L., Frederiksen, J., Naji, A., Ljunggren, H. G., Sonnerborg, A., et al. (2017). Perturbed CD8(+) T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals. *Sci. Rep*. 7:40354. doi: 10.1038/srep40354

Tremblay-McLean, A., Bruneau, J., Lebouche, B., Lisovsky, I., Song, R., and Bernard, N. F. (2017). Expression profiles of ligands for activating natural killer cell receptors on HIV infected and uninfected CD4(+) T cells. *Viruses* 9:295. doi: 10.3390/v9100295

Vassena, L., Giuliani, E., Matusali, G., Cohen, E. A., and Doria, M. (2013). The human immunodeficiency virus type 1 Vpr protein upregulates PVR via activation of the ATR-mediated DNA damage response pathway. *J. Gen. Virol*. 94, 2664–2669. doi: 10.1099/vir.0.055541-0

Vendrame, E., Seiler, C., Ranganath, T., Zhao, N. Q., Vergara, R., Alary, M., et al. (2020). TIGIT is upregulated by HIV-1 infection and marks a highly functional adaptive and mature subset of natural killer cells. *AIDS* 34, 801–813. doi: 10.1097/QAD.0000000000002488

Vilches, C., Gardiner, C. M., and Parham, P. (2000a). Gene structure and promoter variation of expressed and nonexpressed variants of the KIR2DL5 gene. *J. Immunol*. 165, 6416–6421. doi: 10.4049/jimmunol.165.11.6416

Vilches, C., Rajalingam, R., Uhrberg, M., Gardiner, C. M., Young, N. T., and Parham, P. (2000b). KIR2DL5, a novel killer-cell receptor with a D0-D2 configuration of Ig-like domains. *J. Immunol*. 164, 5797–5804. doi: 10.4049/jimmunol.164.11.5797

Wang, F., Hou, H., Wu, S., Tang, Q., Liu, W., Huang, M., et al. (2015). TIGIT expression levels on human NK cells correlate with functional heterogeneity among healthy individuals. *Eur. J. Immunol*. 45, 2886–2897. doi: 10.1002/eji.201545480

Whelan, S., Ophir, E., Kotturi, M. F., Levy, O., Ganguly, S., Leung, L., et al. (2019). PVRIG and PVRL2 are induced in cancer and inhibit CD8(+) T-cell function. *Cancer Immunol. Res*. 7, 257–268. doi: 10.1158/2326-6066.CIR-18-0442

Wherry, E. J., Ha, S. J., Kaech, S. M., Haining, W. N., Sarkar, S., Kalia, V., et al. (2007). Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* 27:6. doi: 10.1016/j.immuni.2007.09.006

Wherry, E. J., and Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol*. 15, 486–499. doi: 10.1038/nri3862

Wong, J. K., Hezareh, M., Gunthard, H. F., Havlir, D. V., Ignacio, C. C., Spina, C. A., et al. (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 278, 1291–1295. doi: 10.1126/science.278.5341.1291

Wong, J. K., and Yukl, S. A. (2016). Tissue reservoirs of HIV. *Curr. Opin. HIV AIDS* 11, 362–370. doi: 10.1097/COH.0000000000000293

Wong, M. E., Jaworowski, A., and Hearps, A. C. (2019). The HIV reservoir in monocytes and macrophages. *Front. Immunol*. 10:1435. doi: 10.3389/fimmu.2019.01435

Wu, H., Chen, Y., Liu, H., Xu, L. L., Teuscher, P., Wang, S., et al. (2016). Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice. *Eur. J. Immunol*. 46, 1152–1161. doi: 10.1002/eji.201546094

Wykes, M. N., and Lewin, S. R. (2018). Immune checkpoint blockade in infectious diseases. *Nat. Rev. Immunol*. 18, 91–104. doi: 10.1038/nri.2017.112

Xu, F., Sunderland, A., Zhou, Y., Schulick, R. D., Edil, B. H., and Zhu, Y. (2017). Blockade of CD112R and TIGIT signaling sensitizes human natural killer cell functions. *Cancer Immunol. Immunother*. 66, 1367–1375. doi: 10.1007/s00262-017-2031-x

Yin, X., Liu, T., Wang, Z., Ma, M., Lei, J., Zhang, Z., et al. (2018). Expression of the inhibitory receptor TIGIT is up-regulated specifically on NK cells with CD226 activating receptor from HIV-infected individuals. *Front. Immunol*. 9:2341. doi: 10.3389/fimmu.2018.02341

Yu, X., Harden, K., Gonzalez, L. C., Francesco, M., Chiang, E., Irving, B., et al. (2009). The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat. Immunol*. 10, 48–57. doi: 10.1038/ni.1674

Zhang, Q., Bi, J., Zheng, X., Chen, Y., Wang, H., Wu, W., et al. (2018). Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat. Immunol.* 19, 723–732. doi: 10.1038/s41590-018-0132-0

Zhu, W., Lei, R., Le Duff, Y., Li, J., Guo, F., Wainberg, M. A., et al. (2015). The CRISPR/Cas9 system inactivates latent HIV-1 proviral DNA. *Retrovirology* 12:22. doi: 10.1186/s12977-015-0150-z

Zhu, Y., Paniccia, A., Schulick, A. C., Chen, W., Koenig, M. R., Byers, J. T., et al. (2016). Identification of CD112R as a novel checkpoint for human T cells. *J. Exp. Med.* 213, 167–176. doi: 10.1084/jem.20150785

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 08 May 2020
doi: 10.3389/fcimb.2020.00216

Pattern Recognition Receptor Ligands as an Emerging Therapeutic Agent for Latent HIV-1 Infection

Shokichi Takahama[1] *and Takuya Yamamoto*[1,2,3]*

[1] Laboratory of Immunosenescence, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan,
[2] Laboratory of Aging and Immune Regulation, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan,
[3] Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan

Toll-like receptors (TLRs) were first identified as molecular sensors that transduce signals from specific structural patterns derived from pathogens; their underlying molecular mechanisms of recognition and signal transduction are well-understood. To date, more than 20 pattern-recognition receptors (PRRs) have been reported in humans, some of which are membrane-bound, similar to TLRs, whereas others are cytosolic, including retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and stimulator of interferon genes (STING). Clinically, PRR ligands have been developed as vaccine adjuvants to activate innate immunity and enhance subsequent antigen-specific immune responses. Recently, PRR ligands have been used as direct immunostimulators to enhance immune responses against infectious diseases and cancers. HIV-1 remains one of the world's most significant public health challenges. Without the elimination of HIV-1 latently infected cells, patients require lifelong combination antiretroviral therapy (cART), while research aimed at a functional cure for HIV-1 infection continues. Based on the concept of "shock and kill," a latency-reversing agent (LRA) has been developed to reactivate latently infected cells and induce cell death. However, previous research has shown that LRAs have limited efficacy in the eradication of these reservoirs *in vivo*. Besides, PRR ligands with anti-retroviral drugs have been developed for use in HIV treatment for these years. This mini-review summarizes the current understanding of the role of PRR ligands in AIDS research, suggests directions for future research, and proposes potential clinical applications.

Keywords: TLRs, PRRs, latently HIV-1 infected cells, non-human primates, STING, immunostimulators

OPEN ACCESS

Edited by:
Renee Marije Van Der Sluis, Aarhus Institute of Advanced Studies, Denmark

Reviewed by:
Maria Kalamvoki, University of Kansas Medical Center, United States
Logan Banadyga, National Microbiology Laboratory, Canada

***Correspondence:**
Takuya Yamamoto
yamamotot2@nibiohn.go.jp

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *24 January 2020*
Accepted: *20 April 2020*
Published: *08 May 2020*

Citation:
Takahama S and Yamamoto T (2020) Pattern Recognition Receptor Ligands as an Emerging Therapeutic Agent for Latent HIV-1 Infection. Front. Cell. Infect. Microbiol. 10:216. doi: 10.3389/fcimb.2020.00216

INTRODUCTION

Once HIV-1 infected into CD4 T cells, it integrates into the host genome and replicates for a long time. Some of the infected cells are hidden from the immune system, and in case of immunocompromization, virions re-emerge from the latently infected cells. Even under combination antiretroviral therapy (cART), latently infected cells exist in body. Thus, curing HIV-1 infection requires elimination of HIV-1 latently infected cells, mainly located in the lymphoid organs. Several factors affect the effectiveness of viral reservoirs eradication, specifically: (1) reactivation of latently infected cells, (2) prevention of *de novo* infection by re-emerged virus produced by reactivation, (3) killing of these reactivated latently infected cells by inducing

a cytopathic effect (CPE) and subsequent apoptosis and/or anti-HIV immune responses. Extensive research has been done to understand how best to use latency-reversing agents (LRAs) against HIV-1 to achieve a functional cure; these strategies have been referred to as "shock and kill" therapy (Deeks et al., 2016; Sengupta and Siliciano, 2018).

Among a variety of reagents potentially harboring LRA activity, histone-deacetylase inhibitors (HDACi) and PKC agonists have been investigated extensively and are well-documented as LRAs (Spivak and Planelles, 2018). It was initially thought that reactivation of latent HIV by LRAs would be sufficient to eliminate infected cells through CPE. However, recent data have suggested that immune effectors such as HIV-specific CTL, NK cells, or immunotoxins are likely required to recognize and eliminate exposed target cells in the so-called "flush-and-kill" strategy (Deng et al., 2015; Cartwright et al., 2016; Jones and Walker, 2016). In fact, Archin et al. have demonstrated that a single dose of vorinostat (VOR) increased the levels of cellular biomarkers of increased acetylation and simultaneously induced an increase in HIV RNA expression in resting CD4 T cells isolated from donors receiving cART (Archin et al., 2012). However, the authors did not observe any alteration in low-level viremia. This study has suggested that a single, clinically tolerable dose of VOR might be sufficient to induce the desired biological effect (histone acetylation) in PBMCs of HIV-positive, cART-treated patients. These effects were noted as temporary and were associated with increased levels of HIV RNA expression within resting CD4 T cells. Concurrently, concerns were raised about HDACi's negative impact on CTL functions (Jones et al., 2014; Clutton et al., 2016). However, a recent study by Margolis et al. has reported no measurable negative effects of HDACi on NK cell function based on *ex vivo* comprehensive immunological analysis, using PBMCs from participants treated with HDACi in two clinical studies (Garrido et al., 2019). Nevertheless, attenuated immune responses by HDACi remain subject to discussions.

Meanwhile, pattern recognition receptors (PRRs) were first identified as molecular sensors that transduce signals from specific structural patterns derived from pathogens. Their underlying molecular mechanisms of recognition and signal transduction are well-documented (Kawai and Akira, 2010, 2011; Takeuchi and Akira, 2010). To-date, over 20 PRRs have been reported; some of them are potential therapeutic targets against infectious disease or other types of disease for which there is currently no treatment. Indeed, 584 clinical trials on PRR ligands are registered at ClinicalTrials.gov, with the majority of these trials testing PRR ligands as vaccine adjuvants (Coffman et al., 2010; Reed et al., 2013; Del Giudice et al., 2018; Temizoz et al., 2018). Recently, PRR ligands as immunostimulatory drugs have received attention as potential immune therapy agents against infectious diseases and cancer, with an increasing number of trials registered at ClinicalTrials.gov. Moreover, most of the PRRs used for prospective treatment of infectious disease or cancer are agonists of TLR7, TLR8, TLR9, and STING; four clinical trials have been registered for HIV-1 treatment (**Table 1**). The present review summarizes the current state of knowledge regarding PRR agonists as alternative to LRAs and discusses the possible future use of these drugs as potential cure for HIV-1 infection.

POTENTIAL FOR SINGLE USE OF EACH PRR LIGAND

Among the variety of PRR ligands, ligands against TLR7/TLR8/TLR9 were studied extensively as LRA. These PRR agonist's LRA function resulted in reactivation of the latently infected cells, triggering viral gene expression, surface Env expression, and release of virions (**Figure 1A**). TLR7/8 senses single-stranded RNA, on the other hand, TLR9 senses unmethylated CpG-oligodeoxynucleotide-containing DNA on the endosomal membranes. These receptors are known to be expressed in pDCs and consequently produced Type I IFN upon stimulation to prevent viral infection in general. In addition, RIG-I and STING ligands are also of particular interest in the field. Thus, we will summarize the current reports of single use of these ligands.

TLR7 Agonist

The GS-9620 is a potent and selective TLR7 agonist, originally used to treat chronic hepatitis (summarized in **Table 1**). Safety of oral administration of GS-9620 has been shown in phase 1/2 studies for hepatitis B (Gane et al., 2015) and hepatitis C (Lawitz et al., 2015). Jansenn et al. demonstrated the GS-9620 induced type I IFN responses assessed by ISG induction, although no significant serum IFN alpha upregulation or HBsAg level decline was observed (Janssen et al., 2018). In addition, GS-9620 has been used in the trials for HIV-1 infection (NCT02858401). For example, Tsai et al. have demonstrated that GS-9620 might induce reversal of latency in cells from HIV-infected aviremic donors on cART. Moreover, CD8 T cell, NK cell activity, and phagocytic cells activity were enhanced through Type I IFN secretion from pDC (Tsai et al., 2017). Subsequently, Lim et al. demonstrated that GS-9620 induced transient viremia and reduced viral reservoir in acutely cART-treated SIV-infected macaque model (Lim et al., 2018); 2 out of 9 treatment monkeys remained aviremic for >2 years.

TLR8 Agonist

Recently, Meas et al. proposed the new topic of TLR8 as an LRA for HIV-1 cure strategy (Meas et al., 2020). Using human primary CD4 T cells, they demonstrated that endocytosed HIV-1 was recognized by TLR8, which in turn induces an inflammatory response that is suitable for HIV-1 replication and latency reversal. Furthermore, they also demonstrated that TLR8, but not TLR7 or TLR9, stimulation promoted differentiation to Th1/Th17 type cells, that might be contributing for HIV-1 long-term persistence in patients receiving cART (Sun et al., 2015). Thus, agonist of TLR8 would be a unique clinical target. Currently Selgantolimod (GS-9688), a selective TLR8 agonist, is intended as an immunostimulatory drug for hepatitis B (**Table 1**), which has been tested in animal models assessing *in vivo* safety, pharmacokinetics, pharmacodynamics, and efficacy (Daffis et al.,

TABLE 1 | Selected pattern-recognition receptor agonists investigated in clinical trials for HIV, Hepatitis B/C, or cancer treatment.

Type	Target PRRs	Drug	Alternative Name	Disease	Responsible Party	Clinical Stage	Identifier
Single	TLR7	Vesatolimod	GS-9620	Hepatitis B	Gilead Sciences	Phase II	NCT01590641 NCT01590654 NCT02166047 NCT02579382
	TLR7	Vesatolimod	GS-9620	Hepatitis C	Gilead Sciences	Phase I	NCT01591668
	TLR7	Vesatolimod	GS-9620	HIV	Gilead Sciences	Phase II	NCT02858401 NCT03060447
	TLR7	RO7020531		Hepatitis B	Hoffmann-La Roche	Phase I	NCT02956850
	TLR7	TQ-A3334	AL-034	Hepatitis B	Chia Tai Tianqing Pharmaceutical Group Co., Ltd.	Phase II	NCT04180150
	TLR8	Selgantolimod	GS-9688	Hepatitis B	Gilead Sciences	Phase II	NCT03615066 NCT03491553
	TLR9	Lefitolimod	MGN1703	HIV	University of Aarhus	Phase I / II	NCT02443935
	TLR9	SD-101		Hepatitis C	Dynavax Technologies Corporation	Phase I	NCT00823862
	RIG-I	Inarigivir	GS-9992 (SB 9200)	Hepatitis B	Spring Bank Pharmaceuticals, Inc. (with Gilead Sciences)	Phase II	NCT03493698 NCT03932513 NCT04059198 NCT04023721 NCT03434353
	RIG-I	Acitretin		HIV	Ottawa Hospital Research Institute	Phase I	NCT03753867
	STING	E7766		Cancer	Eisai Inc.	Phase I	NCT04109092 NCT04144140
	STING	GSK3745417		Cancer	GlaxoSmithKline	Phase I	NCT03843359
Combination	TLR9	Lefitolimod	MGN1703	HIV	University of Aarhus	Phase I/II	NCT03837756

2017). This agent could be useful as HIV-1 treatment in the future.

TLR9 Agonist

TLR9 agonists have been shown to reactivate latently infected cells in cART-treated patients' PBMC samples. Previous studies have shown that CpG-ODN might cause minor but significant decrease in the HIV-1 proviral reservoir (Scheller et al., 2004; Sogaard et al., 2010; Winckelmann et al., 2013), despite CpG-ODN-associated toxicity (Sogaard et al., 2010; Rynkiewicz et al., 2011; Manegold et al., 2012). In particular, a novel TLR9 agonist MGN1703 was recently developed and the effects on the reactivation of latently infected cells are well-documented (Schmidt et al., 2015; Wittig et al., 2015; Offersen et al., 2016). Søgaard et al. tested a hypothesis that MGN1703 might have dual effects (potential latency reversal and enhancement of immune function) within the "shock-and-kill" HIV-1 eradication approach. MGN1703 has been shown to induce potent antiviral responses in immune effector cells from HIV-1 infected individuals on suppressive cART *in vitro*.

Clinical studies have shown no safety concerns regarding MGN1703 (**Table 1**), supporting its positive effect on anti-HIV1 specific adaptive immunity (Vibholm et al., 2017, 2019). In fact, 24 weeks post-MGN1703 treatment of participants on cART, upregulation of Type I IFN and increase in the number of NK cells or proportion of activated pDC were observed. However, there was no statistically significant reduction in the number of the latently infected cells or viral load values. Furthermore, the positive effects of MGN1703 were reported within the lymph nodes and colon. In a clinical trial, Schleimann et al. demonstrated that MGN1703 enhances B cell differentiation and induction of Type I IFN responses in the lymph nodes (Schleimann et al., 2019). Similarly, Krarup et al. have demonstrated that MGN1703 induced potent type I IFN responses in colon, and that there exists an inverse correlation between the level of TLR9 and the viral DNA in colon (Krarup et al., 2018). It might be helpful to reactivate latent reservoirs that were

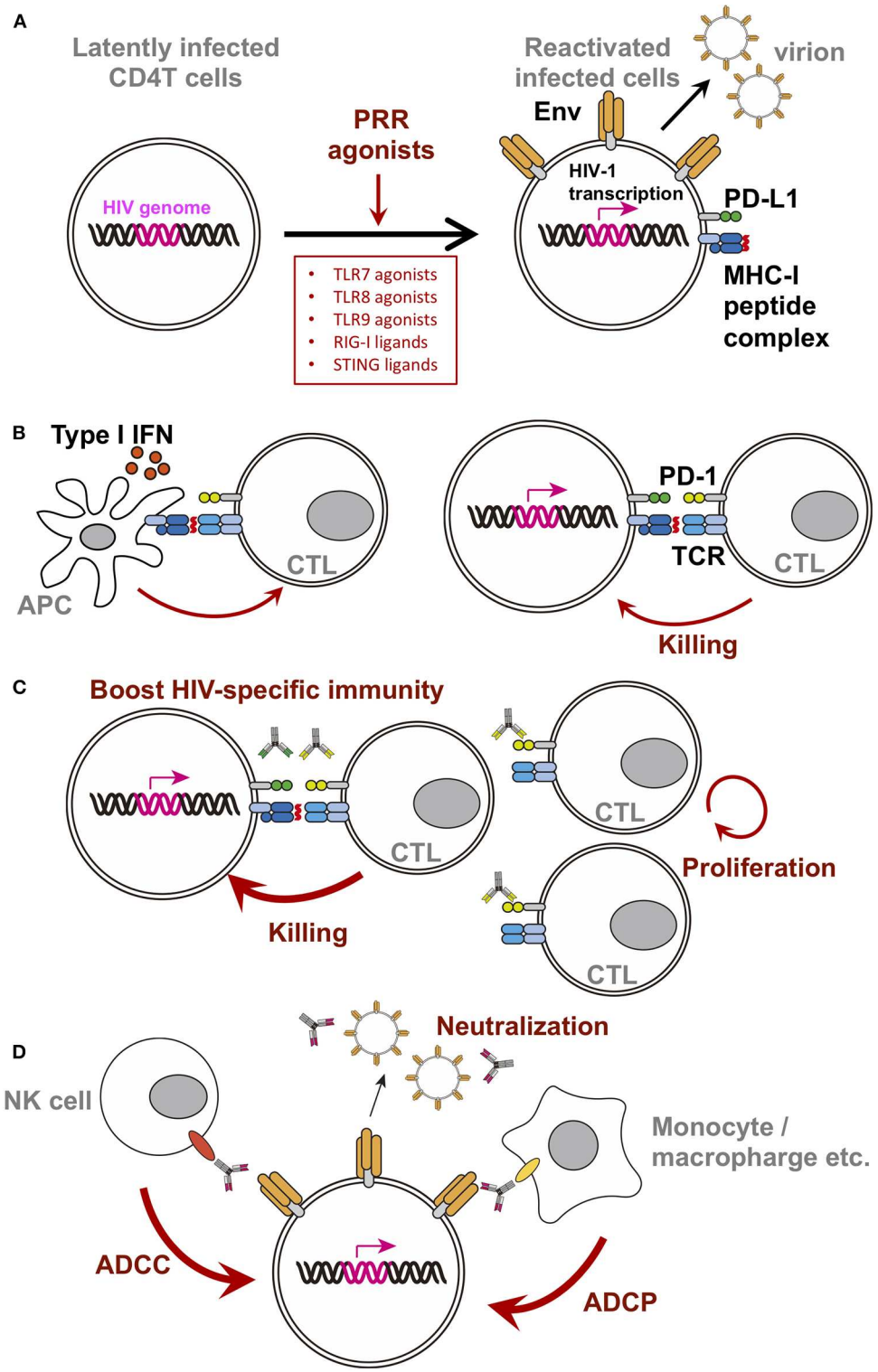


FIGURE 1 | Schematic diagram of PRR agonist-induced changes in latently infected cells or surrounding players. For clarity, only selected molecules/factors are depicted **(B–D)**. Key molecules are color-coded as follows: integrated HIV-genome (magenta), surface Env (tangerine), PD-1 (yellow-green), PD-L1 (green), MHC-I/II (dark blue), peptide on MHC (red), TCR (turquoise blue), CD16 (orange), and CD32 (yellow). Each PRR ligands discussed in this mini review are written in the box. **(A)** Single treatment of PRR agonists. PRR agonist's LRA function resulted in reactivation of the latently infected cells, triggering viral gene expression, surface Env expression, and release of virions. **(B)** A simplified depiction of the consequences of the stimulation using TLR7 agonist with a therapeutic vaccine. **(C)** A simplified depiction of the consequence of using TLR7 agonist with anti-checkpoint molecules monoclonal antibodies. Blocking immune checkpoints by antibodies against PD-1 or PD-L1 revive the CTL, likely resulting in increased activity. **(D)** A simplified depiction of the consequence of using TLR7 agonist with bNab.

mainly accumulated in lymphoid tissues under cART treatment (Banga et al., 2016).

Other TLR Ligands

Data regarding reactivation of latent reservoirs by stimulation with TLR ligands is also available from *in vitro* studies. Sher et al. have demonstrated the importance of TLR2 for the induction of integrated HIV-1 expression by mycobacteria in a mouse model (Bafica et al., 2003, 2004). Subsequently, Bosque's group has demonstrated the reactivation of latently infected cells by TLR1/2 stimulation *in vitro* (Novis et al., 2013). More recently, Bosque's group has reported on the efficacy of a synthetic molecule, CL413 (or CL531/CL572), comprising TLR2 and TLR7 agonist. The compound acted as a dual TLR2/7 agonist and reactivates latency via two distinct mechanisms. As a TLR2 agonist, the compound reactivates HIV by inducing NF-kB activation in memory CD4 T cells; as a TLR7 agonist, it induces secretion of TNF-a by monocytes and pDCs, promoting viral reactivation in CD4 T cells (Macedo et al., 2018). In a cell line model, TLR3 agonist poly (I:C) efficiently reactivates HIV transcription in HIV-infected microglial cells via the IRF3 pathway without activating the NF-kB pathway (Alvarez-Carbonell et al., 2017). This effect is likely cell type-specific, as it was not observed in monocytes or T cells.

Cytosolic PRR Ligands

Type I IFN is the best-studied downstream signaling pathway activated by PRRs. In AIDS research, the amount of Type I IFN produced by PRR ligands has been shown to correlate with the level of reactivation of latently infected cells *in vitro* (Borducchi et al., 2016; Li et al., 2016). Thus, the levels of Type I IFN production might be a marker of reactivation *in vitro*. As the stimulation of cytosolic RNA helicases (RLRs), such as retinoic acid-inducible gene-1 (RIG-I) and melanoma differentiation associated gene 5 (MDA5) enhances production of Type I IFNs, their ligands might be of interest.

In contrast to membrane-bound TLRs, RLRs detect cytosolic RNA derived from viruses (Broz and Monack, 2013; Kell and Gale, 2015). Similar to TLR agonists, the RIG-I agonist GS-9992 for Hepatitis B treatment has been conducted clinical studies (summarized in **Table 1**). RIG-I has been shown to detect HIV-1 RNA (Solis et al., 2011; Wang et al., 2013, 2015). Retinoic acid (RA) is a stimulator of RIG-I signaling, used as RIG-I agonist. Li et al. have reported that acitretin (an RA derivative) enhanced RIG-I signaling and increased HIV transcription, while inducing preferential apoptosis of HIV-infected cells (Li et al., 2016). Concurrently, acitretin decreased proviral DNA levels in CD4 T cells from HIV-positive participants on suppressive cART, an effect that was amplified when combined with SAHA. Acitretin also induced Type-I IFN and chemokine production. A phase-I clinical trial utilizing the oral tablet formulation of acitretin has been planned to investigate its safety and effect on the expression of several markers, including RIG-I, in CD4 T cells (NCT03753867).

Another potential candidate RLR ligand is a synthetic RLR agonist, KIN1148, which has been reported as an influenza vaccine adjuvant (Probst et al., 2017). KIN1148 induces dose-dependent expression of IRF3 and enhances the H1N1 influenza vaccine activity. However, no studies on KIN1148 as an immunostimulatory drug or LRA have been reported to-date.

Stimulator of interferon genes (STING) is another candidate for inducing strong type I IFN responses as a PRR. STING is a type of innate immune sensor for c-di-AMP (Barber, 2011, 2014, 2015; Burdette and Vance, 2013). A STING ligand, cGAMP is a promising immunomodulator, previously shown to improve tumor control in cancer studies (Corrales et al., 2016; Kinkead et al., 2018). Furthermore, several phase I trials for cancer by using STING ligands have been ongoing (selected two in **Table 1**). Using the PBMCs harboring of latently infected cells, previous studies have identified STING ligands as novel type of LRA, which might induce reactivation of latently infected cells and simultaneously enhancing antigen-specific CTLs (Palermo et al., 2019; Yamamoto et al., 2019). Moreover, our group has reported that another STING ligand, 3'3'-cGAMP, might induce HIV-1-specific CD8 T cells with strong effector function from naïve T cells via Type I IFN production (Kuse et al., 2019). These results suggest that STING ligands are potentially an immunostimulatory drugs for HIV.

COMBINATION OF PRR LIGAND AND OTHER IMMUNOTHERAPIES

Combination With Therapeutic Vaccines

Efficacy of TLR7 agonist for HIV infection *in vivo* was first shown in combination with a therapeutic vaccine in SIV-infected macaque model. Ad26/MVA therapeutic vaccine, a vaccine regimen primed by a recombinant adenovirus serotype 26 (Ad26), and boosted by a modified vaccinia Ankara (MVA), was administered in monkeys along with a TLR7 agonist under cART during the acute phase of infection. As a result, the set point viral load after treatment interruption alongside viral DNA in lymph nodes and PBMC were reduced, and delayed rebound was observed (Borducchi et al., 2016). Moreover, the breadth of cellular immune responses induced or boosted by this vaccine regimen inversely correlated with set point viral loads and directly correlated with time to viral rebound. The simplified depiction of the consequence of the stimulation using TLR7 agonist with a therapeutic vaccine was shown in **Figure 1B** (left).

Combination With Checkpoint Inhibitors

Programmed death-1 (PD-1) blockage has been known to restore exhausted T-cell function. For clinical usage, it was first used successfully in the treatment of malignant melanoma cancers, and its effectiveness for other cancers or infectious disease has been tested. Furthermore, as a cancer immunotherapy, a combination of TLR agonists and antibodies against immune checkpoint inhibitors (e.g., PD-1, PD-L1, or CTLA4) has been proposed for clinical trials. Conceptually, immune checkpoint inhibitor compensates the barrier to attack from immune cells, while TLR agonists enhance the immune cell attack, possibly contributing to tumor size reduction.

In HIV research, upregulation of PD-1 expression in chronic HIV-1-infected patients was reported in 2006. The therapeutic vaccine boosts HIV-1-specific CTL (**Figure 1B** left), while CTL

continues to express PD-1, which might limit CTL activity (**Figure 1B** right). Indeed, many groups reported the inverse correlation between the level of PD-1 expression on virus-specific CD8 T cells and VLs during chronic phase of infection (Day et al., 2006; Petrovas et al., 2006; Trautmann et al., 2006). Subsequently, PD-1 blockage enhances SIV-specific immunity *in vivo* in SIV-infected macaque model without cART (Velu et al., 2009; Dyavar Shetty et al., 2012). Furthermore, the blockage of PD-1 has been shown to enhance antiviral CTL activity and reduce the viral reservoir in SIV-infected macaque model with cART (Mylvaganam et al., 2018). These data suggested that blockage of PD-1 signaling might be a mode of treatment for HIV-1 infection (**Figure 1C**).

In contrast, Bekerman et al. have demonstrated the opposite result of a combination of anti-PD-1 with GS-9620 in SIV-infected chronic cART model (Bekerman et al., 2019). These authors reported no delayed rebound or changes to the size of reservoir because of treatment with PD-1 or GS-9620 used separately or concurrently, in a placebo-controlled trial. Due to the duration of cART treatment, the authors suggested PD-1 blockage might be of limited benefit. In addition to the limited benefit, the adverse effects of the checkpoint inhibitors should also be considered. In the cancer patient who has HIV-1 infection, the side effects of the PD-1 blockage appear similar to that in non-HIV patients (Scully et al., 2018). Further research on checkpoint inhibitors is required to achieve a functional cure.

Combination With bNabs

Since the first identification of the broad neutralizing antibodies (bNabs) against HIV-1 from patient blood (Scheid et al., 2009; Walker et al., 2009; Wu et al., 2010; Zhou et al., 2010), tremendous amount of new bNabs have been developed (Kwong and Mascola, 2018; Sok and Burton, 2018; Haynes et al., 2019). In clinical studies, some of them have been shown to delay viral rebound after analytic treatment interruption (ATI) *in vivo* (Bar et al., 2016; Scheid et al., 2016). Based on these data, the Barouch group proposes a direct antiviral effect of bNabs on latently infected cells, alongside the neutralizing effect of antibodies. There might be potentially two advantages. (1) The neutralization activity against *de novo* virion produced concomitantly with the reactivation. (2) bNabs might be helpful to induce FcR-mediated killing (ADCC or ADCP) with surrounding FcR-expressing cells (**Figure 1D**). Using SHIV-infected macaque model treated with cART in the acute phase of infection, they demonstrated that V3 type bNab PGT121 with GS-9620 under cART delayed viral rebound following ATI (Borducchi et al., 2018). In their study, serum antibody concentration was below the detectable limit at the time of ATI, suggesting bNab's direct effect on latently infected cells. Concurrently, a combination of TLR agonists and bNabs is being studied in a Phase II clinical trial. In the study (TITAN), a combination of MGN1703 (TLR9 agonist) and 3BMC117 (anti-Env CD4 binding site bNab) will be tested with cART patients (NCT03837756).

IMPORTANCE OF ANIMAL MODELS FOR THE DEVELOPMENT OF PRR DRUGS

Animal studies are essential to the development of vaccines and their adjuvants. Such experiments generally involve small animals, with mouse and rat models most commonly used for efficacy and safety, respectively. Such experiments have several advantages, including relatively straightforward experimental protocols, and availability of a variety of assays alongside genetically modified mouse models required for mechanistic analyses.

In spite of these advantages, these models have several disadvantages. For example, previous studies have reported differences between mice and humans in PRR expression and innate immune responsiveness, specifically regarding innate immune response, which is relevant to research into PRR ligands as immunostimulatory drugs (Hornung et al., 2002; Pulendran, 2004; Kastenmuller et al., 2014). Moreover, TLRs 7 and 9 have been reported to show expression localized in plasmacytoid dendritic cells (pDCs) and B cells in humans; however, in mice, they are also expressed in CD8+ DC. Moreover, TLR8 expression has been reported in monocytes and conventional DC (cDC) in humans (Kadowaki et al., 2001), while functional TLR8 is not expressed in any type of mice cell, where it could be a pseudogene. These differences between mouse and human level of TLR expression might limit translation of any findings regarding PRR ligands therapeutic agents into clinical use, highlighting the need for a non-human primate model, and subsequent human trials.

Another important aspect of an animal model in HIV-1 cure studies is the timing of cART initiation. For instance, cART starting early after infection has been shown to limit the reservoir size in SIV infected macaque (Okoye et al., 2018).

Del Prete et al. have stressed the importance of cART timing. They observed no increase in viremia after serial administration of GS-9620, or changes to viral DNA associated with GS-9620 in PBMC or tissues. In their study, cART was initiated 13 days after infection and continued for 75 weeks before GS-9620 administration. Nevertheless, they observed transient upregulation of IFN-stimulated genes in blood and tissues, an increase in plasma cytokines level, changes in immune cell population activation, and phenotypes (Del Prete et al., 2019).

In chronic SIV-infected model, despite combination with PD-1 blockage, GS-9620 could not reduce the amount of viral RNA in PBMC or tissues after treatment interruption (Bekerman et al., 2019), which is in contrast to previously reported beneficial results of GS-9620 in SIV- or SHIV-infected macaque models (Borducchi et al., 2016, 2018; Lim et al., 2018). These discrepancies might be due to the timing and duration of cART, which might affect the level of set point viral loads, reservoirs size, and the likelihood of regular immune system preservation.

Altogether, data from previous studies should be carefully considered when evaluating the effect of treatment with PRR

ligands based on the timing of cART initiation and its duration *in vivo*.

CONCLUDING REMARKS

In this mini review, we discussed potential use of PRR agonists as a single agent or in combination with other LRA to eliminate latently infected cells. We focused on agonists of TLRs, STING, and RIG-I, showing that several PRR ligands might help eliminate latently infected cells (**Figure 1A**). However, further research is required to elucidate the underlying mechanisms of action, differences in pathway activation, crosstalk between pathways, and their metabolism *in vivo*. As most of the presented studies involved *in vitro* observations, examination of the efficacy *in vivo* is the next paramount step. In addition, an animal model treated *in vivo* with cART during a chronic phase of infection is necessary, as it would be clinically relevant to the majority of HIV-infected individuals.

In summary, progress toward a functional cure against HIV-1 in humans continues. Some of the pre-clinical studies using innate-immune activators have shown encouraging data, making it a promising candidate for a future HIV-1 cure.

AUTHOR CONTRIBUTIONS

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

FUNDING

This study was supported by JSPS Grant-in-Aid for Young Scientists (A) (grant number 17H05087), Scientific Research (B) (grant number 17H04079), challenging Exploratory Research (19k21601) and AMED (grant number 19fk0410012s0102, 19fk0210057h0001).

ACKNOWLEDGMENTS

We would like to acknowledge all our lab members for their support in writing this manuscript.

REFERENCES

Alvarez-Carbonell, D., Garcia-Mesa, Y., Milne, S., Das, B., Dobrowolski, C., Rojas, R., et al. (2017). Toll-like receptor 3 activation selectively reverses HIV latency in microglial cells. *Retrovirology* 14:9. doi: 10.1186/s12977-017-0335-8

Archin, N. M., Liberty, A. L., Kashuba, A. D., Choudhary, S. K., Kuruc, J. D., Crooks, A. M., et al. (2012). Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 487, 482–485. doi: 10.1038/nature11286

Bafica, A., Scanga, C. A., Schito, M., Chaussabel, D., and Sher, A. (2004). Influence of coinfecting pathogens on HIV expression: evidence for a role of Toll-like receptors. *J. Immunol.* 172, 7229–7234. doi: 10.4049/jimmunol.172.12.7229

Bafica, A., Scanga, C. A., Schito, M. L., Hieny, S., and Sher, A. (2003). Cutting edge: *in vivo* induction of integrated HIV-1 expression by mycobacteria is critically dependent on Toll-like receptor 2. *J. Immunol.* 171, 1123–1127. doi: 10.4049/jimmunol.171.3.1123

Banga, R., Procopio, F. A., Noto, A., Pollakis, G., Cavassini, M., Ohmiti, K., et al. (2016). PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat. Med.* 22, 754–761. doi: 10.1038/nm.4113

Bar, K. J., Sneller, M. C., Harrison, L. J., Justement, J. S., Overton, E. T., Petrone, M. E., et al. (2016). Effect of HIV antibody VRC01 on viral rebound after treatment *interruption. N. Engl. J. Med.* 375, 2037–2050. doi: 10.1056/NEJMoa1608243

Barber, G. N. (2011). STING-dependent signaling. *Nat. Immunol.* 12, 929–930. doi: 10.1038/ni.2118

Barber, G. N. (2014). STING-dependent cytosolic DNA sensing pathways. *Trends Immunol.* 35, 88–93. doi: 10.1016/j.it.2013.10.010

Barber, G. N. (2015). STING: infection, inflammation and cancer. *Nat. Rev. Immunol.* 15, 760–770. doi: 10.1038/nri3921

Bekerman, E., Hesselgesser, J., Carr, B., Nagel, M., Hung, M., Wang, A., et al. (2019). PD-1 blockade and TLR7 activation lack therapeutic benefit in chronic simian immunodeficiency virus-infected macaques on antiretroviral therapy. *Antimicrob. Agents Chemother.* 63:e01163-19. doi: 10.1128/AAC.01163-19

Borducchi, E. N., Cabral, C., Stephenson, K. E., Liu, J., Abbink, P., Ng'ang'a, D., et al. (2016). Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 540, 284–287. doi: 10.1038/nature20583

Borducchi, E. N., Liu, J., Nkolola, J. P., Cadena, A. M., Yu, W. H., Fischinger, S., et al. (2018). Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* 563, 360–364. doi: 10.1038/s41586-018-0600-6

Broz, P., and Monack, D. M. (2013). Newly described pattern recognition receptors team up against intracellular pathogens. *Nat. Rev. Immunol.* 13, 551–565. doi: 10.1038/nri3479

Burdette, D. L., and Vance, R. E. (2013). STING and the innate immune response to nucleic acids in the cytosol. *Nat. Immunol.* 14, 19–26. doi: 10.1038/ni.2491

Cartwright, E. K., Spicer, L., Smith, S. A., Lee, D., Fast, R., Paganini, S., et al. (2016). CD8(+) lymphocytes are required for maintaining viral suppression in SIV-infected macaques treated with short-term antiretroviral therapy. *Immunity* 45, 656–668. doi: 10.1016/j.immuni.2016.08.018

Clutton, G., Xu, Y., Baldoni, P. L., Mollan, K. R., Kirchherr, J., Newhard, W., et al. (2016). The differential short- and long-term effects of HIV-1 latency-reversing agents on T cell function. *Sci. Rep.* 6:30749. doi: 10.1038/srep30749

Coffman, R. L., Sher, A., and Seder, R. A. (2010). Vaccine adjuvants: putting innate immunity to work. *Immunity* 33, 492–503. doi: 10.1016/j.immuni.2010.10.002

Corrales, L., McWhirter, S. M., Dubensky, T. W. Jr., and Gajewski, T. F. (2016). The host STING pathway at the interface of cancer and immunity. *J. Clin. Invest.* 126, 2404–2411. doi: 10.1172/JCI86892

Daffis, S., Chamberlain, J., Zheng, J., Santos, R., Rowe, W., Mish, M., et al. (2017). Sustained efficacy and surface antigen seroconversion in the woodchuck model of chronic hepatitis B with the selective toll-like receptor 8 agonist GS-9688. *J. Hepatol.* 66, S692–S693. doi: 10.1016/S0168-8278(17)31859-7

Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., et al. (2006). PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443, 350–354. doi: 10.1038/nature05115

Deeks, S. G., Lewin, S. R., Ross, A. L., Ananworanich, J., Benkirane, M., Cannon, P., et al. (2016). International AIDS Society global scientific strategy: towards an HIV cure 2016. *Nat. Med.* 22, 839–850. doi: 10.1038/nm.4108

Del Giudice, G., Rappuoli, R., and Didierlaurent, A. M. (2018). Correlates of adjuvanticity: a review on adjuvants in licensed vaccines. *Semin. Immunol.* 39, 14–21. doi: 10.1016/j.smim.2018.05.001

Del Prete, G. Q., Alvord, W. G., Li, Y., Deleage, C., Nag, M., Oswald, K., et al. (2019). TLR7 agonist administration to SIV-infected macaques receiving early initiated cART does not induce plasma viremia. *JCI Insight.* 4:e127717. doi: 10.1172/jci.insight.127717

Deng, K., Pertea, M., Rongvaux, A., Wang, L., Durand, C. M., Ghiaur, G., et al. (2015). Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. *Nature* 517, 381–385. doi: 10.1038/nature14053

Dyavar Shetty, R., Velu, V., Titanji, K., Bosinger, S. E., Freeman, G. J., Silvestri, G., et al. (2012). PD-1 blockade during chronic SIV infection reduces

hyperimmune activation and microbial translocation in rhesus macaques. *J. Clin. Invest.* 122, 1712–1716. doi: 10.1172/JCI60612

Gane, E. J., Lim, Y. S., Gordon, S. C., Visvanathan, K., Sicard, E., Fedorak, R. N., et al. (2015). The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. *J. Hepatol.* 63, 320–328. doi: 10.1016/j.jhep.2015.02.037

Garrido, C., Tolstrup, M., Sogaard, O. S., Rasmussen, T. A., Allard, B., Soriano-Sarabia, N., et al. (2019). *In-vivo* administration of histone deacetylase inhibitors does not impair natural killer cell function in HIV+ individuals. *AIDS* 33, 605–613. doi: 10.1097/QAD.0000000000002112

Haynes, B. F., Burton, D. R., and Mascola, J. R. (2019). Multiple roles for HIV broadly neutralizing antibodies. *Sci. Transl. Med.* 11:eaaz2686. doi: 10.1126/scitranslmed.aaz2686

Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdorfer, B., Giese, T., et al. (2002). Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J. Immunol.* 168, 4531–4537. doi: 10.4049/jimmunol.168.9.4531

Janssen, H. L. A., Brunetto, M. R., Kim, Y. J., Ferrari, C., Massetto, B., Nguyen, A. H., et al. (2018). Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. *J. Hepatol.* 68, 431–440. doi: 10.1016/j.jhep.2017.10.027

Jones, R. B., O'Connor, R., Mueller, S., Foley, M., Szeto, G. L., Karel, D., et al. (2014). Histone deacetylase inhibitors impair the elimination of HIV-infected cells by cytotoxic T-lymphocytes. *PLoS Pathog.* 10:e1004287. doi: 10.1371/journal.ppat.1004287

Jones, R. B., and Walker, B. D. (2016). HIV-specific CD8(+) T cells and HIV eradication. *J. Clin. Invest.* 126, 455–463. doi: 10.1172/JCI80566

Kadowaki, N., Ho, S., Antonenko, S., Malefyt, R. W., Kastelein, R. A., Bazan, F., et al. (2001). Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* 194, 863–869. doi: 10.1084/jem.194.6.863

Kastenmuller, W., Kastenmuller, K., Kurts, C., and Seder, R. A. (2014). Dendritic cell-targeted vaccines–hope or hype? *Nat. Rev. Immunol.* 14, 705–711. doi: 10.1038/nri3727

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384. doi: 10.1038/ni.1863

Kawai, T., and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650. doi: 10.1016/j.immuni.2011.05.006

Kell, A. M., and Gale, M. Jr. (2015). RIG-I in RNA virus recognition. *Virology* 479, 110–121. doi: 10.1016/j.virol.2015.02.017

Kinkead, H. L., Hopkins, A., Lutz, E., Wu, A. A., Yarchoan, M., Cruz, K., et al. (2018). Combining STING-based neoantigen-targeted vaccine with checkpoint modulators enhances antitumor immunity in murine pancreatic cancer. *JCI Insight* 3:e122857. doi: 10.1172/jci.insight.122857

Krarup, A. R., Abdel-Mohsen, M., Schleimann, M. H., Vibholm, L., Engen, P. A., Dige, A., et al. (2018). The TLR9 agonist MGN1703 triggers a potent type I interferon response in the sigmoid colon. *Mucosal. Immunol.* 11, 449–461. doi: 10.1038/mi.2017.59

Kuse, N., Sun, X., Akahoshi, T., Lissina, A., Yamamoto, T., Appay, V., et al. (2019). Priming of HIV-1-specific CD8(+) T cells with strong functional properties from naive T cells. *EBioMedicine* 42, 109–119. doi: 10.1016/j.ebiom.2019.03.078

Kwong, P. D., and Mascola, J. R. (2018). HIV-1 vaccines based on antibody identification, B cell ontogeny, and epitope structure. *Immunity* 48, 855–871. doi: 10.1016/j.immuni.2018.04.029

Lawitz, E., Gruener, D., Marbury, T., Hill, J., Webster, L., Hassman, D., et al. (2015). Safety, pharmacokinetics and pharmacodynamics of the oral toll-like receptor 7 agonist GS-9620 in treatment-naive patients with chronic hepatitis C. *Antivir. Ther.* 20, 699–708. doi: 10.3851/IMP2845

Li, P., Kaiser, P., Lampiris, H. W., Kim, P., Yukl, S. A., Havlir, D. V., et al. (2016). Stimulating the RIG-I pathway to kill cells in the latent HIV reservoir following viral reactivation. *Nat. Med.* 22, 807–811. doi: 10.1038/nm.4124

Lim, S. Y., Osuna, C. E., Hraber, P. T., Hesselgesser, J., Gerold, J. M., Barnes, T. L., et al. (2018). TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci. Transl. Med.* 10:aao4521. doi: 10.1126/scitranslmed.aao4521

Macedo, A. B., Novis, C. L., De Assis, C. M., Sorensen, E. S., Moszczynski, P., Huang, S. H., et al. (2018). Dual TLR2 and TLR7 agonists as HIV latency-reversing agents. *JCI Insight* 3:e122673. doi: 10.1172/jci.insight.122673

Manegold, C., van Zandwijk, N., Szczesna, A., Zatloukal, P., Au, J. S., Blasinska-Morawiec, M., et al. (2012). A phase III randomized study of gemcitabine and cisplatin with or without PF-3512676 (TLR9 agonist) as first-line treatment of advanced non-small-cell lung cancer. *Ann. Oncol.* 23, 72–77. doi: 10.1093/annonc/mdr030

Meas, H. Z., Haug, M., Beckwith, M. S., Louet, C., Ryan, L., Hu, Z., et al. (2020). Sensing of HIV-1 by TLR8 activates human T cells and reverses latency. *Nat. Commun.* 11:147. doi: 10.1038/s41467-019-13837-4

Mylvaganam, G. H., Chea, L. S., Tharp, G. K., Hicks, S., Velu, V., Iyer, S. S., et al. (2018). Combination anti-PD-1 and antiretroviral therapy provides therapeutic benefit against SIV. *JCI Insight* 3:e122940. doi: 10.1172/jci.insight.122940

Novis, C. L., Archin, N. M., Buzon, M. J., Verdin, E., Round, J. L., Lichterfeld, M., et al. (2013). Reactivation of latent HIV-1 in central memory CD4(+) T cells through TLR-1/2 stimulation. *Retrovirology* 10:119. doi: 10.1186/1742-4690-10-119

Offersen, R., Nissen, S. K., Rasmussen, T. A., Ostergaard, L., Denton, P. W., Sogaard, O. S., et al. (2016). A novel toll-like receptor 9 agonist, MGN1703, enhances HIV-1 transcription and NK cell-mediated inhibition of HIV-1-infected autologous CD4+ T cells. *J. Virol.* 90, 4441–4453. doi: 10.1128/JVI.00222-16

Okoye, A. A., Hansen, S. G., Vaidya, M., Fukazawa, Y., Park, H., Duell, D. M., et al. (2018). Early antiretroviral therapy limits SIV reservoir establishment to delay or prevent post-treatment viral rebound. *Nat. Med.* 24, 1430–1440. doi: 10.1038/s41591-018-0130-7

Palermo, E., Acchioni, C., Di Carlo, D., Zevini, A., Muscolini, M., Ferrari, M., et al. (2019). Activation of latent HIV-1 T cell reservoirs with a combination of innate immune and epigenetic regulators. *J. Virol.* 93:e01194-19. doi: 10.1128/JVI.01194-19

Petrovas, C., Casazza, J. P., Brenchley, J. M., Price, D. A., Gostick, E., Adams, W. C., et al. (2006). PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J. Exp. Med.* 203, 2281–2292. doi: 10.1084/jem.20061496

Probst, P., Grigg, J. B., Wang, M., Munoz, E., Loo, Y. M., Ireton, R. C., et al. (2017). A small-molecule IRF3 agonist functions as an influenza vaccine adjuvant by modulating the antiviral immune response. *Vaccine* 35, 1964–1971. doi: 10.1016/j.vaccine.2017.01.053

Pulendran, B. (2004). Modulating vaccine responses with dendritic cells and Toll-like receptors. *Immunol. Rev.* 199, 227–250. doi: 10.1111/j.0105-2896.2004.00144.x

Reed, S. G., Orr, M. T., and Fox, C. B. (2013). Key roles of adjuvants in modern vaccines. *Nat. Med.* 19, 1597–1608. doi: 10.1038/nm.3409

Rynkiewicz, D., Rathkopf, M., Sim, I., Waytes, A. T., Hopkins, R. J., Giri, L., et al. (2011). Marked enhancement of the immune response to BioThrax(R) (Anthrax vaccine adsorbed) by the TLR9 agonist CPG 7909 in healthy volunteers. *Vaccine* 29, 6313–6320. doi: 10.1016/j.vaccine.2011.05.047

Scheid, J. F., Horwitz, J. A., Bar-On, Y., Kreider, E. F., Lu, C. L., Lorenzi, J. C., et al. (2016). HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 535, 556–560. doi: 10.1038/nature18929

Scheid, J. F., Mouquet, H., Feldhahn, N., Seaman, M. S., Velinzon, K., Pietzsch, J., et al. (2009). Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature* 458, 636–640. doi: 10.1038/nature07930

Scheller, C., Ullrich, A., McPherson, K., Hefele, B., Knoferle, J., Lamla, S., et al. (2004). CpG oligodeoxynucleotides activate HIV replication in latently infected human T cells. *J. Biol. Chem.* 279, 21897–21902. doi: 10.1074/jbc.M311609200

Schleimann, M. H., Kobbero, M. L., Vibholm, L. K., Kjaer, K., Giron, L. B., Busman-Sahay, K., et al. (2019). TLR9 agonist MGN1703 enhances B cell differentiation and function in lymph nodes. *EBioMedicine* 45, 328–340. doi: 10.1016/j.ebiom.2019.07.005

Schmidt, M., Hagner, N., Marco, A., Konig-Merediz, S. A., Schroff, M., and Wittig, B. (2015). Design and structural requirements of the potent and safe TLR-9 agonistic immunomodulator MGN1703. *Nucleic Acid Ther.* 25, 130–140. doi: 10.1089/nat.2015.0533

Scully, E. P., Rutishauser, R. L., Simoneau, C. R., Delagreverie, H., Euler, Z., Thanh, C., et al. (2018). Inconsistent HIV reservoir dynamics and immune responses

following anti-PD-1 therapy in cancer patients with HIV infection. *Ann. Oncol.* 29, 2141–2142. doi: 10.1093/annonc/mdy259

Sengupta, S., and Siliciano, R. F. (2018). Targeting the latent reservoir for HIV-1. *Immunity* 48, 872–895. doi: 10.1016/j.immuni.2018.04.030

Sogaard, O. S., Lohse, N., Harboe, Z. B., Offersen, R., Bukh, A. R., Davis, H. L., et al. (2010). Improving the immunogenicity of pneumococcal conjugate vaccine in HIV-infected adults with a toll-like receptor 9 agonist adjuvant: a randomized, controlled trial. *Clin. Infect. Dis.* 51, 42–50. doi: 10.1086/653112

Sok, D., and Burton, D. R. (2018). Recent progress in broadly neutralizing antibodies to HIV. *Nat. Immunol.* 19, 1179–1188. doi: 10.1038/s41590-018-0235-7

Solis, M., Nakhaei, P., Jalalirad, M., Lacoste, J., Douville, R., Arguello, M., et al. (2011). RIG-I-mediated antiviral signaling is inhibited in HIV-1 infection by a protease-mediated sequestration of RIG-I. *J. Virol.* 85, 1224–1236. doi: 10.1128/JVI.01635-10

Spivak, A. M., and Planelles, V. (2018). Novel latency reversal agents for HIV-1 Cure. *Annu. Rev. Med.* 69, 421–436. doi: 10.1146/annurev-med-052716-031710

Sun, H., Kim, D., Li, X., Kiselinova, M., Ouyang, Z., Vandekerckhove, L., et al. (2015). Th1/17 Polarization of CD4 T cells supports HIV-1 persistence during antiretroviral therapy. *J. Virol.* 89, 11284–11293. doi: 10.1128/JVI.01595-15

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* 140, 805–820. doi: 10.1016/j.cell.2010.01.022

Temizoz, B., Kuroda, E., and Ishii, K. J. (2018). Combination and inducible adjuvants targeting nucleic acid sensors. *Curr. Opin. Pharmacol.* 41, 104–113. doi: 10.1016/j.coph.2018.05.003

Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., et al. (2006). Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat. Med.* 12, 1198–1202. doi: 10.1038/nm1482

Tsai, A., Irrinki, A., Kaur, J., Cihlar, T., Kukolj, G., Sloan, D. D., et al. (2017). Toll-like receptor 7 agonist GS-9620 induces HIV expression and HIV-specific immunity in cells from HIV-infected individuals on suppressive antiretroviral therapy. *J. Virol.* 91:e02166-16. doi: 10.1128/JVI.02166-16

Velu, V., Titanji, K., Zhu, B., Husain, S., Pladevega, A., Lai, L., et al. (2009). Enhancing SIV-specific immunity *in vivo* by PD-1 blockade. *Nature* 458, 206–210. doi: 10.1038/nature07662

Vibholm, L., Schleimann, M. H., Hojen, J. F., Benfield, T., Offersen, R., Rasmussen, K., et al. (2017). Short-course toll-like receptor 9 agonist treatment impacts innate immunity and plasma viremia in individuals with human immunodeficiency virus infection. *Clin. Infect. Dis.* 64, 1686–1695. doi: 10.1093/cid/cix201

Vibholm, L. K., Konrad, C. V., Schleimann, M. H., Frattari, G., Winckelmann, A., Klastrup, V., et al. (2019). Effects of 24-week Toll-like receptor 9 agonist treatment in HIV type 1+ individuals. *AIDS* 33, 1315–1325. doi: 10.1097/QAD.0000000000002213

Walker, L. M., Phogat, S. K., Chan-Hui, P. Y., Wagner, D., Phung, P., Goss, J. L., et al. (2009). Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* 326, 285–289. doi: 10.1126/science.1178746

Wang, M. Q., Huang, Y. L., Huang, J., Zheng, J. L., and Qian, G. X. (2015). RIG-I detects HIV-1 infection and mediates type I interferon response in human macrophages from patients with HIV-1-associated neurocognitive disorders. *Genet. Mol. Res.* 14, 13799–13811. doi: 10.4238/2015.October.28.42

Wang, Y., Wang, X., Li, J., Zhou, Y., and Ho, W. (2013). RIG-I activation inhibits HIV replication in macrophages. *J. Leukoc. Biol.* 94, 337–341. doi: 10.1189/jlb.0313158

Winckelmann, A. A., Munk-Petersen, L. V., Rasmussen, T. A., Melchjorsen, J., Hjelholt, T. J., Montefiori, D., et al. (2013). Administration of a Toll-like receptor 9 agonist decreases the proviral reservoir in virologically suppressed HIV-infected patients. *PLoS ONE* 8:e62074. doi: 10.1371/journal.pone.0062074

Wittig, B., Schmidt, M., Scheithauer, W., and Schmoll, H. J. (2015). MGN1703, an immunomodulator and toll-like receptor 9 (TLR-9) agonist: from bench to bedside. *Crit. Rev. Oncol. Hematol.* 94, 31–44. doi: 10.1016/j.critrevonc.2014.12.002

Wu, X., Yang, Z. Y., Li, Y., Hogerkorp, C. M., Schief, W. R., Seaman, M. S., et al. (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* 329, 856–861. doi: 10.1126/science.1187659

Yamamoto, T., Kanuma, T., Takahama, S., Okamura, T., Moriishi, E., Ishii, K. J., et al. (2019). STING agonists activate latently infected cells and enhance SIV-specific responses *ex vivo* in naturally SIV controlled cynomolgus macaques. *Sci. Rep.* 9:5917. doi: 10.1038/s41598-019-42253-3

Zhou, T., Georgiev, I., Wu, X., Yang, Z. Y., Dai, K., Finzi, A., et al. (2010). Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science* 329, 811–817. doi: 10.1126/science.1192819

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 19 May 2020
doi: 10.3389/fcimb.2020.00233

What Can Gamma Delta T Cells Contribute to an HIV Cure?

Jennifer A. Juno[1]* *and Stephen J. Kent*[1,2,3]

[1] Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, VIC, Australia, [2] Department of Infectious Diseases, Melbourne Sexual Health Centre, Alfred Health, Central Clinical School, Monash University, Clayton, VIC, Australia, [3] ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, The University of Melbourne, Melbourne, VIC, Australia

Elimination of the latent HIV reservoir remains a major barrier to achieving an HIV cure. In this review, we discuss the cytolytic nature of human gamma delta T cells and highlight the emerging evidence that they can target and eliminate HIV-infected T cells. Based on observations from human clinical trials assessing gamma delta immunotherapy in oncology, we suggest key questions and research priorities for the study of these unique T cells in HIV cure research.

Keywords: gamma delta T cell, Vδ2, Vδ1, HIV, immunotherapy

OPEN ACCESS

Edited by:
Alfredo Garzino-Demo, University of Maryland, United States

Reviewed by:
Natalia Soriano-Sarabia, George Washington University, United States
Redmond Patrick Smyth, Helmholtz-Institut für RNA-basierte Infektionsforschung (HIRI), Germany

***Correspondence:**
Jennifer A. Juno
jennifer.juno@unimelb.edu.au

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *05 February 2020*
Accepted: *23 April 2020*
Published: *19 May 2020*

Citation:
Juno JA and Kent SJ (2020) What Can Gamma Delta T Cells Contribute to an HIV Cure? Front. Cell. Infect. Microbiol. 10:233. doi: 10.3389/fcimb.2020.00233

INTRODUCTION

Efforts to eliminate the latent HIV reservoir have, to date, been unsuccessful, outside of aggressive chemotherapy and stem cell transplants from genetically resistant donors (Hutter et al., 2009; Gupta et al., 2020). Killing of infected cells with minimal viral replication, or killing of infected cells after latency reversal, is likely to be critical in the long-term containment or eradication of HIV. Novel approaches to clear infected cells now include the study and manipulation of highly cytotoxic lymphocyte subsets beyond traditional CD8+ CTL (Garrido et al., 2018a,b). It has become apparent from both *in vitro* studies and human clinical trials in oncology that gamma delta T (γδT) cells exhibit remarkable cytotoxicity (Simoes et al., 2018) and the potential for safe clinical use in human immunotherapy (Silva-Santos et al., 2019). A number of excellent reviews have recently described the use of human γδT cells in clinical trials for cancer treatment (Lo Presti et al., 2017; Godfrey et al., 2018; Pauza et al., 2018; Silva-Santos et al., 2019). Here, we will discuss how advances in gdT immunology have identified these cells as potential anti-HIV effectors, and what remains to be established regarding the efficacy of γδT cells as components of an HIV cure intervention.

HUMAN GAMMA DELTA T CELL SUBSETS

Human γδT cells are typically classified on the basis of their TCR delta chain, of which there are 8 variants (Hayday, 2000). In peripheral blood, up to 90% of γδT cells express the Vδ2 chain (Triebel et al., 1988). The majority of Vδ2 cells pair with Vγ9 and form the well-studied population of phosphoantigen-reactive γδT cells (Tanaka et al., 1995). In contrast, Vδ2-negative γδT s dominate at many mucosal sites, including the gut (Lundqvist et al., 1995). These Vδ2- γδT cells tend to express either the Vδ1 or Vδ3 chain, with a variety of Vγ chain pairings (Groh et al., 1998). Vδ1 cells typically, but not always (Hviid et al., 2000), form a minor population of the circulating γδT cell compartment.

Vδ2Vγ9 cells (herein referred to as Vδ2 cells) form a polyclonal T cell population that rapidly expands postnatally, most likely due to persistent antigen exposure or other inflammatory stimuli (Pauza and Cairo, 2015; van Der Heiden et al., 2020). The Vδ2Vγ9 TCR recognizes

pyrophosphate antigens, which include isopentenyl pyrophosphate (IPP) and the potent microbial metabolite (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) (Triebel et al., 1988). Like other unconventional T cells, however, Vd2 cells can also respond to TCR-independent stimuli, including cytokines such as IL-12 and IL-18, and various NK cell receptor ligands (Provine et al., 2018). Interestingly, phosphoantigen-reactive γδT cells are found only in humans, non-human primates, and alpacas, with no γδT cells in mice recognizing similar antigens (Fichtner et al., 2020). Owing in part to the ease with which they can be expanded *in vitro*, Vδ2 cells have been well characterized in human health and disease, and many of their defining features have been recently reviewed (Tyler et al., 2015; Davey et al., 2018). Notably, Vδ2 cells are commonly depleted during chronic HIV infection, with incomplete restoration among ART-treated cohorts (Hinz et al., 1994; Poccia et al., 1996; Wesch et al., 1998; Enders et al., 2003; Poles et al., 2003; Li et al., 2014, 2015; Cimini et al., 2015; Strbo et al., 2016; Bhatnagar et al., 2017). We have recently reviewed the impact of HIV infection and treatment on both circulating and tissue-resident γδT cells (Juno and Eriksson, 2019).

In contrast to Vδ2 cells, the antigen specificity of Vδ1 cells remains largely unknown. The Vδ1 cell population includes cells with specificity for R-phycoerythrin and EphA2 (Willcox and Willcox, 2019), CD1c- and CD1d-restricted lipid antigens (Uldrich et al., 2013; Roy et al., 2016; Willcox and Willcox, 2019), and the antigen presenting molecule MR1 (Le Nours et al., 2019). Vδ1 phenotype and frequency is markedly altered by infections such as malaria (Hviid et al., 2019) and human cytomegalovirus (HCMV) (Pitard et al., 2008; van Der Heiden et al., 2020). HCMV seropositivity is typically associated with substantial clonal expansion within the Vδ1 population, and differentiation toward a terminally differentiated (CD27-CD45RA+) phenotype (Davey et al., 2017; van Der Heiden et al., 2020). In contrast to Vδ2 cells, the Vδ1 cell population is significantly expanded during HIV infection and ART (De Paoli et al., 1991; De Maria et al., 1992; Rossol et al., 1998; Wesch et al., 1998; Poles et al., 2003; Poggi et al., 2004; Fausther-Bovendo et al., 2008; Li et al., 2014; Cimini et al., 2015; Olson et al., 2018; Chevalier et al., 2019), the implications of which are largely unknown (Juno and Eriksson, 2019).

ANTI-HIV ACTIVITY OF GAMMA DELTA T CELLS

Efforts to eliminate the HIV reservoir following latency reversal have traditionally focused on the utility of antigen-stimulated conventional CD8+ T cells to kill HIV-infected cells (Shan et al., 2012; Deng et al., 2015). Limitations to this approach include the requirement for autologous CD8+ T cells to be collected and pre-stimulated for each individual (Shan et al., 2012), as well as a high burden of CTL escape variants in the latent reservoir (Deng et al., 2015). More recently, other lymphocyte subsets have been considered for use in HIV cure approaches. Evidence suggests that cytokine-treated NK cells can eliminate HIV-infected T cells following *ex vivo* latency reactivation, although IL-15 treatment downregulates the expression of the key NK cell receptor NKp46, which may be undesirable *in vivo* (Garrido et al., 2018a). With a transcriptional phenotype that blends characteristics of both NK and CD8+ T cells (Gutierrez-Arcelus et al., 2019; Pizzolato et al., 2019), γδT cells are intriguing candidates to mediate anti-HIV effector functions. Indeed, γδT-mediated inhibition of HIV replication has been recognized for more than 20 years (Poccia et al., 1999).

Like NK cells (Fehniger et al., 1998; Oliva et al., 1998), stimulated γδT cells can produce sufficient β-chemokines to block HIV entry into either CCR5+ or CXCR4+ CD4+ T cells (Poccia et al., 1999; Omi et al., 2014). In the context of HIV cure approaches, however, it is the potent cytolytic function of γδT cells that makes them strong candidates for immunotherapy. Early reports suggested that direct cytotoxicity toward HIV-infected cells was largely restricted to Vδ2 cell clones (Wallace et al., 1996; Poccia et al., 1997), with little to no cytotoxicity observed among Vδ1 cell lines (Wallace et al., 1996). More recently, Vδ1 recognition and killing of HIV-infected CD4+ T cells has been demonstrated (Fausther-Bovendo et al., 2008). Although it is challenging to determine the extent to which γδT cells contribute to natural control of HIV infection in cross-sectional studies, elite/viral controllers do exhibit higher frequencies of Vδ2 cells than untreated or antiretroviral treated normal progressors (Riedel et al., 2009; Chevalier et al., 2019). A study in non-human primates identified a relationship between cervical Vδ2 frequency and simian immunodeficiency virus (SIV) viral load (Tuero et al., 2016), which supports the possibility of a protective role for these cells during infection.

Perhaps the strongest proof-of-concept evidence for Vδ2-mediated elimination of infected CD4+ T cells following latency reversal *in vitro* was reported by Garrido et al. (2018b). Despite low frequencies of Vδ2 cells in ART-treated donors *ex vivo*, pamidronate + IL-2 treatment successfully expanded the Vδ2 population in a manner comparable to that of uninfected donors *in vitro* (i.e., a similar fold-increase). The expanded cells expressed CD56 and CD16, as well as relatively low levels of the inhibitory markers PD-1 and CTLA-4. Interestingly, both *ex vivo* isolated and *in vitro* expanded Vδ2 cells were equally capable of inhibiting HIV replication in autologous superinfected CD4+ T cells, with a level of inhibition comparable to that of CD8+ CTL. More importantly, however, expanded Vδ2 cells degranulated in response to co-culture with HIV-infected, but not uninfected, CD4+ T cells. These results were further extended to a latency clearance assay, which demonstrated that expanded Vδ2 cells cleared infected CD4+ T cells derived from ART-treated donors following *in vitro* reactivation with vorinostat. This study provides promising evidence for the ability of Vδ2 cells to contribute to an HIV cure approach that relies on eliminating reactivated virally infected target cells. A number of important questions remain, however, particularly regarding the mechanisms of Vδ2 recognition of HIV-infected cells as well as the optimal conditions for expansion Vδ2 cells with the most potent cytotoxicity. The need to address these gaps in knowledge is evidenced in a recent study by James et al. (2020) which found that the presence of gamma delta T cells in a viral outgrowth assay impacted viral replication in 4 of 15 donors. CD16 expression

on Vδ2 cells correlated with a reduction in HIV recovery, but the key receptors involved in recognition of CD4 target cells and the reason for the heterogeneity in responses among the cohort remains unknown. Below, we explore the potential mechanisms that could be exploited in a gamma delta T-mediated HIV cure strategy (summarized in **Figure 1**).

RECEPTORS MEDIATING DIRECT CYTOTOXICITY

Vδ2 cells express a number of receptors that might facilitate recognition of HIV-infected T cells. A critical receptor for the detection and killing of transformed and virally infected is NKG2D, which is commonly expressed on NK cells. NKG2D is widely expressed by Vδ2 cells both *ex vivo* and following *in vitro* expansion, and can mediate direct killing of target cells (Rincon-Orozco et al., 2005; Wrobel et al., 2007). The NKG2D ligands ULBP-1,−2, and−3 are absent on uninfected CD4+ T cells, but are highly upregulated upon HIV infection and mediate NK-cell killing of HIV-infected cells (Ward et al., 2007). NKG2D is therefore a strong candidate for Vδ2-mediated direct recognition and killing of reactivated HIV-infected cells. NK cells can also recognize infected CD4+ T cells through 2B4-CD48 binding (Ward et al., 2007). Although 2B4 is highly expressed on Vδ2 cells *ex vivo*, triggering of 2B4 in isolation is insufficient to activate γδT effector functions (Nakajima et al., 1999). Whether phosphoantigen-expanded Vδ2 cells can be activated through 2B4 signaling and/or recognition of CD48 on HIV-infected T cells is currently unknown. Similarly, there is a potential for Vδ2-expressed DNAM-1 to recognize its ligand PVR on infected CD4+ T cells, although NK cell killing of infected cells through this mechanism seems to require signaling through both NKG2D and DNAM-1, rather than DNAM-1 alone (Cifaldi et al., 2019). In general, it will be important for future studies to consider the impact of activating receptor ligation both among *ex vivo*-derived Vδ2 cells, as well as *in vitro* expanded cells. For example, NKG2D ligation on resting Vδ2 cells is reported to have either no or minimal functional impact (Rincon-Orozco et al., 2005; Nedellec et al., 2010), but in the context of TCR stimulation can provide robust co-stimulatory activity and effector function (Nedellec et al., 2010).

Expression of cytotoxicity receptors on Vδ1 cells differs substantially from those described on Vδ2 cells. Vδ1 cells commonly express CD94 paired with either the inhibitory receptor NKG2A (CD159a) or the activating receptor NKG2C (CD159c). Chronic HIV infection is associated with a significant increase in NKG2C expression on Vδ1 cells, which can substantially boost Vδ1 cell cytotoxicity when triggered by binding to its ligand HLA-E (Fausther-Bovendo et al., 2008). Interestingly, Vδ1 cells derived *ex vivo* from HIV-infected participants can lyse autologous HIV-infected CD4+ T cells in a manner which is partially dependent on Vδ1 NKG2C expression (Fausther-Bovendo et al., 2008), suggesting a currently unrealized potential for Vδ1 cells in anti-HIV immunotherapy. The *in vitro* expansion of Vδ1 cells is less commonly reported or standardized compared to Vδ2 cells, largely due to the lack of an antigen that can easily stimulate the majority of Vδ1 cells *ex vivo*. Several groups, however, have reported protocols to expand these cells using a combination of γδ TCR stimulus and cytokines. Almeida et al. reported on the production of "Delta One T (DOT)" cells using isolated γδT cells, CD3 engagement and a cocktail of cytokines, which resulted in a 60,000-fold expansion of Vδ1 cells in culture (Almeida et al., 2016). Vδ1 expansion can also be induced through the culture of isolated γδT cells with PHA and IL-7 (Wu et al., 2015). In both studies, expanded Vδ1 cells expressed a number of surface markers related to cytotoxic function, including NKp30, NKp44, NKp46, NKG2D, 2B4, DNAM-1, and CD94. Other approaches to expand Vδ1 cells include the use of artificial APCs (aAPCs) presenting CMV-derived peptides (Polito et al., 2019). Vδ1 cells expanded by this approach recognize an array of tumor cells and virally infected cell lines, but the mechanism underlying this recognition was not assessed. Although the translational potential of DOT cells and other expanded Vδ1 cells are currently focussed on cancer immunotherapy, the expression of traditionally NK cell-associated markers such as NKp44, NKG2D, and 2B4 on expanded Vδ1 cells may also mediate recognition of HIV-infected CD4+ T cells (Ward et al., 2007).

ANTIBODY-DEPENDENT CYTOTOXICITY

In addition to direct recognition and killing of HIV-infected target cells, HIV-specific antibodies can mediate antibody-dependent cellular cytotoxicity (ADCC) via Fc receptor-expressing cells such as NK cells. Naturally occurring anti-HIV ADCC antibodies decline following ART (Madhavi et al., 2015, 2017) and are not boosted following panobinostat administration (as a latency-reversing agent, LRA) and subsequent analytical treatment interruption (ATI) (Lee et al., 2017). This suggests such antibodies may be insufficient to mediate the level of ADCC needed to clear the latent reservoir. The infusion of more potent broadly neutralizing antibodies (BnAbs), however, may be sufficient to allow FcR-expressing cells to recognize Env expression on reactivated infected cells (Bruel et al., 2016). HIV-specific ADCC has been widely studied in the context of NK cells, but less so in relation to unconventional T cells. Both Vδ1 and Vδ2 cells can express FcgRIIIA (CD16) (Angelini et al., 2004; Couzi et al., 2012; He et al., 2013), making them potential mediators of ADCC in the context of latency reactivation and BnAb therapy.

Despite depletion of Vδ2 cells during chronic HIV infection, ART-treated individuals exhibit similar frequencies of CD16+ Vδ2 cells compared to uninfected controls (although there is intra-donor variability in steady-state CD16 expression) (He et al., 2013). As such, *ex vivo* CD16-mediated degranulation responses are comparable between these groups (He et al., 2013), suggesting that primary Vδ2 cells from ART-treated individuals are capable of performing ADCC. Poonia and Pauza (2012) demonstrated that zoledronate can expand Vδ2 cells *in vitro* from both HIV-uninfected and HIV-infected donors, with expression of CD16 detectible on a subset of the expanded cells. Although expanded Vδ2 cells from HIV-infected donors

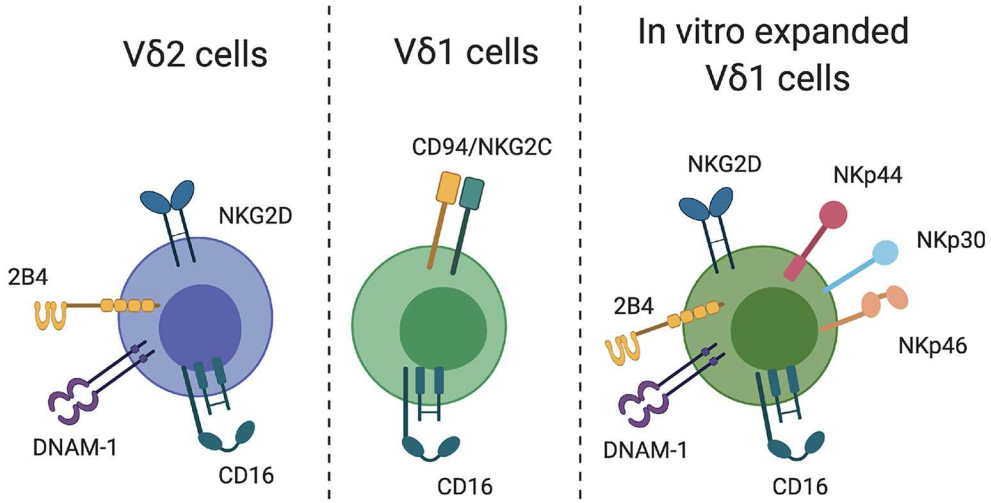


FIGURE 1 | γδT cell recognition of target cells. Relevant receptors that may facilitate γδT cell recognition of HIV-infected CD4 T cells are indicated for Vδ2, Vδ1, and *in vitro*-expanded Vδ1 cells.

exhibited lower ADCC than those from uninfected donors, they remained capable of degranulation and cytokine expression following CD16 cross-linking.

The expression of CD16 on Vδ1 cells has been studied in the context of HCMV infection (Couzi et al., 2012; Bachelet et al., 2014) and cancer immunotherapy (Fisher et al., 2014), but despite the high prevalence of HCMV seropositivity among HIV-infected populations (Gianella and Letendre, 2016), there has been little study of Vδ1 ADCC in the context of HIV infection. The expanded population of Vδ1 cells in HIV-infected individuals exhibits high expression of perforin (Boullier et al., 1997), but whether this translates into antibody-mediated cytotoxicity is unknown. Similar gaps in knowledge exist for *in vitro*-expanded Vδ1 cells. There is no data available to describe CD16 expression on DOT cells (Almeida et al., 2016), while PHA/IL-7-expanded Vδ1 cells express moderate levels of CD16 and high levels of GzmB and Perforin, though they have not been assessed for ADCC activity (Wu et al., 2015).

APPROACHES FOR GAMMA DELTA IMMUNOTHERAPY

As described above, there are many *in vitro* protocols that result in Vd2 or Vd1 T cell expansion from bulk PBMC. To translate these ideas to the clinic, there are two major approaches that can be considered: *in vivo* gamma delta T cell expansion, or adoptive transfer of *in vitro*-expanded cells. Currently, *in vivo* expansion has only been demonstrated for human Vd2 T cells. This method involves the administration of zoledronate and recombinant IL-2, and has successfully expanded Vd2 T cells in HIV-infected participants in a small clinical trial (Poccia et al., 2009). An advantage of this approach is that it is relatively simple and involves an existing, clinically approved drug (under the brand name Zometa). It would not, however, likely be suitable for individuals with substantial Vd2 T cell depletion/exhaustion or Vd2 T cell phenotypes that lack the receptors determined to be key in recognizing HIV-infected target cells. In such instances, it may be more attractive to consider the use of "off-the-shelf," *in vitro*-expanded gamma delta T cells derived from HIV uninfected donors. Such cells could be expanded by any of the protocols described above, and could include both Vd2 and Vd1 T cell subsets. This approach has been tested in clinical trials for cancer patients (Lo Presti et al., 2017) and also used in studies of non-human primate Vd2 T cells (Qaqish et al., 2017).

ACCESSING THE LATENT HIV RESERVOIR

While *in vitro* studies can provide an important assessment of cytotoxic capacity and recognition of infected T cells, they largely ignore one of the major barriers to achieving an HIV cure: the anatomical localization of the latent reservoir. Recent evidence suggests that the B cell follicle is a key site of HIV replication, even during ART (Bronnimann et al., 2018). Germinal center (GC) T follicular helper (Tfh) cells are highly permissive to HIV infection (Kohler et al., 2016; Aid et al., 2018), while follicular dendritic cells (FDCs) can capture HIV virions and maintain an infectious virus reservoir even during ART (Smith et al., 2001; Bronnimann et al., 2018). A major barrier to the clearance of virus from the B cell follicle is the fact that the follicle is partially protected from CTL activity. Of the few CTLs found in the follicle, many may be exhausted or exhibit a regulatory phenotype (Bronnimann et al., 2018). Similarly, there are few NK cells found in the human lymph node, and a high proportion of LN NK cells in HIV-infected individuals exhibit an anergic CD56-CD16+ phenotype (Luteijn et al., 2011). Given these limitations, expanded γδT cells may be strong candidates for B cell follicle targeting and elimination of infected cells. Vδ2 cells can acquire a Tfh-like phenotype, complete with expression of the GC-homing marker CXCR5 (Caccamo et al., 2006; Bansal et al., 2012), and

such cells have been described both *in vitro* and *in vivo* in human lungs (Caccamo et al., 2006). Furthermore, γδT cells have been visualized within GCs in the gastrointestinal mucosa, lymph nodes, tonsil and spleen, likely due to the induction of a LN migration program that occurs following Vδ2 TCR triggering (Brandes et al., 2003). Functional studies of Vδ2 Tfh cells have largely focused on their capacity to provide B cell help, leaving open the question of their cytotoxic potential and expression of cytotoxicity receptors. Nonetheless, the fact that γδT cells appear to access the B cell follicle merits further study of their potential to eliminate HIV-infected cells in this unique environment (Bronnimann et al., 2018).

Beyond the B cell follicle, many other tissues serve as HIV reservoir sites, including the spleen, bone marrow, liver, gut, nervous system, lung, and male and female reproductive tracts (Wong and Yukl, 2016; Cantero-Perez et al., 2019). Infection of tissue-resident T cells may be particularly important, given their longevity and capacity for self-renewal (Cantero-Perez et al., 2019). Both expanded Vδ1 and Vδ2 cells appear to be excellent candidates for tissue trafficking *in vivo*. When administered to mice, human-derived DOT cells seeded, and then further expanded in, various tissues including the spleen, lung, bone marrow, and liver (Almeida et al., 2016). The tissue-resident cells retained function, as assessed by IFNg and TNF production, and exhibited evidence of ongoing proliferation. Similarly, the adoptive transfer of expanded Vδ2 cells has been studied in non-human primates. Infusion of zoledronate-expanded Vδ2 cells resulted in trafficking to and persistence in the airway for at least 7 days (Qaqish et al., 2017).

POTENTIAL ADVANTAGES AND DISADVANTAGES OF γδT CELL IMMUNOTHERAPY

As with any immune-based intervention, there are both key advantages offered by γδT cells over other T cell therapies and issues that will need to be addressed prior to clinical testing.

Advantages of γδT cell-based immunotherapy include:

1) *Lack of MHC restriction*: GMP-compliant protocols have been developed to generate large banks of expanded γδT cells, allowing for an "off-the-shelf" allogeneic product that is not reliant on MHC matching (Deniger et al., 2014; Almeida et al., 2016; Polito et al., 2019). The lack of MHC-restriction of γδT cells is a major advantage that should avoid issues of graft-vs.-host disease in γδT -based immunotherapy. Indeed, adoptive transfer of haploidentical expanded γδT cells from the family members of cancer patients proved to be safe and highly effective toward achieving complete remission (Wilhelm et al., 2014).

2) *Safety*: Clinical trials of human γδT cell therapy, either using *in vivo* Vδ2 expansion or adoptive transfer of expanded Vδ2 cells, have proven to be safe and well-tolerated (Lo Presti et al., 2017). A major disadvantage to current αβ T cell-based chimeric antigen receptor (CAR)-T cell therapies is the potential for serious and/or fatal side effects, including cytokine release syndrome (van Den Berg et al., 2015), off-target effects due to antigen cross-reactivity (Linette et al., 2013), or transgenic TCR mispairing with endogenous TCR (Zhang et al., 2004). Several studies suggest that γδT-based immunotherapy is less likely to result in cytokine release syndrome (CRS) or off-target effects than αβ T cells (Harrer et al., 2017; Pauza et al., 2018; Rotolo et al., 2019). Indeed, expanded γδ T cells can mediate potent cytotoxicity but simultaneously produce lower levels of cytokines than αβ T cells (Harrer et al., 2017).

3) *HIV-associated expansion and differentiation of Vd1 cells*: As discussed in the introduction, Vδ1 cell frequency and absolute counts increase in the circulation during HIV infection. This increase persists during suppressive ART, resulting in a pool of terminally differentiated Vδ1 cells (Olson et al., 2018) that express NCRs such as NKG2C (Fausther-Bovendo et al., 2008). To date, we are not aware of any studies that have assessed the expansion and subsequent anti-HIV cytotoxicity of Vδ1 cells specifically derived from HIV-infected individuals. If expansion of Vδ1 cells from healthy donors does not induce the expression of NKG2C or other NCRs that are upregulated during HIV infection, then it may be advantageous to isolate and expand Vδ1 cells expressing these markers from people living with HIV. Care must be taken, however, to characterize the pro-inflammatory nature of these cells, as discussed below.

Potential challenges to γδT cell-based immunotherapy include:

1) *In vivo potency*: One of the major challenges facing the implementation of γδT cell therapy in cancer is a discrepancy between *in vitro* and *in vivo* γδT cell potency (Pauza et al., 2018). *In vitro*, γδT cell cytotoxicity can be achieved with effector:target ratios of <1, but results of human clinical trials show considerably more heterogeneity in objective responses to γδT immunotherapy (Pauza et al., 2018). Pre-clinical animal studies are hindered by the fact that Vδ2Vg9 phosphoantigen-reactive T cells are found only in humans and non-human primates (NHP). Studies in mouse models are therefore less informative and have more caveats than studies of other lymphocyte subsets. In the context of HIV infection, it will be critical to move studies into NHPs and SIV infection prior to human clinical trials.

2) *γδT cell infection by HIV*: Typically, both Vδ1 and Vδ2 cells lack expression of the HIV co-receptor CD4, seemingly rendering them refractory to direct infection. Concerningly, however, Soriano-Sarabia et al. (2015) reported that replication-competent HIV could be recovered from purified Vδ2 cells in 14 of 18 long-term ART recipients. Thus, the use of expanded autologous Vδ2 cells from HIV-infected patients may risk the reactivation and replication of a latent reservoir. This could be mitigated by the use of haploidentical or third-party Vδ2 cells from HIV-uninfected donors. Even in that scenario, though, consideration must be given to whether large numbers of adoptively transferred Vδ2 cells could be infected by HIV, since γδ immunotherapy would be reliant on the use of LRAs to reactivate the latent reservoir. Several reports now suggest that Vδ2 cells can transiently express CD4 during activation (Imlach et al., 2003; Soriano-Sarabia et al., 2015; Strbo et al., 2016) and can be productively infected with HIV (Wallace et al., 1997), which likely mediates the establishment of the latent reservoir detected by Soriano-Sarabia. For that reason, *in vitro* Vδ2 expansion and

adoptive transfer may be preferable compared to *in vivo* Vδ2 expansion using aminobisphosphonate drugs.

3) *Depletion of Vδ2 cells*: In contrast to Vδ1 cells, Vδ2 cells are depleted during HIV infection and do not fully recover following ART (as discussed previously). Most data suggest that Vδ2 cells expand *in vitro* to a similar extent regardless of the HIV status of the donor, but individuals who exhibit low Vδ2 frequencies *ex vivo* will produce fewer absolute numbers of Vδ2 cells after expansion (Garrido et al., 2018b). Furthermore, the observation that the ADCC activity of Vδ2 cells derived from ART-treated donors was reduced compared to uninfected donors (Poonia and Pauza, 2012) suggests that further attention needs to be paid to functional defects of Vδ2 cells in HIV-infected donors.

4) *Contribution to immune activation*: While the causes of Vδ1 expansion during HIV infection are not conclusively known, it is likely that microbial translocation and loss of epithelial barrier integrity in the gut mucosa drive activation and differentiation of this subset (Harris et al., 2010; Olson et al., 2018). Some studies have shown that Vδ1 cells exhibit proinflammatory cytokine production that correlates with CD8+ T cell activation (Olson et al., 2018) or killing of bystander CD4+ T cells (Sindhu et al., 2003) during HIV infection. Thorough assessment of the characteristics of expanded Vδ1 cells derived from HIV-infected donors will be required to determine the potential pathogenic impact of these cells if adoptively transferred.

CONCLUDING THOUGHTS AND FUTURE DIRECTIONS

Intense interest in γδT cell-based immunotherapy for cancer is continually driving innovative and novel approaches to harness γδT cell cytotoxicity, which should be considered in the context of HIV cure strategies. For example, despite substantial differences in antigen specificity, NK cell receptor expression, and tissue tropism between Vδ1 and Vδ2 cells, it may be more effective to non-specifically expand a diverse population of γδT cells than to focus on the development of pure Vδ1 or Vδ2 populations. Using aAPCs engineered to express a number of co-stimulatory molecules, Deniger et al. (2014) demonstrated the expansion of a mixed population of Vδ1, Vδ2, and Vδ1-Vδ2- cells that recognized tumor cells via NKG2D, DNAM-1, and the γδ TCR. In an *in vivo* murine model, this mixed population was more effective at eliminating ovarian cancer xenografts than any of the individual γδT subsets alone. Similar results were obtained with mixed γδT cells in a model of neuroblastoma, where Vδ2 cells mediated CD16-dependent ADCC and Vd1 cells exhibited direct cytotoxicity (Fisher et al., 2014). Given the utility of both ADCC-based and direct recognition of HIV-infected T cells in cure strategies, mixed γδT expansion may be a potent method by which to generate effector γδT cells. More complex approaches could also engineer such cells to express a CAR, providing an additional mechanism by which to recognize infected cells (Rotolo et al., 2019).

Regardless of the nature of the γδT cells chosen for study, the path forward for γδT immunotherapy in HIV cure is clear: First, incisive mechanistic studies are needed to establish the most efficient mechanisms of γδT recognition of reactivated HIV-infected target cells. Despite the intriguing results of Garrido et al. (2018b), the latency-clearing capacity of Vδ2 cells needs to be replicated and the mechanism of recognition defined. With this information, it may be possible to identify γδT cell subsets whose selective expansion would generate better effector cell populations compared to bulk expansion protocols. Second, *in vivo* animal studies using non-human primates will be critical to addressing questions of tissue targeting and assessment of *in vivo* potency. Reagents exist to characterize both Vδ1 and Vδ2 cells in NHPs, and studies confirm that these cells recapitulate many aspects of human immunology including expression of CD16 (Hodara et al., 2014), NKG2D, NKG2A, GzmB, and CD107a (Tuero et al., 2016), as well as the ability to kill SIV-infected cells (Malkovsky et al., 1992; Wallace et al., 1994). SIV-infected macaques also recapitulate important aspects of HIV-infected human cohorts, including inversion of the Vδ1:Vδ2 ratio and microbial translocation-induced expansion of Vδ1 cells (Harris et al., 2010). Critically, translational studies of immunity to *Mycobacterium tuberculosis* have demonstrated the feasibility of both expanding macaque Vδ2 cells *in vivo* (Ali et al., 2007) or adoptively transferring *in vitro* expanded cells (Qaqish et al., 2017). With these goals in mind, the application of γδT-based immunotherapy to HIV cure strategies represents an exciting and informative research avenue.

AUTHOR CONTRIBUTIONS

JJ and SK wrote and edited the manuscript.

FUNDING

JJ and SK were supported by NHMRC Fellowships (APP1123673 and APP1136322).

ACKNOWLEDGMENTS

The authors would like to acknowledge Wen Shi Lee and Kathleen Wragg for providing feedback on the manuscript.

REFERENCES

Aid, M., Dupuy, F. P., Moysi, E., Moir, S., Haddad, E. K., Estes, J. D., et al. (2018). Follicular CD4 T helper cells as a major HIV reservoir compartment: a molecular perspective. *Front. Immunol.* 9:895. doi: 10.3389/fimmu.2018.00895

Ali, Z., Shao, L., Halliday, L., Reichenberg, A., Hintz, M., Jomaa, H., et al. (2007). Prolonged (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate-driven antimicrobial and cytotoxic responses of pulmonary and systemic Vγ2Vδ2 T cells in macaques. *J. Immunol.* 179, 8287–8296. doi: 10.4049/jimmunol.179.12.8287

Almeida, A. R., Correia, D. V., Fernandes-Platzgummer, A., Da Silva, C. L., Da Silva, M. G., Anjos, D. R., et al. (2016). Δ one T cells for immunotherapy of chronic lymphocytic leukemia: clinical-grade expansion/differentiation and preclinical proof of concept.

Clin. Cancer Res. 22, 5795–5804. doi: 10.1158/1078-0432.CCR-16-0597

Angelini, D. F., Borsellino, G., Poupot, M., Diamantini, A., Poupot, R., Bernardi, G., et al. (2004). FcγRIII discriminates between 2 subsets of Vγ9Vδ2 effector cells with different responses and activation pathways. *Blood* 104, 1801–1807. doi: 10.1182/blood-2004-01-0331

Bachelet, T., Couzi, L., Pitard, V., Sicard, X., Rigothier, C., Lepreux, S., et al. (2014). Cytomegalovirus-responsive γδ T cells: novel effector cells in antibody-mediated kidney allograft microcirculation lesions. *J. Am. Soc. Nephrol.* 25, 2471–2482. doi: 10.1681/ASN.2013101052

Bansal, R. R., Mackay, C. R., Moser, B., and Eberl, M. (2012). IL-21 enhances the potential of human γδ T cells to provide B-cell help. *Eur. J. Immunol.* 42, 110–119. doi: 10.1002/eji.201142017

Bhatnagar, N., Girard, P. M., Lopez-Gonzalez, M., Didier, C., Collias, L., Jung, C., et al. (2017). Potential role of Vδ2(+) γδ T cells in regulation of immune activation in primary HIV infection. *Front. Immunol.* 8:1189. doi: 10.3389/fimmu.2017.01189

Boullier, S., Dadaglio, G., Lafeuillade, A., Debord, T., and Gougeon, M. L. (1997). V δ 1 T cells expanded in the blood throughout HIV infection display a cytotoxic activity and are primed for TNF-alpha and IFN-γ production but are not selected in lymph nodes. *J. Immunol.* 159, 3629–3637.

Brandes, M., Willimann, K., Lang, A. B., Nam, K. H., Jin, C., Brenner, M. B., et al. (2003). Flexible migration program regulates γ δ T-cell involvement in humoral immunity. *Blood* 102, 3693–3701. doi: 10.1182/blood-2003-04-1016

Bronnimann, M. P., Skinner, P. J., and Connick, E. (2018). The B-cell follicle in HIV infection: barrier to a cure. *Front. Immunol.* 9:20. doi: 10.3389/fimmu.2018.00020

Bruel, T., Guivel-Benhassine, F., Amraoui, S., Malbec, M., Richard, L., Bourdic, K., et al. (2016). Elimination of HIV-1-infected cells by broadly neutralizing antibodies. *Nat. Commun.* 7:10844. doi: 10.1038/ncomms10844

Caccamo, N., Battistini, L., Bonneville, M., Poccia, F., Fournie, J. J., Meraviglia, S., et al. (2006). CXCR5 identifies a subset of Vγ9Vδ2 T cells which secrete IL-4 and IL-10 and help B cells for antibody production. *J. Immunol.* 177, 5290–5295. doi: 10.4049/jimmunol.177.8.5290

Cantero-Perez, J., Grau-Exposito, J., Serra-Peinado, C., Rosero, D. A., Luque-Ballesteros, L., Astorga-Gamaza, A., et al. (2019). Resident memory T cells are a cellular reservoir for HIV in the cervical mucosa. *Nat. Commun.* 10:4739. doi: 10.1038/s41467-019-12732-2

Chevalier, M. F., Bhatnagar, N., Didier, C., Lopez-Gonzalez, M., Pavie, J., Bollens, D., et al. (2019). γδ T-cell subsets in HIV controllers: potential role of T γδ 17 cells in the regulation of chronic immune activation. *Aids* 33, 1283–1292. doi: 10.1097/QAD.0000000000002196

Cifaldi, L., Doria, M., Cotugno, N., Zicari, S., Cancrini, C., Palma, P., et al. (2019). DNAM-1 Activating receptor and its ligands: how do viruses affect the NK cell-mediated immune surveillance during the various phases of infection? *Int. J. Mol. Sci.* 20:3715. doi: 10.3390/ijms20153715

Cimini, E., Agrati, C., D'offizi, G., Vlassi, C., Casetti, R., Sacchi, A., et al. (2015). Primary and chronic HIV infection differently modulates mucosal Vδ1 and Vδ2 T-cells differentiation profile and effector functions. *PLoS ONE* 10:e0129771. doi: 10.1371/journal.pone.0129771

Couzi, L., Pitard, V., Sicard, X., Garrigue, I., Hawchar, O., Merville, P., et al. (2012). Antibody-dependent anti-cytomegalovirus activity of human γδ T cells expressing CD16 (FcγRIIIa). *Blood* 119, 1418–1427. doi: 10.1182/blood-2011-06-363655

Davey, M. S., Willcox, C. R., Hunter, S., Oo, Y. H., and Willcox, B. E. (2018). Vδ2(+) T cells-two subsets for the price of one. *Front. Immunol.* 9:2106. doi: 10.3389/fimmu.2018.02106

Davey, M. S., Willcox, C. R., Joyce, S. P., Ladell, K., Kasatskaya, S. A., Mclaren, J. E., et al. (2017). Clonal selection in the human Vδ1 T cell repertoire indicates γδ TCR-dependent adaptive immune surveillance. *Nat. Commun.* 8:14760. doi: 10.1038/ncomms14760

De Maria, A., Ferrazin, A., Ferrini, S., Ciccone, E., Terragna, A., and Moretta, L. (1992). Selective increase of a subset of T cell receptor γ δ T lymphocytes in the peripheral blood of patients with human immunodeficiency virus type 1 infection. *J. Infect. Dis.* 165, 917–919. doi: 10.1093/infdis/165.5.917

De Paoli, P., Gennari, D., Martelli, P., Basaglia, G., Crovatto, M., Battistin, S., et al. (1991). A subset of γ δ lymphocytes is increased during HIV-1 infection. *Clin. Exp. Immunol.* 83, 187–191. doi: 10.1111/j.1365-2249.1991.tb05612.x

Deng, K., Pertea, M., Rongvaux, A., Wang, L., Durand, C. M., Ghiaur, G., et al. (2015). Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations. *Nature* 517, 381–385. doi: 10.1038/nature14053

Deniger, D. C., Maiti, S. N., Mi, T., Switzer, K. C., Ramachandran, V., Hurton, L. V., et al. (2014). Activating and propagating polyclonal γ δ T cells with broad specificity for malignancies. *Clin. Cancer Res.* 20, 5708–5719. doi: 10.1158/1078-0432.CCR-13-3451

Enders, P. J., Yin, C., Martini, F., Evans, P. S., Propp, N., Poccia, F., et al. (2003). HIV-mediated γδ T cell depletion is specific for Vγ2+ cells expressing the Jγ1.2 segment. *AIDS Res. Hum. Retrovir.* 19, 21–29. doi: 10.1089/08892220360473934

Fausther-Bovendo, H., Wauquier, N., Cherfils-Vicini, J., Cremer, I., Debre, P., and Vieillard, V. (2008). NKG2C is a major triggering receptor involved in the V[δ]1 T cell-mediated cytotoxicity against HIV-infected CD4 T cells. *Aids* 22, 217–226. doi: 10.1097/QAD.0b013e3282f46e7c

Fehniger, T. A., Herbein, G., Yu, H., Para, M. I., Bernstein, Z. P., O'brien, W. A., et al. (1998). Natural killer cells from HIV-1+ patients produce C-C chemokines and inhibit HIV-1 infection. *J. Immunol.* 161, 6433–6438.

Fichtner, A. S., Karunakaran, M. M., Gu, S., Boughter, C. T., Borowska, M. T., Starick, L., et al. (2020). Alpaca (*Vicugna pacos*), the first nonprimate species with a phosphoantigen-reactive Vγ9Vδ2 T cell subset. *Proc. Natl. Acad. Sci. U.S.A.* 117, 6697–6707. doi: 10.1073/pnas.1909474117

Fisher, J. P., Yan, M., Heuijerjans, J., Carter, L., Abolhassani, A., Frosch, J., et al. (2014). Neuroblastoma killing properties of Vδ2 and Vδ2-negative γδT cells following expansion by artificial antigen-presenting cells. *Clin. Cancer Res.* 20, 5720–5732. doi: 10.1158/1078-0432.CCR-13-3464

Garrido, C., Abad-Fernandez, M., Tuyishime, M., Pollara, J. J., Ferrari, G., Soriano-Sarabia, N., et al. (2018a). Interleukin-15-stimulated natural killer cells clear HIV-1-infected cells following latency reversal *ex vivo*. *J. Virol.* 92:JVI.00235-18. doi: 10.1128/JVI.00235-18

Garrido, C., Clohosey, M. L., Whitworth, C. P., Hudgens, M., Margolis, D. M., and Soriano-Sarabia, N. (2018b). *Γ*δ T cells: an immunotherapeutic approach for HIV cure strategies. *JCI Insight* 3: e120121. doi: 10.1172/jci.insight.120121

Gianella, S., and Letendre, S. (2016). Cytomegalovirus and HIV: a Dangerous Pas de Deux. *J. Infect. Dis.* 214(Suppl. 2), S67–74. doi: 10.1093/infdis/jiw217

Godfrey, D. I., Le Nours, J., Andrews, D. M., Uldrich, A. P., and Rossjohn, J. (2018). Unconventional T cell targets for cancer immunotherapy. *Immunity* 48, 453–473. doi: 10.1016/j.immuni.2018.03.009

Groh, V., Steinle, A., Bauer, S., and Spies, T. (1998). Recognition of stress-induced MHC molecules by intestinal epithelial γδ T cells. *Science* 279, 1737–1740. doi: 10.1126/science.279.5357.1737

Gupta, R. K., Peppa, D., Hill, A. L., Galvez, C., Salgado, M., Pace, M., et al. (2020). Evidence for HIV-1 cure after CCR5Δ32/Δ32 allogeneic haemopoietic stem-cell transplantation 30 months post analytical treatment interruption: a case report. *Lancet HIV*. 7, e340–347. doi: 10.1016/S2352-3018(20)30069-2

Gutierrez-Arcelus, M., Teslovich, N., Mola, A. R., Polidoro, R. B., Nathan, A., Kim, H., et al. (2019). Lymphocyte innateness defined by transcriptional states reflects a balance between proliferation and effector functions. *Nat. Commun.* 10:687. doi: 10.1038/s41467-019-08604-4

Harrer, D. C., Simon, B., Fujii, S. I., Shimizu, K., Uslu, U., Schuler, G., et al. (2017). RNA-transfection of γ/δ T cells with a chimeric antigen receptor or an alpha/beta T-cell receptor: a safer alternative to genetically engineered alpha/beta T cells for the immunotherapy of melanoma. *BMC Cancer* 17:551. doi: 10.1186/s12885-017-3539-3

Harris, L. D., Klatt, N. R., Vinton, C., Briant, J. A., Tabb, B., Ladell, K., et al. (2010). Mechanisms underlying γδ T-cell subset perturbations in SIV-infected Asian rhesus macaques. *Blood* 116, 4148–4157. doi: 10.1182/blood-2010-05-283549

Hayday, A. C. (2000). [γ][δ] cells: a right time and a right place for a conserved third way of protection. *Annu. Rev. Immunol.* 18, 975–1026. doi: 10.1146/annurev.immunol.18.1.975

He, X., Liang, H., Hong, K., Li, H., Peng, H., Zhao, Y., et al. (2013). The potential role of CD16+ Vγ2Vδ2 T cell-mediated antibody-dependent cell-mediated cytotoxicity in control of HIV type 1 disease. *AIDS Res. Hum. Retrovir.* 29, 1562–1570. doi: 10.1089/aid.2013.0111

Hinz, T., Wesch, D., Friese, K., Reckziegel, A., Arden, B., and Kabelitz, D. (1994). T cell receptor γ δ repertoire in HIV-1-infected individuals. *Eur. J. Immunol.* 24, 3044–3049. doi: 10.1002/eji.1830241219

Hodara, V. L., Parodi, L. M., Chavez, D., Smith, L. M., Lanford, R., and Giavedoni, L. D. (2014). Characterization of γδT cells in naive and HIV-infected

chimpanzees and their responses to T-cell activators *in vitro*. *J. Med. Primatol.* 43, 258–271. doi: 10.1111/jmp.12115

Hutter, G., Nowak, D., Mossner, M., Ganepola, S., Mussig, A., Allers, K., et al. (2009). Long-term control of HIV by CCR5 Δ32/Δ32 stem-cell transplantation. *N Engl. J. Med.* 360, 692–698. doi: 10.1056/NEJMoa0802905

Hviid, L., Akanmori, B. D., Loizon, S., Kurtzhals, J. A., Ricke, C. H., Lim, A., et al. (2000). High frequency of circulating γ δ T cells with dominance of the v(δ)1 subset in a healthy population. *Int. Immunol.* 12, 797–805. doi: 10.1093/intimm/12.6.797

Hviid, L., Smith-Togobo, C., and Willcox, B. E. (2019). Human Vδ1(+) T cells in the immune response to plasmodium falciparum infection. *Front. Immunol.* 10:259. doi: 10.3389/fimmu.2019.00259

Imlach, S., Leen, C., Bell, J. E., and Simmonds, P. (2003). Phenotypic analysis of peripheral blood γδ T lymphocytes and their targeting by human immunodeficiency virus type 1 *in vivo*. *Virology* 305, 415–427. doi: 10.1006/viro.2002.1759

James, K. S., Trumble, I., Clohosey, M. L., Moeser, M., Roan, N. R., Adimora, A. A., et al. (2020). Measuring the contribution of γδ T cells to the persistent HIV reservoir. *Aids* 34, 363–371. doi: 10.1097/QAD.0000000000002434

Juno, J. A., and Eriksson, E. M. (2019). γδ T-cell responses during HIV infection and antiretroviral therapy. *Clin. Transl. Immunol.* 8:e01069. doi: 10.1002/cti2.1069

Kohler, S. L., Pham, M. N., Folkvord, J. M., Arends, T., Miller, S. M., Miles, B., et al. (2016). Germinal center T follicular helper cells are highly permissive to HIV-1 and alter their phenotype during virus replication. *J. Immunol.* 196, 2711–2722. doi: 10.4049/jimmunol.1502174

Le Nours, J., Gherardin, N. A., Ramarathinam, S. H., Awad, W., Wiede, F., Gully, B. S., et al. (2019). A class of γδ T cell receptors recognize the underside of the antigen-presenting molecule MR1. *Science* 366, 1522–1527. doi: 10.1126/science.aav3900

Lee, W. S., Kristensen, A. B., Rasmussen, T. A., Tolstrup, M., Ostergaard, L., Sogaard, O. S., et al. (2017). Anti-HIV-1 ADCC antibodies following latency reversal and treatment interruption. *J. Virol.* 91:e00603-17. doi: 10.1128/JVI.00603-17

Li, Z., Jiao, Y., Hu, Y., Cui, L., Chen, D., Wu, H., et al. (2015). Distortion of memory Vδ2 γδ T cells contributes to immune dysfunction in chronic HIV infection. *Cell Mol. Immunol.* 12, 604–614. doi: 10.1038/cmi.2014.77

Li, Z., Li, W., Li, N., Jiao, Y., Chen, D., Cui, L., et al. (2014). γδ T cells are involved in acute HIV infection and associated with AIDS progression. *PLoS ONE* 9:e106064. doi: 10.1371/journal.pone.0106064

Linette, G. P., Stadtmauer, E. A., Maus, M. V., Rapoport, A. P., Levine, B. L., Emery, L., et al. (2013). Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* 122, 863–871. doi: 10.1182/blood-2013-03-490565

Lo Presti, E., Pizzolato, G., Gulotta, E., Cocorullo, G., Gulotta, G., Dieli, F., et al. (2017). Current advances in γδ T cell-based tumor immunotherapy. *Front. Immunol.* 8:1401. doi: 10.3389/fimmu.2017.01401

Lundqvist, C., Baranov, V., Hammarstrom, S., Athlin, L., and Hammarstrom, M. L. (1995). Intra-epithelial lymphocytes. Evidence for regional specialization and extrathymic T cell maturation in the human gut epithelium. *Int. Immunol.* 7, 1473–1487. doi: 10.1093/intimm/7.9.1473

Luteijn, R., Sciaranghella, G., Van Lunzen, J., Nolting, A., Dugast, A. S., Ghebremichael, M. S., et al. (2011). Early viral replication in lymph nodes provides HIV with a means by which to escape NK-cell-mediated control. *Eur. J. Immunol.* 41, 2729–2740. doi: 10.1002/eji.201040886

Madhavi, V., Ana-Sosa-Batiz, F. E., Jegaskanda, S., Center, R. J., Winnall, W. R., Parsons, M. S., et al. (2015). Antibody-dependent effector functions against HIV decline in subjects receiving antiretroviral therapy. *J. Infect. Dis.* 211, 529–538. doi: 10.1093/infdis/jiu486

Madhavi, V., Kulkarni, A., Shete, A., Lee, W. S., Mclean, M. R., Kristensen, A. B., et al. (2017). Effect of combination antiretroviral therapy on HIV-1-specific antibody-dependent cellular cytotoxicity responses in subtype B- and subtype C-infected cohorts. *J. Acquir. Immune Defic. Syndr.* 75, 345–353. doi: 10.1097/QAI.0000000000001380

Malkovsky, M., Bartz, S. R., Mackenzie, D., Radtke, B. E., Wallace, M., Manning, J., et al. (1992). Are γ δ T cells important for the elimination of virus-infected cells? *J. Med. Primatol.* 21, 113–118.

Nakajima, H., Cella, M., Langen, H., Friedlein, A., and Colonna, M. (1999). Activating interactions in human NK cell recognition: the role of 2B4-CD48. *Eur. J. Immunol.* 29, 1676–1683. doi: 10.1002/(SICI)1521-4141(199905)29:05<1676::AID-IMMU1676>3.0.CO;2-Y

Nedellec, S., Sabourin, C., Bonneville, M., and Scotet, E. (2010). NKG2D costimulates human V gamma 9V delta 2 T cell antitumor cytotoxicity through protein kinase C theta-dependent modulation of early TCR-induced calcium and transduction signals. *J. Immunol.* 185, 55–63. doi: 10.4049/jimmunol.1000373

Oliva, A., Kinter, A. L., Vaccarezza, M., Rubbert, A., Catanzaro, A., Moir, S., et al. (1998). Natural killer cells from human immunodeficiency virus (HIV)-infected individuals are an important source of CC-chemokines and suppress HIV-1 entry and replication *in vitro*. *J. Clin. Invest.* 102, 223–231. doi: 10.1172/JCI2323

Olson, G. S., Moore, S. W., Richter, J. M., Garber, J. J., Bowman, B. A., Rawlings, C. A., et al. (2018). Increased frequency of systemic pro-inflammatory Vδ1(+) γδ T cells in HIV elite controllers correlates with gut viral load. *Sci. Rep.* 8:16471. doi: 10.1038/s41598-018-34576-4

Omi, K., Shimizu, M., Watanabe, E., Matsumura, J., Takaku, C., Shinya, E., et al. (2014). Inhibition of R5-tropic HIV type-1 replication in CD4(+) natural killer T cells by γδ T lymphocytes. *Immunology* 141, 596–608. doi: 10.1111/imm.12221

Pauza, C. D., and Cairo, C. (2015). Evolution and function of the TCR Vγ9 chain repertoire: it's good to be public. *Cell Immunol.* 296, 22–30. doi: 10.1016/j.cellimm.2015.02.010

Pauza, C. D., Liou, M. L., Lahusen, T., Xiao, L., Lapidus, R. G., Cairo, C., et al. (2018). *Γ* δ T cell therapy for cancer: it is good to be local. *Front Immunol* 9, 1305. doi: 10.3389/fimmu.2018.01305

Pitard, V., Roumanes, D., Lafarge, X., Couzi, L., Garrigue, I., Lafon, M. E., et al. (2008). Long-term expansion of effector/memory Vδ2-γδ T cells is a specific blood signature of CMV infection. *Blood* 112, 1317–1324. doi: 10.1182/blood-2008-01-136713

Pizzolato, G., Kaminski, H., Tosolini, M., Franchini, D. M., Pont, F., Martins, F., et al. (2019). Single-cell RNA sequencing unveils the shared and the distinct cytotoxic hallmarks of human TCRVδ1 and TCRVδ2 γδ T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* 116, 11906–11915. doi: 10.1073/pnas.1818488116

Poccia, F., Battistini, L., Cipriani, B., Mancino, G., Martini, F., Gougeon, M. L., et al. (1999). Phosphoantigen-reactive Vγ9Vδ2 T lymphocytes suppress *in vitro* human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines. *J. Infect. Dis.* 180, 858–861. doi: 10.1086/314925

Poccia, F., Boullier, S., Lecoeur, H., Cochet, M., Poquet, Y., Colizzi, V., et al. (1996). Peripheral V γ 9/V δ 2 T cell deletion and anergy to nonpeptidic mycobacterial antigens in asymptomatic HIV-1-infected persons. *J. Immunol.* 157, 449–461.

Poccia, F., Cipriani, B., Vendetti, S., Colizzi, V., Poquet, Y., Battistini, L., et al. (1997). CD94/NKG2 inhibitory receptor complex modulates both anti-viral and anti-tumoral responses of polyclonal phosphoantigen-reactive V gamma 9V delta 2 T lymphocytes. *J. Immunol.* 159, 6009–6017.

Poccia, F., Gioia, C., Martini, F., Sacchi, A., Piacentini, P., Tempestilli, M., et al. (2009). Zoledronic acid and interleukin-2 treatment improves immunocompetence in HIV-infected persons by activating Vgamma9Vδ2 T cells. *Aids* 23, 555–565. doi: 10.1097/QAD.0b013e3283244619

Poggi, A., Carosio, R., Fenoglio, D., Brenci, S., Murdaca, G., Setti, M., et al. (2004). Migration of V δ 1 and V δ 2 T cells in response to CXCR3 and CXCR4 ligands in healthy donors and HIV-1-infected patients: competition by HIV-1 Tat. *Blood* 103, 2205–2213. doi: 10.1182/blood-2003-08-2928elta

Poles, M. A., Barsoum, S., Yu, W., Yu, J., Sun, P., Daly, J., et al. (2003). Human immunodeficiency virus type 1 induces persistent changes in mucosal and blood γδ T cells despite suppressive therapy. *J. Virol.* 77, 10456–10467. doi: 10.1128/JVI.77.19.10456-10467.2003

Polito, V. A., Cristantielli, R., Weber, G., Del Bufalo, F., Belardinilli, T., Arnone, C. M., et al. (2019). Universal ready-to-use immunotherapeutic approach for the treatment of Cancer: expanded and activated polyclonal γδ memory T cells. *Front. Immunol.* 10:2717. doi: 10.3389/fimmu.2019.02717

Poonia, B., and Pauza, C. D. (2012). *Γ* δ T cells from HIV+ donors can be expanded *in vitro* by zoledronate/interleukin-2 to become cytotoxic

effectors for antibody-dependent cellular cytotoxicity. *Cytotherapy* 14, 173–181. doi: 10.3109/14653249.2011.623693

Provine, N. M., Binder, B., Fitzpatrick, M. E. B., Schuch, A., Garner, L. C., Williamson, K. D., et al. (2018). Unique and common features of innate-like human Vδ2(+) γδT cells and mucosal-associated invariant T cells. *Front. Immunol.* 9:756. doi: 10.3389/fimmu.2018.00756

Qaqish, A., Huang, D., Chen, C. Y., Zhang, Z., Wang, R., Li, S., et al. (2017). Adoptive transfer of phosphoantigen-specific γδ T cell subset attenuates mycobacterium tuberculosis infection in nonhuman primates. *J Immunol.* 198, 4753–4763. doi: 10.4049/jimmunol.1602019

Riedel, D. J., Sajadi, M. M., Armstrong, C. L., Cummings, J. S., Cairo, C., Redfield, R. R., et al. (2009). Natural viral suppressors of HIV-1 have a unique capacity to maintain γδ T cells. *Aids* 23, 1955–1964. doi: 10.1097/QAD.0b013e32832ff1ff

Rincon-Orozco, B., Kunzmann, V., Wrobel, P., Kabelitz, D., Steinle, A., and Herrmann, T. (2005). Activation of V gamma 9V delta 2 T cells by NKG2D. *J. Immunol.* 175, 2144–2151. doi: 10.4049/jimmunol.175.4.2144

Rossol, R., Dobmeyer, J. M., Dobmeyer, T. S., Klein, S. A., Rossol, S., Wesch, D., et al. (1998). Increase in Vδ1+ γδ T cells in the peripheral blood and bone marrow as a selective feature of HIV-1 but not other virus infections. *Br. J. Haematol.* 100, 728–734. doi: 10.1046/j.1365-2141.1998.00630.x

Rotolo, R., Leuci, V., Donini, C., Cykowska, A., Γitoni, L., Medico, G., et al. (2019). CAR-based strategies beyond T Lymphocytes: integrative opportunities for cancer adoptive immunotherapy. *Int. J. Mol. Sci.* 20:2839. doi: 10.3390/ijms20112839

Roy, S., Ly, D., Castro, C. D., Li, N. S., Hawk, A. J., Altman, J. D., et al. (2016). Molecular analysis of lipid-reactive Vδ1 γδ T cells identified by CD1c tetramers. *J. Immunol.* 196, 1933–1942. doi: 10.4049/jimmunol.1502202

Shan, L., Deng, K., Shroff, N. S., Durand, C. M., Rabi, S. A., Yang, H. C., et al. (2012). Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity* 36, 491–501. doi: 10.1016/j.immuni.2012.01.014

Silva-Santos, B., Mensurado, S., and Coffelt, S. B. (2019). γδ T cells: pleiotropic immune effectors with therapeutic potential in cancer. *Nat. Rev. Cancer* 19, 392–404. doi: 10.1038/s41568-019-0153-5

Simoes, A. E., di Lorenzo, B., and Silva-Santos, B. (2018). Molecular determinants of target cell recognition by human γδ T cells. *Front. Immunol.* 9:929. doi: 10.3389/fimmu.2018.00929

Sindhu, S. T., Ahmad, R., Morisset, R., Ahmad, A., and Menezes, J. (2003). Peripheral blood cytotoxic γδ T lymphocytes from patients with human immunodeficiency virus type 1 infection and AIDS lyse uninfected CD4+ T cells, and their cytocidal potential correlates with viral load. *J. Virol.* 77, 1848–1855. doi: 10.1128/JVI.77.3.1848-1855.2003

Smith, B. A., Gartner, S., Liu, Y., Perelson, A. S., Stilianakis, N. I., Keele, B. F., et al. (2001). Persistence of infectious HIV on follicular dendritic cells. *J. Immunol.* 166, 690–696. doi: 10.4049/jimmunol.166.1.690

Soriano-Sarabia, N., Archin, N. M., Bateson, R., Dahl, N. P., Crooks, A. M., Kuruc, J. D., et al. (2015). Peripheral Vγ9Vδ2 T cells are a novel reservoir of latent HIV infection. *PLoS Pathog.* 11:e1005201. doi: 10.1371/journal.ppat.1005201

Strbo, N., Alcaide, M. L., Romero, L., Bolivar, H., Jones, D., Podack, E. R., et al. (2016). Loss of intra-epithelial endocervical gamma delta (GD) 1 T cells in HIV-infected women. *Am. J. Reprod. Immunol.* 75, 134–145. doi: 10.1111/aji.12458

Tanaka, Y., Morita, C. T., Tanaka, Y., Nieves, E., Brenner, M. B., and Bloom, B. R. (1995). Natural and synthetic non-peptide antigens recognized by human γ δ T cells. *Nature* 375, 155–158. doi: 10.1038/375155a0

Triebel, F., Faure, F., Graziani, M., Jitsukawa, S., Lefranc, M. P., and Hercend, T. (1988). A unique V-J-C-rearranged gene encodes a γ protein expressed on the majority of CD3+ T cell receptor-alpha/beta- circulating lymphocytes. *J. Exp. Med.* 167, 694–699. doi: 10.1084/jem.167.2.694

Tuero, I., Venzon, D., and Robert-Guroff, M. (2016). Mucosal and systemic γδ+ T cells associated with control of simian immunodeficiency virus infection. *J. Immunol.* 197, 4686–4695. doi: 10.4049/jimmunol.1600579

Tyler, C. J., Doherty, D. G., Moser, B., and Eberl, M. (2015). Human Vγ9/Vδ2 T cells: Innate adaptors of the immune system. *Cell Immunol.* 296, 10–21. doi: 10.1016/j.cellimm.2015.01.008

Uldrich, A. P., Le Nours, J., Pellicci, D. G., Gherardin, N. A., Mcpherson, K. G., Lim, R. T., et al. (2013). CD1d-lipid antigen recognition by the γδ TCR. *Nat. Immunol.* 14, 1137–1145. doi: 10.1038/ni.2713

van Den Berg, J. H., Gomez-Eerland, R., van De Wiel, B., Hulshoff, L., Van Den Broek, D., Bins, A., et al. (2015). Case report of a fatal serious adverse event upon administration of T cells transduced With a MART-1-specific T-cell receptor. *Mol. Ther.* 23, 1541–1550. doi: 10.1038/mt.2015.60

van Der Heiden, M., Bjorkander, S., Rahman Qazi, K., Bittmann, J., Hell, L., Jenmalm, M. C., et al. (2020). Characterization of the γδ T-cell compartment during infancy reveals clear differences between the early neonatal period and 2 years of age. *Immunol. Cell Biol.* 98, 79–87. doi: 10.1111/imcb.12303

Wallace, M., Bartz, S. R., Chang, W. L., Mackenzie, D. A., Pauza, C. D., and Malkovsky, M. (1996). Γ δ T lymphocyte responses to HIV. *Clin. Exp. Immunol.* 103, 177–184. doi: 10.1046/j.1365-2249.1996.d01-625.x

Wallace, M., Gan, Y. H., Pauza, C. D., and Malkovsky, M. (1994). Antiviral activity of primate gamma delta T lymphocytes isolated by magnetic cell sorting. *J. Med. Primatol.* 23, 131–135. doi: 10.1111/j.1600-0684.1994.tb00113.x

Wallace, M., Scharko, A. M., Pauza, C. D., Fisch, P., Imaoka, K., Kawabata, S., et al. (1997). Functional γ δ T-lymphocyte defect associated with human immunodeficiency virus infections. *Mol Med.* 3, 60–71. doi: 10.1007/BF03401668

Ward, J., Bonaparte, M., Sacks, J., Guterman, J., Fogli, M., Mavilio, D., et al. (2007). HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T-cell blasts. *Blood* 110, 1207–1214. doi: 10.1182/blood-2006-06-028175

Wesch, D., Hinz, T., and Kabelitz, D. (1998). Analysis of the TCR Vγ repertoire in healthy donors and HIV-1-infected individuals. *Int. Immunol.* 10, 1067–1075. doi: 10.1093/intimm/10.8.1067

Wilhelm, M., Smetak, M., Schaefer-Eckart, K., Kimmel, B., Birkmann, J., Einsele, H., et al. (2014). Successful adoptive transfer and *in vivo* expansion of haploidentical γδ T cells. *J. Transl. Med.* 12:45. doi: 10.1186/1479-5876-12-45

Willcox, B. E., and Willcox, C. R. (2019). γδ TCR ligands: the quest to solve a 500-million-year-old mystery. *Nat. Immunol.* 20, 121–128. doi: 10.1038/s41590-018-0304-y

Wong, J. K., and Yukl, S. A. (2016). Tissue reservoirs of HIV. *Curr. Opin. HIV AIDS* 11, 362–370. doi: 10.1097/COH.0000000000000293

Wrobel, P., Shojaei, H., Schittek, B., Gieseler, F., Wollenberg, B., Kalthoff, H., et al. (2007). Lysis of a broad range of epithelial tumour cells by human γ δ T cells: involvement of NKG2D ligands and T-cell receptor- versus NKG2D-dependent recognition. *Scand. J. Immunol.* 66, 320–328. doi: 10.1111/j.1365-3083.2007.01963.x

Wu, D., Wu, P., Wu, X., Ye, J., Wang, Z., Zhao, S., et al. (2015). *Ex vivo* expanded human circulating Vδ1 γδT cells exhibit favorable therapeutic potential for colon cancer. *Oncoimmunology* 4:e992749. doi: 10.4161/2162402X.2014.992749

Zhang, T., He, X., Tsang, T. C., and Harris, D. T. (2004). Transgenic TCR expression: comparison of single chain with full-length receptor constructs for T-cell function. *Cancer Gene. Ther.* 11, 487–496. doi: 10.1038/sj.cgt.7700703

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 26 May 2020
doi: 10.3389/fcimb.2020.00249

Plasmacytoid Dendritic Cells as Cell-Based Therapeutics: A Novel Immunotherapy to Treat Human Immunodeficiency Virus Infection?

Renée M. van der Sluis[1,2], *Johanne H. Egedal*[2] *and Martin R. Jakobsen*[2]*

[1] *Aarhus Institute of Advanced Studies, Aarhus University, Aarhus, Denmark,* [2] *Department of Biomedicine, Aarhus University, Aarhus, Denmark*

Dendritic cells (DCs) play a critical role in mediating innate and adaptive immune responses. Since their discovery in the late 1970's, DCs have been recognized as the most potent antigen-presenting cells (APCs). DCs have a superior capacity for acquiring, processing, and presenting antigens to T cells and they express costimulatory or coinhibitory molecules that determine immune activation or anergy. For these reasons, cell-based therapeutic approaches using DCs have been explored in cancer and infectious diseases but with limited success. In humans, DCs are divided into heterogeneous subsets with distinct characteristics. Two major subsets are $CD11c^+$ myeloid (m)DCs and $CD11c^-$ plasmacytoid (p)DCs. pDCs are different from mDCs and play an essential role in the innate immune system via the production of type I interferons (IFN). However, pDCs are also able to take-up antigens and effectively cross present them. Given the rarity of pDCs in blood and technical difficulties in obtaining them from human blood samples, the understanding of human pDC biology and their potential in immunotherapeutic approaches (e.g. cell-based vaccines) is limited. However, due to the recent advancements in cell culturing systems that allow for the generation of functional pDCs from $CD34^+$ hematopoietic stem and progenitor cells (HSPC), studying pDCs has become easier. In this mini-review, we hypothesize about the use of pDCs as a cell-based therapy to treat HIV by enhancing anti-HIV-immune responses of the adaptive immune system and enhancing the anti-viral responses of the innate immune system. Additionally, we discuss obstacles to overcome before this approach becomes clinically applicable.

Keywords: HIV, HIV latency, dendritic cells, DC vaccine, plasmacytoid DC, pDC, CD8+ T cells, NK cells

OPEN ACCESS

Edited by:
Heather Shannon Smallwood, The University of Tennessee Health Sciences Center, United States

Reviewed by:
Victor Hugo Aquino, University of São Paulo, Brazil
Damien Ferhadian, National Institutes of Health (NIH), United States
Christopher Lupfer, Missouri State University, United States

***Correspondence:**
Martin R. Jakobsen
mrj@biomed.au.dk

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *09 February 2020*
Accepted: *29 April 2020*
Published: *26 May 2020*

Citation:
van der Sluis RM, Egedal JH and Jakobsen MR (2020) Plasmacytoid Dendritic Cells as Cell-Based Therapeutics: A Novel Immunotherapy to Treat Human Immunodeficiency Virus Infection? Front. Cell. Infect. Microbiol. 10:249. doi: 10.3389/fcimb.2020.00249

INTRODUCTION

Antiretroviral therapy (ART) successfully suppresses human immunodeficiency virus (HIV) replication but it does not cure an individual from having the infection (Finzi et al., 1997). The major barrier to clearing the virus is the persistence of a latent reservoir in long-lived resting and proliferating memory $CD4^+$ T cells (Chun et al., 1997; Wong et al., 1997; Hosmane et al., 2017). Different molecular mechanisms have been described that contribute to HIV latency, reviewed in Dahabieh et al. (2015) and Vanhamel et al. (2019). Accordingly, different strategies that aim at HIV clearance are investigated, reviewed in Margolis et al. (2020). One strategy is to induce HIV

expression, combined with enhanced immune function to clear infected cells. Here, we hypothesize if therapeutic vaccination with pDCs can enhance immune function to clear HIV infected cells.

Plasmacytoid DCs: A Small but Significant Cell Population

Dendritic cells (DCs) play a pivotal role in mediating innate and adaptive immune responses by various cellular mechanisms. In humans, DCs are categorized into heterogeneous subsets with distinct characteristics. Two major subsets are $CD11c^+$ myeloid (m)DCs and $CD11c^-$ plasmacytoid (p)DCs. Although both subsets are classified as DCs, they are in fact very different cell types. Initially, the primary role of pDCs was defined as type I Interferon (IFN)-producing cells in response to a viral infection (Cella et al., 1999; Siegal et al., 1999). PDCs were described as a cell type that effectively senses viral RNA or DNA via toll-like receptors (TLRs) 7 and 9 and subsequently produce vast amounts of type I and type III IFNs (Jarrossay et al., 2001; Kadowaki et al., 2001; Yin et al., 2012). However, the function of pDCs is more diverse, and amongst other functions, pDCs have been described to serve as antigen presenting cells (APC) that stimulate $CD8^+$ and $CD4^+$ T cells, or become "killer-pDCs" with cytotoxic properties, reviewed in Swiecki and Colonna (2015), Musumeci et al. (2019), and Reizis (2019).

DC-based vaccination has been of interest in the fields of oncology and infectious diseases for two decades. It is safe with few adverse effects but despite inducing favorable immune responses in preclinical studies, DC-based immunotherapies have not successfully induced significant clinical responses, reviewed in Mastelic-Gavillet et al. (2019) and da Silva et al. (2018). Because pDCs are key mediators for both innate and adaptive immune cells, interest in using pDCs for cell-based therapeutics is increasing. In this mini-review, we will discuss if therapeutic vaccination with pDCs could be beneficial for the treatment of HIV infection and focus on literature describing observations with pDCs. As the available studies are based on cells from several different species, we will try to simplify it by primarily referring to observations with human pDCs where possible. In doing so, we aim to answer the question: Can therapeutic vaccination with pDCs achieve a functional cure for HIV?

pDC Vaccination in Humans

To this date, only two studies have tested peripheral blood-derived pDCs as cell-based cancer therapy. In the first trial, Tel et al., explored pDCs activated with inactivated Frühsommer-Meningoenzephalitis (FSME) and loaded with tumor antigen-associated peptides *ex vivo*, followed by administration to patients with metastatic melanoma (Tel et al., 2013a). The pDCs distributed over multiple lymph nodes (LNs), mounted both $CD4^+$ and $CD8^+$ T cell responses, and an IFN response was observed after each vaccination. This study showed that vaccination with pDCs was safe and induced favorable immune responses. The second study by Westdorp et al., investigated patients with chemo-naïve castration-resistant prostate cancer receiving vaccinations with mature $CD1c^+$ mDCs, pDCs or a combination of the two, that were stimulated with protamine/RNA and loaded with tumor-associated antigens (Westdorp et al., 2019). The immunotherapy was feasible, well-tolerated with low-grade toxicity and induced functional antigen-specific T cells, which correlated with improved progression-free survival. However, no difference in T cell functionality was observed between the administration of mDCs, pDCs, or mDCs plus pDCs. This strategy is currently under evaluation in a phase I/II clinical trial for stage III melanoma patients (clinicaltrials.gov Identifier: NCT02574377). Additionally, the combined administration of mDCs and pDCs in metastatic endometrial cancer patients is under evaluation (NCT04212377). See **Table 1** for an overview of the clinical trials.

A limiting factor in using peripheral blood-derived pDCs is the number of pDCs available for vaccination. Only a small fraction of peripheral blood mononuclear cells (PBMCs) consists of pDCs (<1%). In the two before-mentioned clinical trials, leukapheresis was used to obtain pDCs and a maximum of three million pDCs could be administered per infusion, which was repeated on three occasions (Tel et al., 2013a; Westdorp et al., 2019). Overcoming the limitation in pDC numbers would greatly improve the application of pDCs as cell-based therapy. One way to get around this is to use an allogeneic pDC cell line. Allogeneic HLA-A*02:01 pDCs can induce melanoma antigen-specific and functional cytotoxic T cell responses *ex vivo* and have been shown to inhibit tumor growth in a humanized mouse model (Aspord et al., 2010, 2012). The safety and tolerability of using the irradiated HLA-A*02:01 pDC cell line loaded with four melanoma peptides (GeniusVac-Mel4) is currently under evaluation in a phase I clinical trial (NCT01863108). Similarly, a pDC cell line (PDC*lung01, PDC*line Pharma) is currently in a phase I/II study for the treatment of non-small-cell lung cancer (NCT03970746). However, the allogeneic pDC vaccine approach has some challenges; it is restricted to HLA-A2 patients and irradiation of the cells impairs the possibility to initiate an innate immune response via the secretion of IFN.

One possibility to obtain a continuous source of pDCs applicable for vaccination is to generate them *ex vivo* from hematopoietic stem cells. Cord blood $CD34^+$ hematopoietic stem and progenitor cells (HSPC) have been shown to be suitable for the differentiation into functional pDCs *in vitro* (Blom et al., 2000; Chen et al., 2004; Olivier et al., 2006; Demoulin et al., 2012; Thordardottir et al., 2014) and can yield clinically relevant cell numbers: up to 81 (±20) pDCs per single HSPC (Laustsen et al., 2018). $CD34^+$ stem cells can also be isolated from peripheral blood after mobilization with G-CSF and the generated pDCs can induce Ag-specific activation of autologous $CD8^+$ memory T cells *ex vivo* (Thordardottir et al., 2017). Although using autologous stem cell-derived pDCs for vaccination is a promising avenue for personalized pDC therapeutics, the HSPC differentiation into pDCs still requires long-term culturing, implying that the field still needs to make several advancements before it has clinical potential.

pDCs as Therapeutic Vaccine for the Treatment of Infectious Diseases

As of today, there are two reports that describe the use of pDCs as therapeutic vaccine for the treatment of an infectious disease. In the first study, the HLA-A*02:01 pDC line was used for the

TABLE 1 | Overview of plasmacytoid dendritic cell vaccination in clinical trials and animal models.

	Pathology	Status	General description	Intervention and method	Outcome measures	Results
NCT01690377 (Tel et al., 2013a)	Melanoma (stage IV)	Phase I Completed (November 2014)	**Participants**: 15 melanoma patients **Type of cells used**: Naturally occurring, autologous pDCs. The maximum feasible dose was 3×10^6 pDCs per injection, which was given to 12/15 patients. **Primary objective**: Generate mature pDCs for a vaccine and determine a safe and effective dosage of pDCs to initiate anti-tumor T cell responses in patients with stage IV melanoma.	**Intervention**: pDCs were activated with inactivated FSME vaccination and loaded with HLA-A2.1-binding tumor antigen-associated peptides (gp100 and tyrosinase) *ex vivo* and administered through intranodal injections. **Vaccination scheme**: Vaccine consists of three intranodal injections given once every 2 weeks. If well-tolerated and no disease progression, then patients were eligible for a maximum of two maintenance cycles of 3 biweekly vaccinations—each with a 6-months interval. **DTH***: DTH challenge following every vaccine administration.	**Primary endpoint**: Intervention-related toxicity. **Secondary endpoint**: Immunological response.	**Safety**: Vaccine is safe and well tolerated with no signs of severe toxicity. **Immunological effect**: pDCs migrate *in vivo*. Vaccination induces a temporal systemic induction of type I IFNs and activation of T cells responses. **PFS/OS****: Median OS improved compared with matched controls. PFS was also increased in the vaccine group, however this was not significant.
NCT02692976 (Westdorp et al., 2019)	Prostate cancer (castration resistant, CRPC)	Phase II Completed (March 2019)	**Participants**: 21 chemo-naïve CRPC patients. **Type of cells used**: Naturally occurring, autologous mDCs, pDCs or a combination of mDCs plus pDCs. Vaccinations had a dose of $1–8 \times 10^6$ cells for injection. **Primary objective**: To show immunologic efficacy of tumor-peptide loaded natural DC in metastatic castration-resistant prostate cancer patients (mCRPC).	**Intervention**: The DCs were stimulated with protamine/mRNA and loaded with tumor-associated antigens. Patients received maximally nine vaccinations. **Vaccination scheme**: Patients were randomly assigned 1:1:1 to receive one of the three vaccination arms. One cycle of vaccination consisted of 3 biweekly vaccinations administered intranodally in a clinically tumor-free lymph node. 1. Intranodal mDC vaccination ($2–5 \times 10^6$ cells) 2. Intranodal pDC vaccination ($1–3 \times 10^6$ cells) 3. Intranodal mDC/pDC vaccination ($3–8 \times 10^6$ cells) **DTH***: DTH challenge performed 1–2 weeks after the third vaccination.	**Primary endpoint**: Immunological response by monitoring: 1. Functional response ($IFNy^+$) and tetramer (dm^+) analysis of DTH infiltrating lymphocytes against tumor peptides. 2. Type I IFN gene expression in PBMCs. 3. Proliferative, effector cytokine-, and humoral responses to KLH****. **Secondary endpoint**: Safety, feasibility and efficacy of natural DC vaccinations, and the quality of life during treatment.	**Safety**: Vaccinations were well-tolerated with grade 1–2 toxicity (CTCAE***). **Immunological effect**: The vaccination enhanced dm^+ and $IFNy^+$ antigen specific T cells. **PFS/OS****: The overall median radiological PFS was 9.5 months and highest in patients with dm^+ and $IFNy^+$ antigen specific T cells.
NCT04212377	Endometrial cancer	Phase II Recruiting (Estimated completion February 2022)	**Participants**: Estimated enrollment is 8 patients. **Type of cells used**: Naturally occurring, autologous pDCs and mDCs loaded with tumor lysate and MUC1 and surviving PepTivators. **Primary objective**: To investigate the hypothesis that pDCs and mDCs are optimal for vaccines to combat cancer. To investigate the hypothesis that the combination of mDC and pDC may induce stronger anti-tumor immune responses as compared to pDC or mDC alone, or moDC.	**Intervention**: Metastatic endometrial cancer (mEC) patients who receive Carboplatin/Paclitaxel chemotherapy will get the vaccine. **Vaccination scheme**: The chemotherapy is given in a weekly schedule (weeks 1–3 and 5–7), and the patients will receive the vaccine by intranodal injection in week 8. **DTH***:	**Primary endpoint**: The anti-tumor response in the mEC patients evaluated by type I IFN expression, response to KLH (proliferative, effector cytokine, and humoral responses) **Secondary endpoint**: Safety by number of participants with adverse effects and change from baseline in pain scores.	**Safety**: **Immunological effect**: **PFS/OS****:

(Continued)

TABLE 1 | Continued

	Pathology	Status	General description	Intervention and method	Outcome measures	Results
NCT02574377	Melanoma (stage III)	Phase I/II Unknown recruitment status (Estimated completion December 2019)	**Participants**: Estimated enrollment is 30 patients. **Type of cells used**: Naturally occurring, autologous mDCs and pDCs, or combined, loaded with. tumor peptides. **Primary objective**: To the immunogenicity of combined adjuvant mDC and pDC vaccination vs. adjuvant mDC or pDC vaccination alone in stage III melanoma patients.	**Intervention**: 2 biweekly vaccinations of intranodal injections with pDC, mDC or combination of pDC and mDC. **Vaccination scheme**: Three-arm study, where stage III melanoma patients will receive: 1. A: pDC ($n = 10$), 2. B: mDC ($n = 10$), 3 C: Combined pDC/mDC ($n = 10$). If patients remain disease free, the cycle will be repeated up to three times with a 6 months interval. **DTH***: A challenge with peptide loaded blood DC is performed from which biopsies are taken for T cell analysis.	**Primary endpoint**: Immune response evaluated by type I IFN expression, response to KLH (proliferative, effector cytokine and humoral responses) and functional response and tetramer analysis of DTH infiltrating T cells against tumor peptides. **Secondary endpoint**: Distribution of mDC and pDC in the lymph node, toxicity, quality of life, PFS, and OS.	**Safety**: **Immunological effect**: **PFS/OS****:
NCT03970746	Non-Small Cell Lung Cancer (NSCLC)	Phase I/II Recruiting (Estimated completion August 2022)	**Participants**: Estimated enrollment is 66 patients. **Type of cells used**: Vaccine is based on the pDC cell line HLA-A*02:01 that is lethally irradiated and then pulsed with peptides from target tumor antigens. **Primary objective**: To assess the tolerability, the immunogenicity and the preliminary clinical activity of the therapeutic cancer vaccine, PDC*lung01, either alone or associated with anti-PD-1 treatment in patients with NSCLC.	**Intervention**: Administration of the vaccine either alone (cohort A) or together with Pembrolizumab (anti-PD-1, cohort B). **Vaccination scheme**: Four-arm vaccination study, consisting of: 1. A1: Low dose PDC*lung01 as single agent or during maintenance treatment by Pemetrexed (chemotherapy). 2. A2: High dose PDC*lung01 as single agent or during maintenance treatment by Pemetrexed (chemotherapy). 3. B1: Low dose PDC*lung01 added to Pembrolizumab. 4. B2: High dose PDC*lung01 added to Pembrolizumab. Cohort A1/2 will receive vaccine at six visits; subcutaneously followed by intravenous route. Cohort B1/2 will receive first vaccine injection 48 h after first infusion of anti-PD-1. The fourth vaccine injection will occur within 48 h after the second infusion of Pembrolizumab. **DTH***:	**Primary endpoint**: Occurrence of dose-limiting toxicity related to administration of the vaccine. **Secondary endpoint**: Evaluation of adverse events induced by the vaccination. Evaluation of the cellular and humoral immunological response to the vaccine.	**Safety**: **Immunological effect**: **PFS/OS****:

(Continued)

TABLE 1 | Continued

References	Infectious disease	Animal model	General description	Experimental design and method	Measurements	Results
Martinet et al. (2012)	Hepatitis B Virus (HBV)	NOD/SCID $\beta 2m^{-/-}$ mice	**Type of cells used**: Vaccine is based on the human pDC cell line HLA-A*02:01 that is lethally irradiated and then pulsed with HBV peptides or control peptides.	**Design:** mice are reconstituted with 50×10^6 PBMCs from a resolved HBV patient and xenotransplanted with 25×10^6 human HBV-transfected or untransfected hepatocytes. **Vaccination scheme:** Mice were treated with pDCs 3 days before or 3 days after challenge with hepatocytes. Mice received 5×10^6 pDCs per vaccination injected at the peritoneal lavage. One injection per week, two injections in total.	**T cells:** After two vaccination, HBV-specific T cells were analyzed via tetramer labeling at the site of injection, draining lymph node, spleen and blood. **Hepatocytes**: size was measured every 2–3 days **Viral load**: measured in serum	Vaccination before and after hepatocyte challenge amplified HBV-specific T cells, inhibited expansion of transfected hepatocytes, and reduced systemic viral load.
Remer et al. (2007)	*Leishmania major* (*L. major*)	BALB/c mice	**Type of cells used**: Splenic murine pDCs pulsed with *L. major* lysates or no lysate	**Design:** 1 week or 4 weeks after pDC vaccination, mice are challenged with *L. major* intradermal in the footpad. Five weeks after challenge, splenic T cells from protected mice are transferred to naïve mice that were then challenged. **Vaccination scheme:** single vaccination in the tail vein of 5×10^4 pDCs.	**Footpad:** swelling, inflammation, ulceration **Parasites:** Parasitic burden in footpad 6 weeks after challenge **Cytokine profile**: LN and spleen cells 6 weeks after challenge (IFNγ, IL-4, IL-10) **IgG subclass profile:** *L. major* specific IgG1 and IgG2a antibodies 5 weeks after challenge.	A single vaccination and adoptive T cell transfer of vaccinated mice onto naïve mice protected against *L. major* infection. Protection was not accompanied by a Th1 cytokine profile but protected animals had lower ratios of IgG1 to IgG2a in sera.

**Delayed-type hypersensitivity challenge.*

***Progression free survival/Overall survival.*

****Common terminology criteria for adverse events.*

*****KLH, Keyhole Limpet Hemocyanin (a protein providing T cell help).*

treatment of Hepatitis B Virus (HBV) (Martinet et al., 2012). Immunodeficient NOD/SCID $\beta 2m^{-/-}$ mice, reconstituted with HBV patient's PBMCs and xenotransplanted with human HBV-transfected hepatocytes, received two vaccinations of irradiated HBV-peptide pulsed pDCs per treatment. Vaccination elicited HBV-specific T cells that were able to lyse the transfected hepatocytes and reduce systemic viral load. In the second study, pDCs were used to vaccinate BALB/c mice to provide protection against the parasitic infection *Leishmania major* (*L. major*) (Remer et al., 2007). Mice received a single dose of splenic murine pDCs that were pulsed with *L. major* lysate. Vaccination provided complete protection when mice were challenged 1 or 4 weeks after vaccination. Additionally, adoptive T cell transfer of protected mice onto naïve susceptible mice provided complete protection to *L. major* challenge.

These studies show that pDC vaccination can provide protection against two different infectious diseases in murine models.

HIV Infection Negatively Impacts pDC Numbers and Functionality

Similar to the decline in $CD4^+$ T cell counts following acute HIV infection, a decline in circulating pDCs can be observed. Upon initiation of antiretroviral therapy (ART) the pDC numbers can increase but are not fully restored (Donaghy et al., 2001; Chehimi et al., 2002; Barron et al., 2003; Finke et al., 2004; Almeida et al., 2005; Kamga et al., 2005; Lichtner et al., 2008; Centlivre et al., 2011; Boichuk et al., 2015; Marquez-Coello et al., 2019). Additionally, pDCs that remain in circulation seem to be dysfunctional (further discussed below).

The reason for the decline in pDC numbers is not fully understood. PDCs express CD4, CCR5 and CXCR4, which theoretically makes them susceptible to infection with HIV. When isolated from peripheral blood or the thymus, pDCs can indeed be infected with both X4 and R5-tropic HIV *in vitro* (Patterson et al., 2001; Fong et al., 2002; Yonezawa et al., 2003; Lore et al., 2005; Smed-Sorensen et al., 2005; Evans et al., 2011). Whether this occurs *in vivo* is not clear, as conflicting observations have been reported. HIV DNA has been detected in pDCs isolated from people living with HIV (PLWH) and pDCs from the tonsil have been found to be positive for the CA-p24 protein (Fong et al., 2002; Donaghy et al., 2003; Centlivre et al., 2011). In contrast, others have not been able to demonstrate the presence of HIV RNA or DNA in peripheral blood pDCs, although this has been explored in samples from PLWH on suppressive ART (Otero et al., 2003) whereas in the other studies the PLWH were therapy naïve.

Yet, the infection of pDCs by HIV seems counterintuitive since they are the major producers of type I IFNs, and IFNs are well-known to have a potent anti-viral effect by increasing the expression of virus restriction and inhibition factors (Kluge et al., 2015; Colomer-Lluch et al., 2018). However, the HIV-induced IFN production by pDCs is delayed and is up to 20-fold lower than IFN production induced by Influenza, Sendai, and HIV-2 (Lo et al., 2012). Furthermore, pDCs do express the restriction factors SAMHD1 (Bloch et al., 2014), tetherin/CD317 (Tavano et al., 2013), and APOBEC3G (Wang et al., 2008) but, similar to other cell types, the expression of these is enhanced by IFN signaling. Thus, it remains a possibility that pDCs can be infected with HIV prior to this delay in IFN production and subsequent delay in viral restriction factor expression. PDC depletion may also occur in people living with HIV-2 in the absence of detectable viremia (Cavaleiro et al., 2009). This indicates that mechanisms other than direct viral infection determine the pDC depletion during persistent infections. A second potential mechanism is that type I IFN negatively controls pDC cell numbers as this has been observed using mice. Viral infection induced the upregulation of pro-apoptotic molecules in pDCs in a type I IFN-dependent manner, resulting in caspase activation and subsequent pDC death (Swiecki et al., 2011). A third possibility to explain the decrease in pDC cell numbers in the periphery is that pDCs no longer circulate. HIV-activated pDCs enhance expression of the lymphoid homing receptor CCR7 and during progressive infection or in the absence of ART, they have been reported to accumulate in LNs and lymphoid tonsillar tissue (Fonteneau et al., 2004; Schmidt et al., 2005; Herbeuval et al., 2006; Lehmann et al., 2010). Another study reported that in the presence of ART, pDCs also home to the LNs but compared to people living without HIV, the pDC numbers in LNs were significantly reduced (Biancotto et al., 2007). This indicates that both circulatory and LN pDC numbers decline, but that the decrease of pDCs in circulation is not necessarily due to homing to lymphoid tissue.

Besides reduced cell numbers, pDCs are also dysfunctional in PLWH and persistently produce low levels of IFNs (O'Brien et al., 2011). During ART, the TLR7/8 response appears to remain intact (Chang et al., 2012; Bam et al., 2017; Tsai et al., 2017) but IFN production upon TLR9 signaling is reduced, potentially due to continuous HIV-induced activation of pDCs *in vivo* or the interaction of CD40 with CD40L (Feldman et al., 2001; Tilton et al., 2008; Cavaleiro et al., 2009; Donhauser et al., 2012). Additionally, pDCs in the blood of PLWH display an exhausted phenotype and this may interfere with TLR signaling and subsequent IFN production (Cavaleiro et al., 2009; Schwartz et al., 2017; Font-Haro et al., 2018). However, administration of the TLR9 agonist MGN1703 to PLWH on ART enhances pDC activation, as measured by activation markers CD86 and CD40. Plasma IFNα-2a levels increased as well, indicating that some TLR9 responsiveness remains or that other cells may produce IFN in response to MGN1703 (Vibholm et al., 2017).

Would There Be Benefit in Restoring pDC Cell Numbers With a Functional IFN Response?

There are observations that indicate a beneficial role of maintaining pDC numbers during HIV infection. First, PLWH on ART with a low baseline pDC blood count are more likely to have an increase of HIV-RNA compared to individuals with high pDC counts during a 30 months follow-up (Lichtner et al., 2008). Second, elite controllers and long-term non-progressors (LTNPs) have preserved pDC counts and functionality (Almeida

et al., 2005; Machmach et al., 2012). It is unclear whether the pDCs from elite controllers are better equipped to suppress viral replication or if the pDC cell numbers are maintained because the viral load is suppressed by other immune cells, like CTLs or natural killer (NK) cells. Third, expansion of pDCs during acute infection delays onset of viremia and reduces HIV replication in humanized mice (Pham et al., 2019).

pDCs Can Potentially Control HIV via Three Mechanisms

PDCs can contribute to suppression of virus replication by fulfilling three different functions: (i) as a professional IFN-producing cell; (ii) as a professional APC and; (iii) as a "killer-pDC" with cytotoxic properties (**Figure 1**). Each mechanism is discussed individually below.

Controlling HIV as a Professional IFN-Producing Cell

PDCs produce abundant type I IFNs (Kadowaki et al., 2000) and type III IFNs (Yin et al., 2012) when sensing viral products such as single stranded RNA by TLR7 or unmethylated DNA molecules by TLR9, reviewed in Swiecki and Colonna (2015). In the context of HIV, pDCs produce type I IFNs in response to cell-free HIV and to HIV infected cells; both require endocytosis and likely initiate the TLR7 signaling pathway (Beignon et al., 2005; Schmidt et al., 2005). IFNs function as the first line of defense against viruses because they have a broad antiviral effect. IFNs can induce inflammation, activate specific immune cells including NK cells, $CD8^+$ T cells, and macrophages, and prime antigen-specific responses, reviewed in Hoffmann et al. (2015) and Kotenko and Durbin (2017). IFNs can also directly inhibit HIV replication by enhancing the expression of virus restriction and inhibition factors, reviewed in Colomer-Lluch et al. (2018) (**Figure 1A**).

However, chronic inflammation and long-term exposure to IFNs have clear detrimental effects (Acchioni et al., 2015). In the context of HIV, the timing of IFN seems to be critical, as demonstrated in a study with SIV-infected rhesus macaques. Blocking type I IFN signaling during acute SIV infection enhanced virus replication and $CD4^+$ T cell loss, whereas administering IFNα-2a during acute SIV infection induced an antiviral state and limited viral spread. However, when IFNα-2a treatment continued, cells became unresponsive to IFN, resulting in decreased viral inhibition and subsequent enhanced $CD4^+$ T cell loss (Sandler et al., 2014). Thus, ideally pDCs should produce IFNs for a limited time only.

In addition to upregulating virus restriction and inhibition factors, IFNs provide an immunostimulatory environment for other immune cells (Gonzalez-Navajas et al., 2012). This is particularly apparent for the cytolytic capacity of natural killer (NK) cells (**Figure 1A**). IFNs produced by stem-cell derived pDCs can trigger NK cell activation and increase the capacity of NK cell-mediated killing of acute lymphoblastic leukemia (Diaz-Rodriguez et al., 2017). Similarly, IFN produced by TLR9-stimulated peripheral blood pDCs can enhance NK-mediated lysis of autologous $CD4^+$ T cells infected with HIV *in vitro* (Tomescu et al., 2007). Additionally, NK-mediated antibody-dependent cellular cytotoxicity (ADCC) via autologous and heterologous HIV serum antibodies can be enhanced by IFNα (Lee et al., 2015; Tomescu et al., 2017). ADCC is a mechanism of cell-mediated cytotoxicity where the effector cell, here the NK cell, lyses a target cell via the recognition of an antibody that is bound to a viral antigen on the target cell. Via CD16, the effector cell binds the Fc portion of the antibody followed by the release of cytotoxic factors (Perussia and Trinchieri, 1984; Perussia et al., 1984).

Controlling HIV as an Antigen-Presenting Cell

Multiple studies have shown that pDCs can process and present endogenous and exogenous antigens on MHC I and II molecules and induce antigen-specific activation of both $CD8^+$ and $CD4^+$ T cells (Fonteneau et al., 2003; Benitez-Ribas et al., 2006; Young et al., 2008; Tel et al., 2010, 2013b). Important for therapeutic purposes, pDCs can be loaded with synthetic long and short peptides to trigger $CD4^+$ and $CD8^+$ T cell responses *in vitro* (Aspord et al., 2014) and *in vivo* (Tel et al., 2013a; Westdorp et al., 2019). This suggests that, similar to monocyte-derived DCs (moDCs), pDCs could be loaded with HIV antigens *in vitro* to enhance T cell responses *in vivo* (**Figure 1B**). To date, 17 clinical trials using moDC vaccination for HIV infection have been published and the clinical outcomes were variable, reviewed in da Silva et al. (2018). The studies all had in common the use of moDCs, but differed regarding moDC preparation and maturation, HIV antigen, route of administration, the number of cells administered per dose, and the number of doses administered. Regardless of these variations, all trials were safe and well tolerated. Seven of the seventeen studies demonstrated a prolonged suppression of HIV RNA in plasma and this correlated with HIV-specific T cell responses. In these studies the moDCs were exposed to autologous aldrithiol-2-inactivated HIV (Lu et al., 2004), autologous heat-inactivated HIV (Garcia et al., 2005, 2011, 2013), autologous inactivated HIV-infected apoptotic cells (Macatangay et al., 2016), or HIV peptides (Kloverpris et al., 2009; Levy et al., 2014). If these strategies are appropriate for pDC vaccination remains to be determined.

Besides inducing inflammatory T cells, pDCs have been reported to induce the formation of regulatory T cells (Tregs) and expression of indoleamine 2,3-dioxygenase (IDO) by pDCs is one of the reported mechanisms (Chen et al., 2008). Additionally, HIV-stimulated pDCs have been reported to differentiate naïve $CD4^+$ T cells into Tregs (Manches et al., 2008). Tregs suppress immune activation and limit viral clearance, reviewed in Kleinman et al. (2018). Thus, in the context of using pDCs as a therapy to induce elimination of HIV-infected cells, the induction of Tregs would likely be undesirable. However, whether pDCs exposed to inactivated HIV or HIV peptides also induce Treg formation remains to be determined.

Controlling HIV as a Killer-pDC

In a mouse model for melanoma, TRL7-stimulated pDCs have been shown to directly kill tumor cells via the secretion of TNF-related apoptosis-inducing ligand (TRAIL) and granzymes (Drobits et al., 2012). Similarly, HIV-exposed pDCs also gain cytotoxic properties, thereby creating so-called "killer-pDCs," which can induce apoptosis of $CD4^+$ T cell lines via the

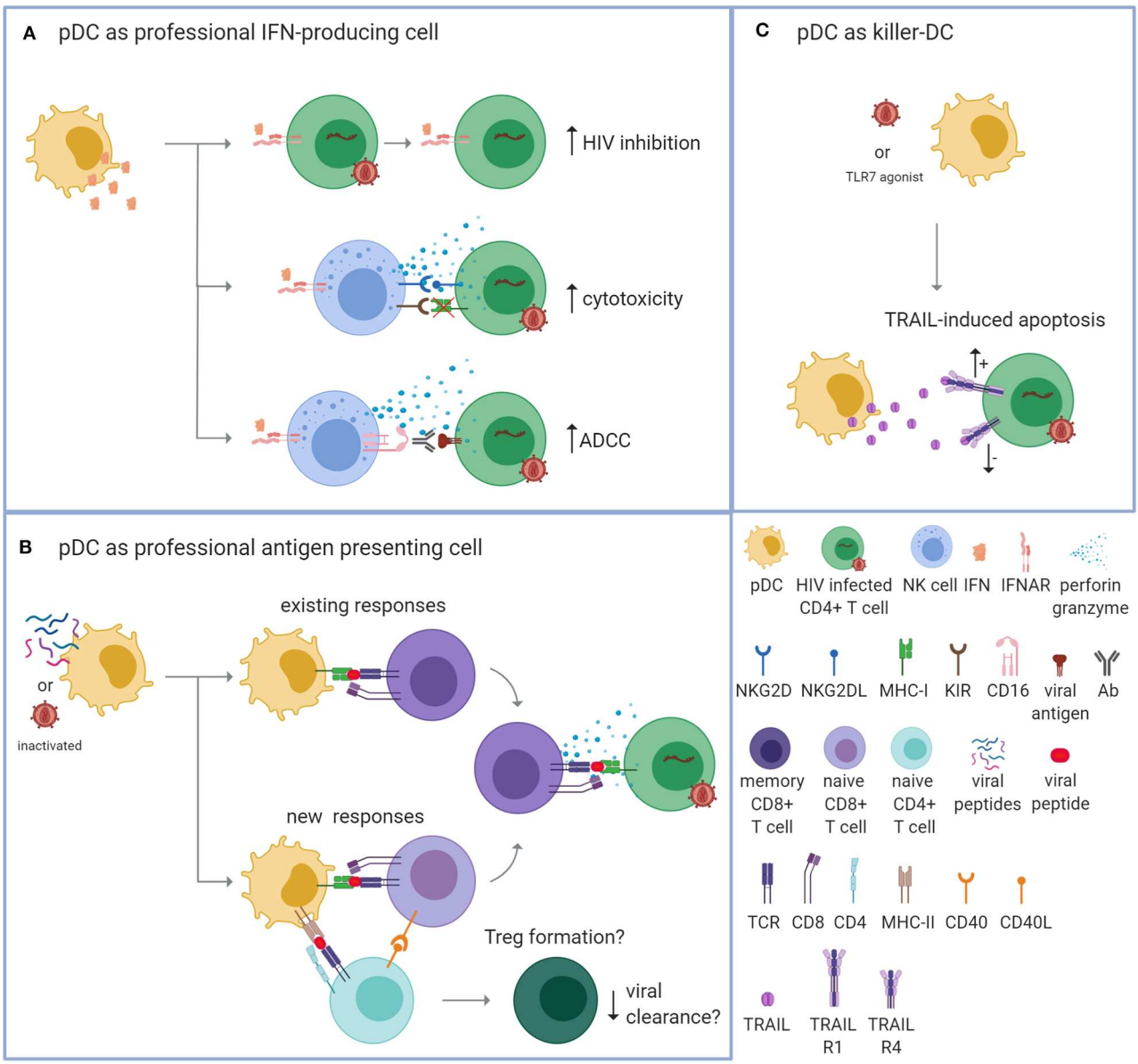


FIGURE 1 | Plasmacytoid dendritic cells can potentially control HIV via three mechanisms. Plasmacytoid dendritic cells (pDCs) can contribute to the suppression of virus replication by fulfilling three different functions. **(A)** As a professional interferon (IFN)-producing cell. PDCs can produce vast amounts of IFNs upon TLR7/9 signaling. IFNs can directly inhibit HIV replication by enhancing the expression of virus restriction and inhibition factors within the $CD4^+$ T cell (top; increased HIV inhibition). Additionally, IFNs can provide an immunostimulatory environment that enhances the cytotoxic function of natural killer (NK) cells. Apoptosis of HIV-infected cells can be induced by the release of cytotoxic perforins and granzymes after detecting reduced expression of MHC-I molecules in combination with activating signals, such as the binding of NKG2D to NKG2DL (middle; enhanced cytotoxicity). Apoptosis can also be induced by detecting the Fc tail of an antibody (Ab) that is bound to a viral antigen on the surface of the infected cell [bottom; enhanced antibody-dependent cellular cytotoxicity (ADCC)]. **(B)** As a professional antigen presenting cell (APC). PDCs can process endogenous and exogenous antigens for the antigen-specific stimulation of T cells. PDCs could be loaded with autologous inactivated HIV or HIV peptides that are presented on MHC-I molecules to activate existing HIV-specific memory $CD8^+$ T cells (top; existing responses). Upon repeated exposure, this strategy could be applied to induce new responses by activating naïve $CD8^+$ T cells with the help of $CD4^+$ T cells (bottom; new responses). The activated HIV-specific $CD8^+$ T cells are then able to recognize and eliminate HIV-infected cells via a cognate TCR-MHC interaction. PDCs may also induce the formation of regulatory T cells (Tregs) that could potentially suppress immune activation and counteract viral clearance. **(C)** As a "killer-pDC." TLR7-stimulated or HIV-exposed pDCs can obtain cytotoxic properties through the expression and secretion of TNF-related apoptosis-inducing ligand (TRAIL) that can bind to the apoptosis-transmitting TRAIL receptor 1 (R1). However, HIV-infected $CD4^+$ T cells from PLWH who receive antiretroviral treatment express the TRIAL R1 receptor and the decoy receptor TRAIL R4, which makes the T cells likely resistant to pDC-mediated killing.

TRAIL pathway (Hardy et al., 2007; Chehimi et al., 2010; Barblu et al., 2012; **Figure 1C**). This seems to have great potential for eliminating virus-infected cells but expression of the apoptosis-transmitting TRAIL receptor 1 (R1) is not restricted to HIV-infected cells and seems to be the result of chronic immune activation, possibly resulting in the killing of bystander

T cells as well (Stary et al., 2009). $CD4^+$ T cells express TRAIL R1 during detectable viremia but initiation of ART enhances expression of the decoy receptor TRAIL R4, making the T cells resistant to pDC-mediated killing (Stary et al., 2009; Chehimi et al., 2010). Unless expression of the decoy TRAIL R4 can be downregulated on HIV-infected cells, the TRAIL-expressing pDC approach seems like a rather unattractive strategy to treat HIV infection because it would require pausing therapy and would not be limited to HIV-infected cells only.

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, pDCs are multifaceted cells and could have the potential to enhance immune function to inhibit HIV replication and clear infected cells (**Figure 1**). Similar to moDCs, pDCs have the capacity to function as APC and activate existing memory T cells or induce the activation of naïve T cells. Whether pDCs can achieve this *in vivo* after exposure to HIV Ag needs to be evaluated. Additionally, a key point to address in this regard is the pDCs' capacity to induce Tregs. If pDCs were to be used as therapeutic vaccine, it would be critical to evaluate the formation of Tregs as these anti-inflammatory cells could limit viral clearance.

IFNs are key mediators of an anti-viral response and although pDCs are potent producers of type I IFNα it is important to emphasize that IFNα comprises 13 different subtypes. They all bind to the same receptor but have distinct biological activities, and display differences in their HIV inhibiting properties (Gibbert et al., 2013; Harper et al., 2015; Lavender et al., 2016). Depending on the type of TLR stimulation, pDCs can produce a broad range of IFNα subtypes (Puig et al., 2012; Harper et al., 2015). There may be a benefit in using pDCs as a tool to deliver a broad range of IFNs, rather than administering a single subtype, which is the current approach with pegylated (peg)IFNα-2a. An additional benefit of using pDCs for the production of IFNs could be the local delivery of IFNs. As pDC can travel to lymphoid organs and other peripheral tissues, this may allow for more local and targeted delivery of IFNs rather than systemic administration via intravenous injection.

Lastly, there are currently three possibilities to obtain pDCs for therapeutic vaccination. pDCs can be obtained from peripheral blood via leukapheresis, generated from stem cells obtained from peripheral blood after mobilization, or provided as irradiated pDC cell lines. Each source has its advantages and disadvantages, and the choice may depend on the function that the pDCs are required to fulfill in order for the therapy to be effective. As APCs, pDCs would ideally be from an autologous source, but could also be from an allogeneic HLA-matched source.

However, as professional IFN-producing cells there would be no requirement for HLA-matching and it would perhaps be possible to generate a pDC vaccine from allogeneic origin. While this is an intriguing avenue of research, there are several advancements required within the field before pDC vaccination is able to reach its clinical potential for the treatment of HIV.

AUTHOR CONTRIBUTIONS

RS and MJ conceptualized the idea for this manuscript. RS outlined the manuscript and wrote the first draft. JE conceptualized **Table 1** and contributed together with MJ to editing the manuscript. All authors approved the final version.

FUNDING

RS was an AIAS fellow and this project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 754513 and The Aarhus University Research Foundation. JE was supported by a Ph.D. fellowship from the Graduate School of Health, Aarhus University. MJ was funded by the Lundbeck Foundation (R238–2016–2708).

ACKNOWLEDGMENTS

We thank Rasmus O. Bak, Christian Krapp, and Luke Ring for critical reading of the manuscript. The figure in this manuscript was created using BioRender 2020 software.

REFERENCES

Acchioni, C., Marsili, G., Perrotti, E., Remoli, A. L., Sgarbanti, M., and Battistini, A. (2015). Type I IFN–a blunt spear in fighting HIV-1 infection. *Cytokine Growth Factor Rev.* 26, 143–158. doi: 10.1016/j.cytogfr.2014.10.004

Almeida, M., Cordero, M., Almeida, J., and Orfao, A. (2005). Different subsets of peripheral blood dendritic cells show distinct phenotypic and functional abnormalities in HIV-1 infection. *AIDS* 19, 261–271.

Aspord, C., Charles, J., Leccia, M. T., Laurin, D., Richard, M. J., Chaperot, L., et al. (2010). A novel cancer vaccine strategy based on HLA-A*0201 matched allogeneic plasmacytoid dendritic cells. *PLoS ONE* 5:e10458. doi: 10.1371/journal.pone.0010458

Aspord, C., Leccia, M. T., Salameire, D., Laurin, D., Chaperot, L., Charles, J., et al. (2012). HLA-A(*)0201(+) plasmacytoid dendritic cells provide a cell-based immunotherapy for melanoma patients. *J. Invest. Dermatol.* 132, 2395–2406. doi: 10.1038/jid.2012.152

Aspord, C., Leloup, C., Reche, S., and Plumas, J. (2014). pDCs efficiently process synthetic long peptides to induce functional virus- and tumour-specific T-cell responses. *Eur. J. Immunol.* 44, 2880–2892. doi: 10.1002/eji.201444588

Bam, R. A., Hansen, D., Irrinki, A., Mulato, A., Jones, G. S., Hesselgesser, J., et al. (2017). TLR7 agonist GS-9620 is a potent inhibitor of acute HIV-1 infection in human peripheral blood mononuclear cells. *Antimicrob. Agents Chemother.* 61:e01369–16. doi: 10.1128/AAC.01369-16

Barblu, L., Machmach, K., Gras, C., Delfraissy, J. F., Boufassa, F., Leal, M., et al. (2012). Plasmacytoid dendritic cells (pDCs) from HIV controllers produce interferon-α and differentiate into functional killer pDCs under HIV activation. *J. Infect. Dis.* 206, 790–801. doi: 10.1093/infdis/jis384

Barron, M. A., Blyveis, N., Palmer, B. E., Mawhinney, S., and Wilson, C. C. (2003). Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. *J. Infect. Dis.* 187, 26–37. doi: 10.1086/345957

Beignon, A. S., Mckenna, K., Skoberne, M., Manches, O., Dasilva, I., Kavanagh, D. G., et al. (2005). Endocytosis of HIV-1 activates plasmacytoid dendritic cells

via toll-like receptor-viral RNA interactions. *J. Clin. Invest.* 115, 3265–3275. doi: 10.1172/JCI26032

Benitez-Ribas, D., Adema, G. J., Winkels, G., Klasen, I. S., Punt, C. J., Figdor, C. G., et al. (2006). Plasmacytoid dendritic cells of melanoma patients present exogenous proteins to CD4+ T cells after Fc gamma RII-mediated uptake. *J. Exp. Med.* 203, 1629–1635. doi: 10.1084/jem.20052364

Biancotto, A., Grivel, J. C., Iglehart, S. J., Vanpouille, C., Lisco, A., Sieg, S. F., et al. (2007). Abnormal activation and cytokine spectra in lymph nodes of people chronically infected with HIV-1. *Blood* 109, 4272–4279. doi: 10.1182/blood-2006-11-055764

Bloch, N., O'Brien, M., Norton, T. D., Polsky, S. B., Bhardwaj, N., and Landau, N. R. (2014). HIV type 1 infection of plasmacytoid and myeloid dendritic cells is restricted by high levels of SAMHD1 and cannot be counteracted by Vpx. *AIDS Res. Hum. Retroviruses* 30, 195–203. doi: 10.1089/aid.2013.0119

Blom, B., Ho, S., Antonenko, S., and Liu, Y. J. (2000). Generation of interferon alpha-producing predendritic cell (Pre-DC)2 from human CD34(+) hematopoietic stem cells. *J. Exp. Med.* 192, 1785–1796. doi: 10.1084/jem.192.12.1785

Boichuk, S. V., Khaiboullina, S. F., Ramazanov, B. R., Khasanova, G. R., Ivanovskaya, K. A., Nizamutdinov, E. Z., et al. (2015). Gut-associated plasmacytoid dendritic cells display an immature phenotype and upregulated granzyme B in subjects with HIV/AIDS. *Front. Immunol.* 6:485. doi: 10.3389/fimmu.2015.00485

Cavaleiro, R., Baptista, A. P., Soares, R. S., Tendeiro, R., Foxall, R. B., Gomes, P., et al. (2009). Major depletion of plasmacytoid dendritic cells in HIV-2 infection, an attenuated form of HIV disease. *PLoS Pathog.* 5:e1000667. doi: 10.1371/journal.ppat.1000667

Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., et al. (1999). Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* 5, 919–923. doi: 10.1038/11360

Centlivre, M., Legrand, N., Steingrover, R., Van Der Sluis, R., Grijsen, M. L., Bakker, M., et al. (2011). Altered dynamics and differential infection profiles of lymphoid and myeloid cell subsets during acute and chronic HIV-1 infection. *J. Leukoc. Biol.* 89, 785–795. doi: 10.1189/jlb.0410231

Chang, J. J., Lacas, A., Lindsay, R. J., Doyle, E. H., Axten, K. L., Pereyra, F., et al. (2012). Differential regulation of toll-like receptor pathways in acute and chronic HIV-1 infection. *AIDS* 26, 533–541. doi: 10.1097/QAD.0b013e32834f3167

Chehimi, J., Campbell, D. E., Azzoni, L., Bacheller, D., Papasavvas, E., Jerandi, G., et al. (2002). Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J. Immunol.* 168, 4796–4801. doi: 10.4049/jimmunol.168.9.4796

Chehimi, J., Papasavvas, E., Tomescu, C., Gekonge, B., Abdulhaqq, S., Raymond, A., et al. (2010). Inability of plasmacytoid dendritic cells to directly lyse HIV-infected autologous CD4+ T cells despite induction of tumor necrosis factor-related apoptosis-inducing ligand. *J. Virol.* 84, 2762–2773. doi: 10.1128/JVI.01350-09

Chen, W., Antonenko, S., Sederstrom, J. M., Liang, X., Chan, A. S., Kanzler, H., et al. (2004). Thrombopoietin cooperates with FLT3-ligand in the generation of plasmacytoid dendritic cell precursors from human hematopoietic progenitors. *Blood* 103, 2547–2553. doi: 10.1182/blood-2003-09-3058

Chen, W., Liang, X., Peterson, A. J., Munn, D. H., Blazar, B. R. (2008). The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. *J. Immunol.* 181, 5396–5404. doi: 10.4049/jimmunol.181.8.5396

Chun, T. W., Carruth, L., Finzi, D., Shen, X., Digiuseppe, J. A., Taylor, H., et al. (1997). Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387, 183–188. doi: 10.1038/387183a0

Colomer-Lluch, M., Ruiz, A., Moris, A., and Prado, J. G. (2018). Restriction factors: from intrinsic viral restriction to shaping cellular immunity against HIV-1. *Front. Immunol.* 9:2876. doi: 10.3389/fimmu.2018.02876

da Silva, L. T., Santillo, B. T., De Almeida, A., Duarte, A., and Oshiro, T. M. (2018). Using dendritic cell-based immunotherapy to treat HIV: how can this strategy be improved? *Front. Immunol.* 9:2993. doi: 10.3389/fimmu.2018.02993

Dahabieh, M. S., Battivelli, E., and Verdin, E. (2015). Understanding HIV latency: the road to an HIV cure. *Annu. Rev. Med.* 66, 407–421. doi: 10.1146/annurev-med-092112-152941

Demoulin, S., Roncarati, P., Delvenne, P., and Hubert, P. (2012). Production of large numbers of plasmacytoid dendritic cells with functional activities from CD34(+) hematopoietic progenitor cells: use of interleukin-3. *Exp. Hematol.* 40, 268–278. doi: 10.1016/j.exphem.2012.01.002

Diaz-Rodriguez, Y., Cordeiro, P., Belounis, A., Herblot, S., and Duval, M. (2017). *In vitro* differentiated plasmacytoid dendritic cells as a tool to induce anti-leukemia activity of natural killer cells. *Cancer Immunol. Immunother.* 66, 1307–1320. doi: 10.1007/s00262-017-2022-y

Donaghy, H., Gazzard, B., Gotch, F., and Patterson, S. (2003). Dysfunction and infection of freshly isolated blood myeloid and plasmacytoid dendritic cells in patients infected with HIV-1. *Blood* 101, 4505–4511. doi: 10.1182/blood-2002-10-3189

Donaghy, H., Pozniak, A., Gazzard, B., Qazi, N., Gilmour, J., Gotch, F., et al. (2001). Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. *Blood* 98, 2574–2576. doi: 10.1182/blood.V98.8.2574

Donhauser, N., Pritschet, K., Helm, M., Harrer, T., Schuster, P., Ries, M., et al. (2012). Chronic immune activation in HIV-1 infection contributes to reduced interferon alpha production via enhanced CD40:CD40 ligand interaction. *PLoS ONE* 7:e33925. doi: 10.1371/journal.pone.0033925

Drobits, B., Holcmann, M., Amberg, N., Swiecki, M., Grundtner, R., Hammer, M., et al. (2012). Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells. *J. Clin. Invest.* 122, 575–585. doi: 10.1172/JCI61034

Evans, V. A., Lal, L., Akkina, R., Solomon, A., Wright, E., Lewin, S. R., et al. (2011). Thymic plasmacytoid dendritic cells are susceptible to productive HIV-1 infection and efficiently transfer R_5 HIV-1 to thymocytes *in vitro*. *Retrovirology* 8:43. doi: 10.1186/1742-4690-8-43

Feldman, S., Stein, D., Amrute, S., Denny, T., Garcia, Z., Kloser, P., et al. (2001). Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin. Immunol.* 101, 201–210. doi: 10.1006/clim.2001.5111

Finke, J. S., Shodell, M., Shah, K., Siegal, F. P., and Steinman, R. M. (2004). Dendritic cell numbers in the blood of HIV-1 infected patients before and after changes in antiretroviral therapy. *J. Clin. Immunol.* 24, 647–652. doi: 10.1007/s10875-004-6250-5

Finzi, D., Hermankova, M., Pierson, T., Carruth, L. M., Buck, C., Chaisson, R. E., et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295–1300. doi: 10.1126/science.278.5341.1295

Fong, L., Mengozzi, M., Abbey, N. W., Herndier, B. G., and Engleman, E. G. (2002). Productive infection of plasmacytoid dendritic cells with human immunodeficiency virus type 1 is triggered by CD40 ligation. *J. Virol.* 76, 11033–11041. doi: 10.1128/JVI.76.21.11033-11041.2002

Fonteneau, J. F., Gilliet, M., Larsson, M., Dasilva, I., Munz, C., Liu, Y. J., et al. (2003). Activation of influenza virus-specific CD4+ and CD8+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity. *Blood* 101, 3520–3526. doi: 10.1182/blood-2002-10-3063

Fonteneau, J. F., Larsson, M., Beignon, A. S., Mckenna, K., Dasilva, I., Amara, A., et al. (2004). Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. *J. Virol.* 78, 5223–5232. doi: 10.1128/JVI.78.10.5223-5232.2004

Font-Haro, A., Janovec, V., Hofman, T., Machala, L., Jilich, D., Melkova, Z., et al. (2018). Expression of TIM-3 on plasmacytoid dendritic cells as a predictive biomarker of decline in HIV-1 RNA level during ART. *Viruses* 10:154. doi: 10.3390/v10040154

Garcia, F., Climent, N., Assoumou, L., Gil, C., Gonzalez, N., Alcami, J., et al. (2011). A therapeutic dendritic cell-based vaccine for HIV-1 infection. *J. Infect. Dis.* 203, 473–478. doi: 10.1093/infdis/jiq077

Garcia, F., Climent, N., Guardo, A. C., Gil, C., Leon, A., Autran, B., et al. (2013). A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. *Sci. Transl. Med.* 5:166ra2. doi: 10.1126/scitranslmed.3004682

Garcia, F., Lejeune, M., Climent, N., Gil, C., Alcami, J., Morente, V., et al. (2005). Therapeutic immunization with dendritic cells loaded with heat-inactivated

autologous HIV-1 in patients with chronic HIV-1 infection. *J. Infect. Dis.* 191, 1680–1685. doi: 10.1086/429340

Gibbert, K., Schlaak, J. F., Yang, D., and Dittmer, U. (2013). IFN-α subtypes: distinct biological activities in anti-viral therapy. *Br. J. Pharmacol.* 168, 1048–1058. doi: 10.1111/bph.12010

Gonzalez-Navajas, J. M., Lee, J., David, M., and Raz, E. (2012). Immunomodulatory functions of type I interferons. *Nat. Rev. Immunol.* 12, 125–135. doi: 10.1038/nri3133

Hardy, A. W., Graham, D. R., Shearer, G. M., and Herbeuval, J. P. (2007). HIV turns plasmacytoid dendritic cells (pDC) into TRAIL-expressing killer pDC and down-regulates HIV coreceptors by toll-like receptor 7-induced IFN-alpha. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17453–17458. doi: 10.1073/pnas.0707244104

Harper, M. S., Guo, K., Gibbert, K., Lee, E. J., Dillon, S. M., Barrett, B. S., et al. (2015). Interferon-alpha subtypes in an *ex vivo* model of acute HIV-1 infection: expression, potency and effector mechanisms. *PLoS Pathog.* 11:e1005254. doi: 10.1371/journal.ppat.1005254

Herbeuval, J. P., Nilsson, J., Boasso, A., Hardy, A. W., Kruhlak, M. J., Anderson, S. A., et al. (2006). Differential expression of IFN-alpha and TRAIL/DR5 in lymphoid tissue of progressor versus nonprogressor HIV-1-infected patients. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7000–7005. doi: 10.1073/pnas.0600363103

Hoffmann, H. H., Schneider, W. M., and Rice, C. M. (2015). Interferons and viruses: an evolutionary arms race of molecular interactions. *Trends Immunol.* 36, 124–138. doi: 10.1016/j.it.2015.01.004

Hosmane, N. N., Kwon, K. J., Bruner, K. M., Capoferri, A. A., Beg, S., Rosenbloom, D. I., et al. (2017). Proliferation of latently infected CD4+ T cells carrying replication-competent HIV-1: potential role in latent reservoir dynamics. *J. Exp. Med.* 214, 959–972. doi: 10.1084/jem.20170193

Jarrossay, D., Napolitani, G., Colonna, M., Sallusto, F., and Lanzavecchia, A. (2001). Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur. J. Immunol.* 31, 3388–3393. doi: 10.1002/1521-4141(200111)31:11<3388::aid-immu3388>3.0.co;2-q

Kadowaki, N., Antonenko, S., Lau, J. Y., and Liu, Y. J. (2000). Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J. Exp. Med.* 192, 219–226. doi: 10.1084/jem.192.2.219

Kadowaki, N., Ho, S., Antonenko, S., Malefyt, R. W., Kastelein, R. A., Bazan, F., et al. (2001). Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* 194, 863–869. doi: 10.1084/jem.194.6.863

Kamga, I., Kahi, S., Develioglu, L., Lichtner, M., Maranon, C., Deveau, C., et al. (2005). Type I interferon production is profoundly and transiently impaired in primary HIV-1 infection. *J. Infect. Dis.* 192, 303–310. doi: 10.1086/430931

Kleinman, A. J., Sivanandham, R., Pandrea, I., Chougnet, C. A., and Apetrei, C. (2018). Regulatory T cells as potential targets for HIV cure research. *Front. Immunol.* 9:734. doi: 10.3389/fimmu.2018.00734

Kloverpris, H., Karlsson, I., Bonde, J., Thorn, M., Vinner, L., Pedersen, A. E., et al. (2009). Induction of novel CD8+ T-cell responses during chronic untreated HIV-1 infection by immunization with subdominant cytotoxic T-lymphocyte epitopes. *AIDS* 23, 1329–1340. doi: 10.1097/QAD.0b013e32832d9b00

Kluge, S. F., Sauter, D., Kirchhoff, F. (2015). SnapShot: antiviral restriction factors. *Cell* 163, 774–774.e1. doi: 10.1016/j.cell.2015.10.019

Kotenko, S. V., and Durbin, J. E. (2017). Contribution of type III interferons to antiviral immunity: location, location, location. *J. Biol. Chem.* 292, 7295–7303. doi: 10.1074/jbc.R117.777102

Laustsen, A., Bak, R. O., Krapp, C., Kjaer, L., Egedahl, J. H., Petersen, C. C., et al. (2018). Interferon priming is essential for human CD34+ cell-derived plasmacytoid dendritic cell maturation and function. *Nat. Commun.* 9:3525. doi: 10.1038/s41467-018-05816-y

Lavender, K. J., Gibbert, K., Peterson, K. E., Van Dis, E., Francois, S., Woods, T., et al. (2016). Interferon alpha subtype-specific suppression of HIV-1 infection *in vivo*. *J. Virol.* 90, 6001–6013. doi: 10.1128/JVI.00451-16

Lee, W. S., Richard, J., Lichtfuss, M., Smith, A. B., 3rd, Park, J., Courter, J. R., et al. (2015). Antibody-dependent cellular cytotoxicity against reactivated HIV-1-infected cells. *J. Virol.* 90, 2021–2030. doi: 10.1128/JVI.02717-15

Lehmann, C., Lafferty, M., Garzino-Demo, A., Jung, N., Hartmann, P., Fatkenheuer, G., et al. (2010). Plasmacytoid dendritic cells accumulate and secrete interferon alpha in lymph nodes of HIV-1 patients. *PLoS ONE* 5:e11110. doi: 10.1371/journal.pone.0011110

Levy, Y., Thiebaut, R., Montes, M., Lacabaratz, C., Sloan, L., King, B., et al. (2014). Dendritic cell-based therapeutic vaccine elicits polyfunctional HIV-specific T-cell immunity associated with control of viral load. *Eur. J. Immunol.* 44, 2802–2810. doi: 10.1002/eji.201344433

Lichtner, M., Rossi, R., Rizza, M. C., Mengoni, F., Sauzullo, I., Massetti, A. P., et al. (2008). Plasmacytoid dendritic cells count in antiretroviral-treated patients is predictive of HIV load control independent of CD4+ T-cell count. *Curr. HIV Res.* 6, 19–27. doi: 10.2174/157016208783571937

Lo, C. C., Schwartz, J. A., Johnson, D. J., Yu, M., Aidarus, N., Mujib, S., et al. (2012). HIV delays IFN-α production from human plasmacytoid dendritic cells and is associated with SYK phosphorylation. *PLoS ONE* 7:e37052. doi: 10.1371/journal.pone.0037052

Lore, K., Smed-Sorensen, A., Vasudevan, J., Mascola, J. R., and Koup, R. A. (2005). Myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific CD4+ T cells. *J. Exp. Med.* 201, 2023–2033. doi: 10.1084/jem.20042413

Lu, W., Arraes, L. C., Ferreira, W. T., and Andrieu, J. M. (2004). Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nat. Med.* 10, 1359–1365. doi: 10.1038/nm1147

Macatangay, B. J., Riddler, S. A., Wheeler, N. D., Spindler, J., Lawani, M., Hong, F., et al. (2016). Therapeutic vaccination with dendritic cells loaded with autologous HIV type 1-infected apoptotic cells. *J. Infect. Dis.* 213, 1400–1409. doi: 10.1093/infdis/jiv582

Machmach, K., Leal, M., Gras, C., Viciana, P., Genebat, M., Franco, E., et al. (2012). Plasmacytoid dendritic cells reduce HIV production in elite controllers. *J. Virol.* 86, 4245–4252. doi: 10.1128/JVI.07114-11

Manches, O., Munn, D., Fallahi, A., Lifson, J., Chaperot, L., Plumas, J., et al. (2008). HIV-activated human plasmacytoid DCs induce tregs through an indoleamine 2,3-dioxygenase-dependent mechanism. *J. Clin. Invest.* 118, 3431–3439. doi: 10.1172/JCI34823

Margolis, D. M., Archin, N. M., Cohen, M. S., Eron, J. J., Ferrari, G., Garcia, J. V., et al. (2020). Curing HIV: seeking to target and clear persistent infection. *Cell* 181, 189–206. doi: 10.1016/j.cell.2020.03.005

Marquez-Coello, M., Montes De Oca Arjona, M., Martin-Aspas, A., Guerrero Sanchez, F., Fernandez-Gutierrez Del Alamo, C., and Giron-Gonzalez, J. A. (2019). Antiretroviral therapy partially improves the abnormalities of dendritic cells and lymphoid and myeloid regulatory populations in recently infected HIV patients. *Sci. Rep.* 9:11654. doi: 10.1038/s41598-019-48185-2

Martinet, J., Leroy, V., Dufeu-Duchesne, T., Larrat, S., Richard, M. J., Zoulim, F., et al. (2012). Plasmacytoid dendritic cells induce efficient stimulation of antiviral immunity in the context of chronic hepatitis B virus infection. *Hepatology* 56, 1706–1718. doi: 10.1002/hep.25879

Mastelic-Gavillet, B., Balint, K., Boudousquie, C., Gannon, P. O., and Kandalaft, L. E. (2019). Personalized dendritic cell vaccines-recent breakthroughs and encouraging clinical results. *Front. Immunol.* 10:766. doi: 10.3389/fimmu.2019.00766

Musumeci, A., Lutz, K., Winheim, E., and Krug, A. B. (2019). What makes a pDC: recent advances in understanding plasmacytoid DC development and heterogeneity. *Front. Immunol.* 10:1222. doi: 10.3389/fimmu.2019.01222

O'Brien, M., Manches, O., Sabado, R. L., Baranda, S. J., Wang, Y., Marie, I., et al. (2011). Spatiotemporal trafficking of HIV in human plasmacytoid dendritic cells defines a persistently IFN-α-producing and partially matured phenotype. *J. Clin. Invest.* 121, 1088–1101. doi: 10.1172/JCI44960

Olivier, A., Lauret, E., Gonin, P., and Galy, A. (2006). The notch ligand delta-1 is a hematopoietic development cofactor for plasmacytoid dendritic cells. *Blood* 107, 2694–2701. doi: 10.1182/blood-2005-03-0970

Otero, M., Nunnari, G., Leto, D., Sullivan, J., Wang, F. X., Frank, I., et al. (2003). Peripheral blood dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. *AIDS Res. Hum. Retroviruses* 19, 1097–1103. doi: 10.1089/088922203771881194

Patterson, S., Rae, A., Hockey, N., Gilmour, J., and Gotch, F. (2001). Plasmacytoid dendritic cells are highly susceptible to human immunodeficiency virus type 1 infection and release infectious virus. *J. Virol.* 75, 6710–6713. doi: 10.1128/JVI.75.14.6710-6713.2001

Perussia, B., and Trinchieri, G. (1984). Antibody 3G8, specific for the human neutrophil Fc receptor, reacts with natural killer cells. *J. Immunol.* 132, 1410–1415.

Perussia, B., Trinchieri, G., Jackson, A., Warner, N. L., Faust, J., Rumpold, H., et al. (1984). The Fc receptor for IgG on human natural killer cells: phenotypic, functional, and comparative studies with monoclonal antibodies. *J. Immunol.* 133, 180–189.

Pham, T. N. Q., Meziane, O., Miah, M. A., Volodina, O., Colas, C., Beland, K., et al. (2019). Flt3L-mediated expansion of plasmacytoid dendritic cells suppresses HIV infection in humanized mice. *Cell Rep.* 29, 2770–2782.e5. doi: 10.1016/j.celrep.2019.10.094

Puig, M., Tosh, K. W., Schramm, L. M., Grajkowska, L. T., Kirschman, K. D., Tami, C., et al. (2012). TLR9 and TLR7 agonists mediate distinct type I IFN responses in humans and nonhuman primates *in vitro* and *in vivo*. *J. Leukoc. Biol.* 91, 147–158. doi: 10.1189/jlb.0711371

Reizis, B. (2019). Plasmacytoid dendritic cells: development, regulation, and function. *Immunity* 50, 37–50. doi: 10.1016/j.immuni.2018.12.027

Remer, K. A., Apetrei, C., Schwarz, T., Linden, C., and Moll, H. (2007). Vaccination with plasmacytoid dendritic cells induces protection against infection with *Leishmania major* in mice. *Eur. J. Immunol.* 37, 2463–2473. doi: 10.1002/eji.200636780

Sandler, N. G., Bosinger, S. E., Estes, J. D., Zhu, R. T., Tharp, G. K., Boritz, E., et al. (2014). Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature* 511, 601–605. doi: 10.1038/nature13554

Schmidt, B., Ashlock, B. M., Foster, H., Fujimura, S. H., and Levy, J. A. (2005). HIV-infected cells are major inducers of plasmacytoid dendritic cell interferon production, maturation, and migration. *Virology* 343, 256–266. doi: 10.1016/j.virol.2005.09.059

Schwartz, J. A., Clayton, K. L., Mujib, S., Zhang, H., Rahman, A. K., Liu, J., et al. (2017). Tim-3 is a marker of plasmacytoid dendritic cell dysfunction during HIV infection and is associated with the recruitment of IRF7 and p85 into lysosomes and with the submembrane displacement of TLR9. *J. Immunol.* 198, 3181–3194. doi: 10.4049/jimmunol.1601298

Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., et al. (1999). The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284, 1835–1837. doi: 10.1126/science.284.5421.1835

Smed-Sorensen, A., Lore, K., Vasudevan, J., Louder, M. K., Andersson, J., Mascola, J. R., et al. (2005). Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells. *J. Virol.* 79, 8861–8869. doi: 10.1128/JVI.79.14.8861-8869.2005

Stary, G., Klein, I., Kohlhofer, S., Koszik, F., Scherzer, T., Mullauer, L., et al. (2009). Plasmacytoid dendritic cells express TRAIL and induce CD4+ T-cell apoptosis in HIV-1 viremic patients. *Blood* 114, 3854–3863. doi: 10.1182/blood-2009-04-217927

Swiecki, M., and Colonna, M. (2015). The multifaceted biology of plasmacytoid dendritic cells. *Nat. Rev. Immunol.* 15, 471–85. doi: 10.1038/nri3865

Swiecki, M., Wang, Y., Vermi, W., Gilfillan, S., Schreiber, R. D., and Colonna, M. (2011). Type I interferon negatively controls plasmacytoid dendritic cell numbers *in vivo*. *J. Exp. Med.* 208, 2367–2374. doi: 10.1084/jem.20110654

Tavano, B., Galao, R. P., Graham, D. R., Neil, S. J., Aquino, V. N., Fuchs, D., et al. (2013). Ig-like transcript 7, but not bone marrow stromal cell antigen 2 (also known as HM1.24, tetherin, or CD317), modulates plasmacytoid dendritic cell function in primary human blood leukocytes. *J. Immunol.* 190, 2622–2630. doi: 10.4049/jimmunol.1202391

Tel, J., Aarntzen, E. H., Baba, T., Schreibelt, G., Schulte, B. M., Benitez-Ribas, D., et al. (2013a). Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. *Cancer Res.* 3, 1063–1075. doi: 10.1158/0008-5472.CAN-12-2583

Tel, J., Lambeck, A. J., Cruz, L. J., Tacken, P. J., De Vries, I. J., and Figdor, C. G. (2010). Human plasmacytoid dendritic cells phagocytose, process, and present exogenous particulate antigen. *J. Immunol.* 184, 4276–4283. doi: 10.4049/jimmunol.0903286

Tel, J., Schreibelt, G., Sittig, S. P., Mathan, T. S., Buschow, S. I., Cruz, L. J., et al. (2013b). Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets. *Blood* 121, 459–467. doi: 10.1182/blood-2012-06-435644

Thordardottir, S., Hangalapura, B. N., Hutten, T., Cossu, M., Spanholtz, J., Schaap, N., et al. (2014). The aryl hydrocarbon receptor antagonist StemRegenin 1 promotes human plasmacytoid and myeloid dendritic cell development from CD34+ hematopoietic progenitor cells. *Stem Cells Dev.* 23, 955–967. doi: 10.1089/scd.2013.0521

Thordardottir, S., Schaap, N., Louer, E., Kester, M. G., Falkenburg, J. H., Jansen, J., et al. (2017). Hematopoietic stem cell-derived myeloid and plasmacytoid DC-based vaccines are highly potent inducers of tumor-reactive T cell and NK cell responses *ex vivo*. *Oncoimmunology* 6:e1285991. doi: 10.1080/2162402X.2017.1285991

Tilton, J. C., Manion, M. M., Luskin, M. R., Johnson, A. J., Patamawenu, A. A., Hallahan, C. W., et al. (2008). Human immunodeficiency virus viremia induces plasmacytoid dendritic cell activation *in vivo* and diminished alpha interferon production *in vitro*. *J. Virol.* 82, 3997–4006. doi: 10.1128/JVI.01545-07

Tomescu, C., Chehimi, J., Maino, V. C., and Montaner, L. J. (2007). NK cell lysis of HIV-1-infected autologous CD4 primary T cells: requirement for IFN-mediated NK activation by plasmacytoid dendritic cells. *J. Immunol.* 179, 2097–2104. doi: 10.4049/jimmunol.179.4.2097

Tomescu, C., Tebas, P., and Montaner, L. J. (2017). IFN-alpha augments natural killer-mediated antibody-dependent cellular cytotoxicity of HIV-1-infected autologous CD4+ T cells regardless of major histocompatibility complex class 1 downregulation. *AIDS* 31, 613–622. doi: 10.1097/QAD.0000000000001380

Tsai, A., Irrinki, A., Kaur, J., Cihlar, T., Kukolj, G., Sloan, D. D., et al. (2017). Toll-like receptor 7 agonist GS-9620 induces HIV expression and HIV-specific immunity in cells from HIV-infected individuals on suppressive antiretroviral therapy. *J. Virol.* 91:e02166–16. doi: 10.1128/JVI.02166-16

Vanhamel, J., Bruggemans, A., and Debyser, Z. (2019). Establishment of latent HIV-1 reservoirs: what do we really know? *J. Virus Erad.* 5, 3–9.

Vibholm, L., Schleimann, M. H., Hojen, J. F., Benfield, T., Offersen, R., Rasmussen, K., et al. (2017). Short-course toll-like receptor 9 agonist treatment impacts innate immunity and plasma viremia in individuals with human immunodeficiency virus infection. *Clin. Infect. Dis.* 64, 1686–1695. doi: 10.1093/cid/cix201

Wang, F. X., Huang, J., Zhang, H., Ma, X., and Zhang, H. (2008). APOBEC3G upregulation by alpha interferon restricts human immunodeficiency virus type 1 infection in human peripheral plasmacytoid dendritic cells. *J. Gen. Virol.* 89, 722–730. doi: 10.1099/vir.0.83530-0

Westdorp, H., Creemers, J. H. A., Van Oort, I. M., Schreibelt, G., Gorris, M. A. J., Mehra, N., et al. (2019). Blood-derived dendritic cell vaccinations induce immune responses that correlate with clinical outcome in patients with chemo-naive castration-resistant prostate cancer. *J. Immunother. Cancer* 7:302. doi: 10.1186/s40425-019-0787-6

Wong, J. K., Hezareh, M., Gunthard, H. F., Havlir, D. V., Ignacio, C. C., Spina, C. A., et al. (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science* 278, 1291–1295. doi: 10.1126/science.278.5341.1291

Yin, Z., Dai, J., Deng, J., Sheikh, F., Natalia, M., Shih, T., et al. (2012). Type III IFNs are produced by and stimulate human plasmacytoid dendritic cells. *J. Immunol.* 189, 2735–2745. doi: 10.4049/jimmunol.1102038

Yonezawa, A., Morita, R., Takaori-Kondo, A., Kadowaki, N., Kitawaki, T., Hori, T., et al. (2003). Natural alpha interferon-producing cells respond to human immunodeficiency virus type 1 with alpha interferon production and maturation into dendritic cells. *J. Virol.* 77, 3777–3784. doi: 10.1128/JVI.77.6.3777-3784.2003

Young, L. J., Wilson, N. S., Schnorrer, P., Proietto, A., Ten Broeke, T., Matsuki, Y., et al. (2008). Differential MHC class II synthesis and ubiquitination confers distinct antigen-presenting properties on conventional and plasmacytoid dendritic cells. *Nat. Immunol.* 9, 1244–1252. doi: 10.1038/ni.1665

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 13 August 2020
doi: 10.3389/fcimb.2020.00410

Engineering CAR T Cells to Target the HIV Reservoir

Wenli Mu†, *Mayra A. Carrillo*† and *Scott G. Kitchen**†

Division of Hematology and Oncology, Department of Medicine, University of California, Los Angeles, Los Angeles, CA, United States

The HIV reservoir remains to be a difficult barrier to overcome in order to achieve a therapeutic cure for HIV. Several strategies have been developed to purge the reservoir, including the "kick and kill" approach, which is based on the notion that reactivating the latent reservoir will allow subsequent elimination by the host anti-HIV immune cells. However, clinical trials testing certain classes of latency reactivating agents (LRAs) have so far revealed the minimal impact on reducing the viral reservoir. A robust immune response to reactivated HIV expressing cells is critical for this strategy to work. A current focus to enhance anti-HIV immunity is through the use of chimeric antigen receptors (CARs). Currently, HIV-specific CARs are being applied to peripheral T cells, NK cells, and stem cells to boost recognition and killing of HIV infected cells. In this review, we summarize current developments in engineering HIV directed CAR-expressing cells to facilitate HIV elimination. We also summarize current LRAs that enhance the "kick" strategy and how new generation and combinations of LRAs with HIV specific CAR T cell therapies could provide an optimal strategy to target the viral reservoir and achieve HIV clearance from the body.

Keywords: HIV, latency, chimeric antigen receptor, gene therapy, LRA (latency reversing agents), immunotherapy, viral reservoir

OPEN ACCESS

Edited by:
Renee Marije Van Der Sluis, Aarhus Institute of Advanced Studies, Denmark

Reviewed by:
R. Brad Jones, Cornell University, United States
Olivier Rohr, Université de Strasbourg, France

***Correspondence:**
Scott G. Kitchen
skitchen@ucla.edu

†*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *27 March 2020*
Accepted: *03 July 2020*
Published: *13 August 2020*

Citation:
Mu W, Carrillo MA and Kitchen SG (2020) Engineering CAR T Cells to Target the HIV Reservoir. Front. Cell. Infect. Microbiol. 10:410. doi: 10.3389/fcimb.2020.00410

INTRODUCTION

HIV-specific CD8+ cytotoxic T lymphocyte (CTL) response plays a critical role in limiting the virus replication *in vivo* by recognizing viral antigens presented by human leukocyte antigen (HLA) class I and killing infected cells. However, the CTL response fails to durably control HIV replication in the absence of combination antiretroviral therapy (cART) (Jones and Walker, 2016). Intriguingly, very rare HIV-infected individuals, called "elite controllers," are able to spontaneously control and suppress viral replication in the absence of cART. Elite controllers exhibit the core feature that defines a HIV "functional cure": a long-term drug-free viral remission. There is compelling evidence from large genetic and functional immunology studies that robust CTL responses and protective HLA alleles are crucial for the natural control of HIV-infection (International et al., 2010; Walker and Yu, 2013). Even though natural CTL responses are imperfect and ultimately fail to clear the virus, they still drive partial control of viremia and, in the rare cases of elite controllers, is the dominant component of immune defense in successful long term suppression of viral replication. It is clear that a strong cellular immune response is essential in suppressing the virus and would be an essential component in therapeutic attempts to clear the virus from the body.

Despite the current cART to delay disease progression and prolong life expectancy, HIV remains to be an incurable disease for most. The inability for the host immune system to clear HIV from the body is partially due to the reduced present or absent viral antigen expression on latently infected

CD4+ T cells that harbor integrated replication-competent virus (viral reservoir) that contribute to viral rebound once ART is discontinued (Churchill et al., 2016). Thus, one strategy that proposes to target the viral reservoir is referred to as "kick and kill" (also known as "shock and kill") which postulates that inducing the virus from these latently infected cells (kick or shock) will facilitate "killing" by HIV mediated cell death or by the surrounding immune surveillance and lead to a clearance of the viral reservoir (Kim et al., 2018). However, clinical trials applying this strategy using latency reversal agents (LRAs) came short of promising results (Rasmussen et al., 2014; Spivak et al., 2014; Sogaard et al., 2015), suggesting that natural CTLs appear incapable of clearing this reservoir even after reactivating antigen expression. Although new strategies are improving the "kick" to induce virus, other studies have highlighted reasons for lack of "killing" from the host immune cells, likely due to immune evasion by HIV and dysfunctional HIV-specific T cells (Collins et al., 1998; Fenwick et al., 2019). A promising new approach to enhance the targeting and killing of HIV expressing cells is using chimeric antigen receptors (CARs) (Kuhlmann et al., 2018). T cells modified with new anti-HIV CAR technology can potentially overcome the limitations and barriers that natural HIV-specific T cells are currently facing. Compared with natural conventional effector T cells, CARs can prevent or limit viral immune escape since they directly recognize antigens irrespective of MHC presentation. CAR T cells can also be generated and allowed to expand several orders of magnitude *in vitro* or *in vivo* in a patient, which provides large numbers of engineered antigen specific cells. Ideally, CAR-expressing cells can be engineered to confer a stable and durable immune surveillance to HIV reservoirs.

However, it is still unclear how CAR-modified T cells will perform under a very low HIV antigen environment, therefore combining CAR T cell therapy with LRAs might increase CAR T cell response to latently infected cells. In this review, we summarize current developments to enhance HIV-specific CAR T cell therapy to target the HIV reservoir. In addition, we discuss how future investigation of the "kick and kill" strategy in combination with anti-HIV CAR T cell therapy can lead to synergistic effects to deplete the viral reservoir and setup a closer step to achieve a functional cure of HIV infection.

CAR T Cells to Combat HIV Infection

The concept of using adoptive T cell therapy for treating HIV infection had been proposed decades ago. The earliest study on HIV CAR T cell therapy was designed by transferring adoptive T cells expressing a CAR that was a fusion of CD4 extracellular domain (the primary HIV cellular receptor) to the CD3ζ signaling domain (CD4ζ) (Mitsuyasu et al., 2000; Walker et al., 2000; Deeks et al., 2002). The advantage of choosing CD4 as the reactive ligand for anti-HIV CAR design is that, as the natural HIV envelope recognition moiety, CD4 ensures broad targeting of all HIV isolates. Moreover, CD4 binding sites on the envelope protein are relatively well conserved (Wang et al., 2019), as it mutation would diminish CD4 binding and have a direct effect on decreasing viral fitness. Several clinical trials were performed to test the efficiency and safety of the first-generation CD4-based CAR in HIV patients (Mitsuyasu et al., 2000; Walker et al., 2000; Deeks et al., 2002). It was demonstrated that the treatment did not result in durable control of viral replication; however, there were no overt toxicities associated with the treatment, and the modified cells persisted for >10 years (Mitsuyasu et al., 2000). The reasons for the lack of viral control could be due to several factors: (1) CD4-based CARs render the gene-modified T cells susceptible to HIV infection and elimination of activated cells, (2) lack of efficient activation signaling from costimulatory signals, (3) suboptimal T cell handling and expansion, and/or (4) lack of viral antigen stimulation. Nevertheless, the CD4ζ T cell therapy was confirmed safe and sustained stable levels of engraftment (Scholler et al., 2012).

Further progress in CAR design with the aim of optimizing CAR T cell effector function and persistence in the cancer field have led to rapid advancement in CAR T cell therapy in recent years. Four generations of CARs have been developed so far (**Figure 1**). The first-generation of CARs linked an extracellular antigen recognition moiety to a lymphocyte-stimulating intracellular endodomain, such as the signal-transducing subunit of the TCR CD3ζ chains (Eshhar et al., 1993). First-generation CAR T cells tended to have limited *in vivo* expansion and cytotoxicity and were highly prone to apoptosis (Heuser et al., 2003; Zhao et al., 2009). The addition of costimulatory molecule domains, such as CD28 or 4-1BB, with the cytoplasmic tail of CD3ζ-containing first-generation constructs had generated second-generation CARs. Optimized anti-HIV second-generation CAR T cells that contained the costimulatory 4-1BB domain were at least 50-fold more potent at suppressing HIV replication *in vitro* than T cells expressing first-generation CARs only (Leibman et al., 2017). Animal studies also demonstrated that secondary generation CARs were superior at expanding in response to antigen, protecting CD4+T cells from HIV infection and reducing CD4 decline compared to the CAR without costimulatory molecules (Leibman et al., 2017). Further, comparable studies demonstrated that the 4-1BB costimulatory domain is superior to the CD28 domain for reducing viral rebound after ART treatment and promoting T cell persistence *in vivo* in the absence of antigen (Zhang et al., 2007; Leibman et al., 2017). Third-generation CARs were created by incorporating multiple costimulatory molecules into secondary generation CARs. Third-generation CARs have been developed with enhanced effector function, proliferation, survival, and ultimately enhanced tumor killing in the cancer field (Savoldo et al., 2011). A third-generation anti-gp120 CAR moiety, combining multiple intracellular signaling domains (CD3ζ-CD28-41BB), displayed augmented potency in lysing Env-expressing cells *in vitro* compared to the CD4ζ-CAR (Liu et al., 2016). Fourth-generation CAR T cells, known as T cells redirected for universal cytokine-mediated killing (TRUCKs), contained a third stimulatory signal which produces cytokines, such as IL-7, IL12, IL-15, or IL-18, in secreted or in a membrane-tethered form that aims to improve CAR T cells expansion and persistence and are under investigation in the oncology field to target solid tumors (Chmielewski et al., 2014; Chmielewski and Abken, 2015; Hurton et al., 2016).

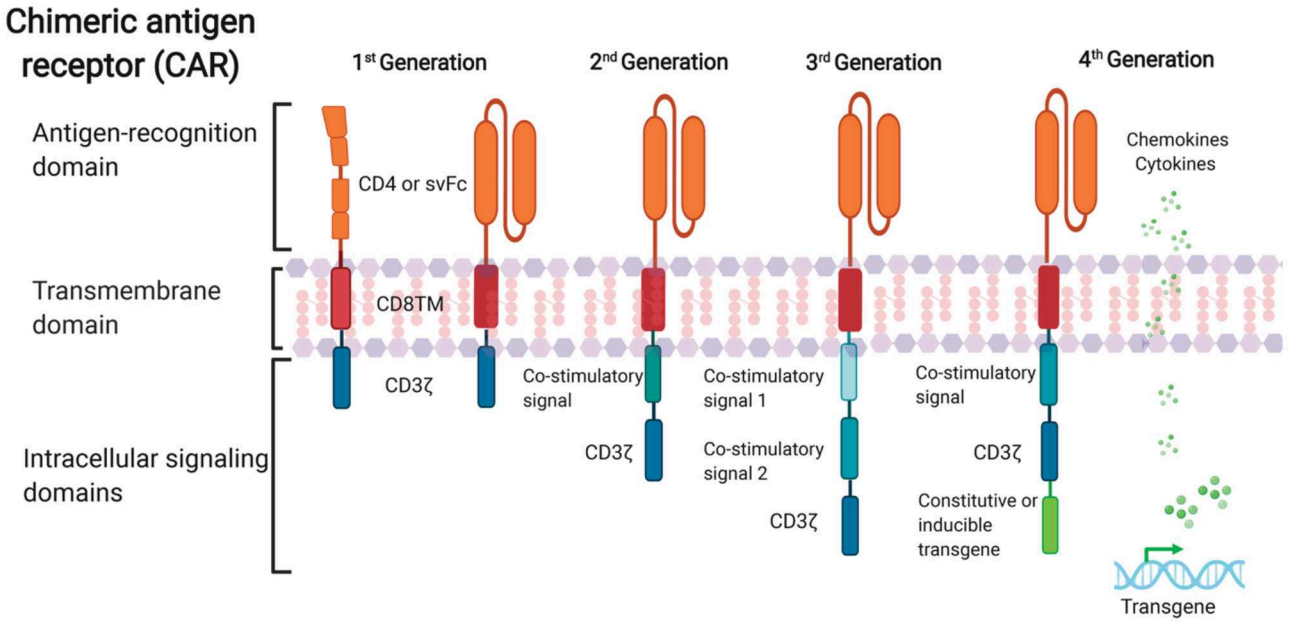


FIGURE 1 | Schematic representation of anti-HIV CAR structure. First-generation CAR consists of a single anti-HIV env domain (CD4 or svFc), the transmembrane domain region, and the T cell receptor CD3-ζ domain. Second-generation CAR incorporates an additional costimulatory signaling domain to the basic first-generation receptor configuration. Third-generation contain more than one co-stimulatory domain. Fourth-generation CARs are characterized by addition of constitutive or inducible transgenes like cytokines or chemokines.

Successful development and application of CAR T cell therapy in clinical cancer studies have also fostered investigations of CAR therapy into the HIV cure field. CAR therapy to target HIV reservoirs is promising for several reasons. Firstly, CAR T cells are capable of long-term immune surveillance. Effector function of CAR-modified peripheral T cells can be retained for 6 months (Kalos et al., 2011; Kochenderfer et al., 2012; Maude et al., 2014). Moreover, animal studies suggest that hematopoietic stem cell (HSPC)-derived CAR cells can persist even longer, providing continuous production of CAR T cells (Zhen et al., 2017b). Importantly, CAR T cells can be reprogrammed and differentiated into effector memory or central memory T cells. As a result, they can potentially provide long-lived immunological memory (Kawalekar et al., 2016). Secondly, CAR T cells are capable of trafficking to the different types of tissue reservoirs, including the central nervous system, which is a potentially important sanctuary for latent HIV (Marban et al., 2016). Evidence that CD19-targeted CARs can traffic to the brain and eliminate cancer supports the notion that CAR T cells may show effective against HIV reservoirs in brain tissue, where it is difficult for drugs to penetrate the blood-brain barrier (Grupp et al., 2013; Maude et al., 2014). Importantly, HSPC-based CAR expressing cells were found in multiple lymphoid tissues, including various lymph nodes, gut, and bone marrow, which are major viral replication sites in a simian-human immunodeficiency virus (SHIV) infected non-human primate (NHP) model (Zhen et al., 2017b). Moreover, CARs can also be further engineered with homing receptors to enhance CAR T cell penetration into the B cell follicle, another major viral reservoir that is difficult to target by CTLs (Haran et al., 2018). Thirdly, CAR T cells are capable of targeting antigen in a major histocompatibility complex (MHC)-independent fashion, which potentially allows therapeutic use by all. In addition, lack of MHC-restriction could make these cells less susceptible to immune escape due to viral downregulation of MHC-I in HIV infected cells (Collins et al., 1998; Goulder and Walker, 1999; Wonderlich et al., 2011). The properties discussed above could allow CAR T cells to confer stable and durable immune surveillance to HIV reservoirs if viral antigen is reactivated at these sites. Therefore, CAR T cell therapy offers a promising approach to eradicate HIV reservoirs.

Strategies for Optimizing CARs Design Against HIV

One of the potential reasons the first CAR T cell trials for HIV infection did not result in impactful reductions in viral load is the notion that the CD4 based anti-HIV CAR design rendered the CAR-expressing T cells susceptible to direct HIV infection (Zhen et al., 2017a). In addition, antibody-based anti-HIV specific CARs could also potentially facilitate HIV infection (Leibman et al., 2017). Effective CAR-based therapeutic approaches would benefit from adding protective mechanisms in the CAR design against HIV infection. Many approaches have been developed for engineering T cells to become resistant to HIV infection utilizing an anti-HIV gene therapy reagent (Carrillo et al., 2017; Zhen et al., 2017a). These approaches have utilized small hairpin RNA (shRNA)-targeted knockdown of the HIV coreceptor CCR5 (Shimizu et al., 2010; Zhen et al., 2015) or shRNA mediated degradation of HIV RNA by targeting specific HIV long terminal

repeated sequences (Ringpis et al., 2012; Kamata et al., 2015). Variations of gp41 heptad repeat 2 domain (Leslie et al., 2016), such as anti-HIV fusion peptide C46, have also been used to protect cells from infection (Younan et al., 2013; Zhen et al., 2015, 2017a). C46 has also been used in combination with the anti-CCR5 shRNA to prevent infection by dual tropic viruses (Zhen et al., 2015, 2017a). Another strategy linked CD4 portion to the carbohydrate recognition domain (CRD) of a human C-type lectin, and the bispecific CARs were completely devoid of the undesired activity of rendering CCR5+ CAR-transduced cells susceptible to HIV-1 infection. The possible reason could be CRD blocks the unwanted HIV entry receptor activity by binding to high-mannose glycans on the viral envelope spike (Feinberg et al., 2001; Ghanem et al., 2018).

In addition to CD4-ligand based CARs, antibody-based CARs can be used to target HIV infection. Most CARs currently in clinical trials for various malignancies have antibody-based antigen recognition regions (Sommermeyer et al., 2017; Guedan et al., 2019). For HIV infection, single-chain variable fragment (scFv) derived from broadly neutralizing antibodies (bNAbs) have been employed to generate HIV-specific CARs. The exponential growth in HIV bNAb identification has provided great opportunities for creating bNAb-CARs that can be potentially more effective in HIV elimination (Burton et al., 1994; Scheid et al., 2011; Huang et al., 2012; Sok and Burton, 2018). bNAb-based CARs contain scFvs derived from bNAbs, which target conserved sites within the Env protein and have been shown to broadly recognize over 95% of HIV-1 strains (Walker et al., 2011; Doria-Rose et al., 2014; Huang et al., 2016). Another study tested a panel of seven HIV-specific CARs based on well-defined HIV-1 bNAbs, which have shown variance in their breadth of HIV-1 sequence diversity coverage. Each scFv-CAR endowed CD8+ T cells with the capacity to proliferate and kill infected cells, and suppress viral replication *in vitro* (Ali et al., 2016). However, additional studies will be necessary to understand and evaluate *in vivo* prosperities and functions for bNAb-CARs.

Nevertheless, a major limitation for single antigen recognition domain-based CAR design, especially for scFV-based CARs, is a high potential for antigen escape and/or lack of antigen expression that can render the CAR T cell therapy inefficient. Recent clinical trials using monotherapy of bNAbs 3BNC117 (Scheid et al., 2016) or VRC01(Bar et al., 2016) showed a decrease in plasma viremia and a delay, but not a prevented viral rebound. However, treatment with a combination of two or more bNAbs can significantly reduce the viral reservoir and showed prolonged viral suppression (Bar-On et al., 2018; Mendoza et al., 2018). Thus, a single bNAb CAR might not be sufficient to suppress HIV-1 for the long term since escape mutants that emerge *in vivo* will allow viral rebound. The use of a combination of antigen recognition domains in multiple CARs or the use of a CAR with multiple antigen recognition domains would likely provide far greater suppression. Approaches utilizing multiple antigen recognition domains are currently under development, termed dual-, bi-, or tri-specific CARs (Ruella et al., 2016; Fry et al., 2018). Dual or bispecific CARs have been recently developed that connect an extracellular CD4 domain to either a bNAb-based scFv (Liu et al., 2016; Anthony-Gonda et al., 2019) or the CRD of a human C-type lectin (Ghanem et al., 2018; Haran et al., 2018). These have been shown to have antiviral effects in redirecting T cells *in vitro* to kill HIV infected cells and, in some cases, *in vivo* in model systems; however, their effects *in vivo* in humans are not well characterized.

In addition to CAR T cell-mediated immunotherapy, an anti-HIV CAR-based approach with natural killer (NK) cells has also been considered as a strategy for antiviral immunotherapy. A key feature of NK cells is that they, in addition to T cells, express the intracellular signaling machinery to allow a CAR expressing the CD3ζ signaling domain to function in redirecting killing activity to the antigen of interest (Liu et al., 2017; Mehta and Rezvani, 2018). Unique anti-HIV features of NK cells makes them an attractive and effective tool for immunotherapy. HIV nef-mediated MHC-1 downregulation can potentially expose the HIV infected cells to be more susceptible to NK cells lacking inhibitory receptors to HLA-C and HLA-E (Bonaparte and Barker, 2004). Moreover, HIV-infected target cells can increase the expression of ligands, such as unique long binding protein ULBP-1 and−2, which can be recognized by NK cells (Richard et al., 2010). These upregulated ligands can induce NK activating receptor (natural killer group 2, member D, NKG2D) -mediated effector functions such as cytotoxicity and cytokine production in human and mouse NK cells (Ogasawara and Lanier, 2005; Bryceson and Ljunggren, 2008; Le Bert and Gasser, 2014; Stojanovic et al., 2018). T follicular helper cells (Tfh cell), a critical reservoir that is established during HIV infection, can also be eliminated by NK cells (Rydyznski et al., 2015). In addition, NK cells can identify and remove HIV infected cells through antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism (Chung et al., 2008, 2011). Therefore, these properties make genetically modified NK-cells an appealing tool to be tested as a novel strategy to control HIV replication and reduce HIV reservoir by directing them to HIV through the use of a CAR. Clinical trials are ongoing using CAR-modified NK cells for cancer immunotherapy and the safety and efficacy of these therapies will be evaluated. A Phase I clinical trial was completed using CD19-41BB-TCRζ CAR NK cells to target B-lineage acute lymphoblastic leukemia (ALL) malignancy (NCT00995137). Another Phase II clinical trial with NK cells has been approved for B-lymphoid malignancies using a CD28-TCRζ CAR with an inducible suicide gene and an activating cytokine (NCT03056339). Recently, this Phase II trial reported that 73% of patients exhibited a clinical response without the development of major toxic effects after administration of NK cells engineered with a CAR targeting CD19 and co-expressing IL-15 (Liu et al., 2020). These trials suggest a promising aspect in the use of redirected immunity using CAR-NK cells.

In early research of NK-based CAR against HIV, CD4-TCRζ CAR-modified NK cells were shown to effectively kill either NK-resistant tumor cells expressing the relevant ligand, gp120, or CD4+ T cells infected with HIV *in vitro* (Tran et al., 1995). We later demonstrated *in vivo* in humanized mice that CAR-modified HSPCs can differentiate into multiple hematopoietic lineages, including functional NK cells (Zhen et al., 2015). These NK cells are resistant to HIV infection and may have played

a role in suppressing HIV replication. CAR-modified NK cells derived from CAR-HSPCs were also detected in NHP models (Zhen et al., 2017b). CAR-modified HSPCs appear to possess the ability to produce functional CAR NK cells continuously over time, overcoming one of the limitations of NK cell-mediated immunotherapy in the relatively short life span of NK cells isolated directly from patient's peripheral blood (Liu et al., 2017). The production of CAR NK cells from HSPCs in addition to T cells could provide added immune surveillance benefits by targeting different tissues and reservoirs and by supplying a different type of cellular immunity.

Strategies to Enhance CAR T Cell Engraftment, Function, and Persistence

The use of CAR or TCR modified autologous peripheral blood T lymphocytes for B-lineage malignancies therapy has shown tremendous clinical success (Ramos et al., 2016; June et al., 2018). However, it remains unclear if CAR T cells can respond to malignancies that may recur after treatment, as antitumor activities of these cells appear to diminish over time (Mueller et al., 2017). As described above, there was limited functionality in autologous peripheral CAR T cells in suppressing HIV in past clinical trials. These studies revealed that a CAR T cell approach is safe and feasible and demonstrated the persistence of gene-modified T cells for years after infusion. However, the reasons for the long-term maintenance of this (albeit small) population of cells are not clear and could be due to homeostatic persistence mechanisms. To achieve a sustainable virologic control after ART cessation, strategies to enhance CAR-expressing cell engraftment, function, and persistence *in vivo* seem to be required.

Strategies to enhance CAR T cell function and persistence have been thoroughly investigated for CD19-based and other tumor-specific antigen targeting CARs (Fesnak et al., 2016; Labanieh et al., 2018). For example, the use of cytokines IL-7 and IL-15 for culturing and expanding CD19CAR T cells resulted in superior expansion and generation of naïve and memory populations that resulted in better persistence and antitumor efficacy *in vivo* compared to CD19CAR T cells cultured in IL-2 alone (Xu et al., 2014; Zhou et al., 2019). The endogenous expression of cytokines by CAR-expressing T cells has also been evaluated to boost persistence and efficacy *in vivo*. A vector containing a CD19CAR co-expressing IL-15 and the suicide gene iC9 has been evaluated in a preclinical study (Hoyos et al., 2010). The iC9/CAR.19/IL-15 not only persisted better but also had greater antitumor efficacy *in vivo* compared to CD19CAR. In addition, the IL-15 had other beneficial effects such as greater expansion, lower cell death, and lower programmed cell death-1 (PD-1) expression in response to antigen stimulation. Other cytokines such as IL-12, IL-7, IL-21, and IL-18 have found to have similar improved antitumor effects (Chinnasamy et al., 2012; Pegram et al., 2015; Hu et al., 2017; Adachi et al., 2018; Batra et al., 2020). Whether culturing conditions, endogenous expression, or administration of immunostimulatory cytokines have similar effects *in vivo* for HIV-specific CARs has yet to be determined. It is cautionary to use such cytokines in HIV targeted therapies during infection and/or in the absence of ART as some of these cytokines, specifically IL-7 and IL-15, may boost immune activation which can promote virus production and lead to higher levels of viremia (Managlia et al., 2006; Mueller et al., 2008; Vassena et al., 2012; Coiras et al., 2016; Manganaro et al., 2018). Another approach to boost CAR T cell function *in vivo* is by blocking immune checkpoint molecules. Strategies to block PD-1 by CRISPR, shRNAs, or PD-1 antibody blockade have been implemented and tested into cancer-specific CARs and observed to improve antitumor responses *in vivo* (Cherkassky et al., 2016; Rupp et al., 2017; Rafiq et al., 2018; Hu et al., 2019a,b). Although PD-1 blockade has been tested in HIV infection to improve T cell responses and suppress viremia, it remains to be seen whether endogenously blocking PD-1 or other immune checkpoint molecules will boost HIV specific CAR T cell responses *in vivo* (Palmer et al., 2013; Seung et al., 2013). Overall, many strategies to improve peripheral CAR T cell function and persistence *in vivo* have been tested and confirmed in the cancer field. However, it is not yet clear whether any of these strategies will show similar outcomes with HIV-specific CARs.

Another approach, as mentioned above, to solve issues with engraftment, function, and persistence is to engineer the expression of CARs in an HSPC-based approach. Despite the adaptation of improved T cell handling techniques and inclusion of anti-HIV reagents, we found that CD4-CAR T cells made from peripheral blood T cells persisted at low levels and had limited antiviral effects in HIV-infected humanized mice (unpublished data). In contrast, proof of concept studies conducted in our group demonstrated that HSPCs are capable of lifelong engraftment and allow normal development of CAR T cells *in vivo* (Kitchen et al., 2012; Zhen and Kitchen, 2013; Zhen et al., 2017b). This includes thymic selection, eliminating potentially self-reactive T cells, and increasing the potential for the development of immunological memory (Kitchen et al., 2012; Zhen and Kitchen, 2013; Gschweng et al., 2014). Most importantly, our previous data showed that autologous HSPCs modified with a TCR molecular clone (Kitchen et al., 2012) or CD4-CAR (Zhen et al., 2015) against HIV resulted in successful T cell differentiation and significant suppression of HIV replication in humanized bone-marrow-thymus-live mice (huBLT). Further, we demonstrated the feasibility, safety, and potential efficacy of the overall HSPC-based CAR approach in NHPs (Zhen et al., 2017b). In the NHP study, we observed normal hematopoietic recovery, and long-term maintenance of CAR-modified cells (over 2 years) in the absence of any adverse events such as oligoclonal expansion of cells, cytokine storms, self-reactivity, or any other health alterations in transplanted animals. Importantly, we found that CAR-HSPCs transplanted animals have a reduced magnitude of rebound viremia after ART cessation as compared to controls (Zhen et al., 2017b). CAR cells were found in multiple lymphoid tissues, resulting in decreased viral RNA levels in tissues and protection of CD4+ T cells in the gut, which is one of the primary replications and reservoir sites for HIV. Moreover, CAR-engineered HSPCs in both huBLT and NHP models can produce myeloid and NK cells in addition to T cells expressing the CAR, suggesting that CAR-engineered immune cells derived from HSPCs can provide broader immune responses to HIV reactivation after ART interruption. Therefore, stem cell-based

CAR offers a promising approach to generate long-term and effective anti-HIV immunity.

Improving CAR Design and Targeting the HIV Reservoir

Several groups have been studying new generations of CAR T cell therapies and their effects on targeting the HIV reservoir. One study engineered potent bNAb-based single-chain variable fragments fused to second-generation CAR signaling domains (Hale et al., 2017). bNAb-based CAR T cells showed specific activation and killing of HIV-infected vs. uninfected cells in the absence of HIV replication. The study also demonstrated that homology-directed recombination of the CAR gene expression cassette into the *CCR5* locus enhances the suppression of replicating viruses compared with CAR expression alone. Therefore, this work suggested that HIV immunotherapy utilizing an approach that directly delivered the CAR into the *CCR5* locus of T cells by homology-directed gene editing is feasible and effective. A CD4-based CAR T cell therapy with CCR5 disruption by zinc-finger nucleases is in phase I clinical trials to treat HIV and examine effects on the reservoir (NCT03617198).

A third-generation anti-HIV CAR molecule (CD3ζ-CD28-CD137) has been developed that consists of a scFv region derived from the bNAb VRC01 capable of redirecting the antigen specificity of primary CD8+ T cell populations against gp120 (Liu et al., 2016). Interestingly, the bNAb-based CAR T cells were able to effectively kill the reactivated HIV-infected CD4+ T lymphocytes isolated from HIV-infected individuals receiving cART, suggesting CAR T could be a potential therapeutic strategy to eradicate HIV (Liu et al., 2016). Moreover, this research is entering clinical trials (NCT03240328) to evaluate bNAb (VRC01)-based CAR for latent reservoir eradication.

Another strategy to improve targeting of HIV reservoir is to modify bispecific CARs with a homing chemokine receptor CXCR5. CD4+ CXCR5+ TfH cells in B cell follicles of lymphoid tissue have been reported to represent a major HIV reservoir compartment harboring intact and infective proviruses (Perreau et al., 2013; Banga et al., 2016). However, HIV-specific CTLs that recognize and kill virus-producing T cells are found in low numbers within the follicle due to reduced expression of CXCR5 (Mylvaganam et al., 2017; Reuter et al., 2017). Thus, the expression of CXCR5 on HIV-specific CAR T cells might promote their homing to lymph nodes to target latently infected TfH cells. Recent research successfully designed a bispecific anti-SIV CAR co-expressing the rhesus macaque follicular homing chemokine receptor CXCR5 to enhance CAR T cell trafficking to B cell follicles. The functionality of the CAR/CXCR5 T cells was demonstrated through their potent suppression of SIV replication *in vitro* and migration to B cell follicles in lymphoid tissues *ex vivo* (Haran et al., 2018).

Most recently, a universal CAR T cell platform, *convertible*CAR T cells, was designed to redirect CTLs by binding a broadly neutralizing anti-HIV antibody or antibodies. *convertible*CAR was modified with the NKG2D receptor, which can turn the T cell into a potent killer, but only when bound to its partner MIC (MHC-class I-like Complex, natural ligands for NK2G)-bNAbs. *convertible*CAR T cells effectively kill HIV-infected, but not uninfected, CD4 T cells from blood, tonsil, or spleen and only when armed with anti-HIV bNAbs. *convertible*CAR T cells can also result in a 50% reduction in the amount of HIV RNA expression in cultured T cells derived from HIV-infected individuals after reactivation (Herzig et al., 2019). The modularity of *convertible*CAR T cell system, which allows multiplexing with several anti-HIV antibodies yielding greater breadth and control, makes it a promising tool for attacking the latent HIV reservoir.

Another recent study engineered T cells with up to three functionally distinct HIV envelope-binding domains to form bi-specific or tri-specific targeting anti-HIV CAR T cells. Three putative targets included the gp120 CD4-binding site, gp120 coreceptor–binding site, and gp41 near the membrane-proximal external region. Bi-and tri-specific CAR T cells showed the capacity to potently reduce cellular HIV infection both *in vitro* and *in vivo*. The multi-specific CARs efficiently killed HIV-infected cells in a humanized mouse model while protecting the CAR T cells from genetically diverse HIV infection (Anthony-Gonda et al., 2019). Despite the lack of evidence whether multi-specific CAR T cells can effectively migrate to a variety of different tissue sites where established HIV reservoir exist and whether they can target HIV latently infected cells after ART interruption, these data strongly support multi-specific anti-HIV CAR as a promising approach for HIV functional cure.

In spite of the recent success of CAR T therapies in targeting HIV, none of the research has shown an effective and significantly durable reduction in HIV viral load after the adoptive transfer of CARs in HIV/SIV infected animals. None of the recent studies so far have tested different types of new generation CAR T cells in killing reactivated HIV *in vivo*. Thus, further research on the capacity of new generation CARs to eradicate the HIV reservoir should be evaluated in HIV/SHIV infected animal models. The application of CAR therapy in HIV cure strategies is just beginning to be explored, and more work is needed. Better designed CARs should also be considered to increase the cytotoxicity/efficacy, improve the proliferation/persistence, prevent exhaustion/senescence, and lower the potential for resistance/escape.

"Kicking" the HIV Reservoir for CAR T Cell "Killing"

HIV persistence despite ongoing, long term antiretroviral therapy is largely due to the ability of the virus to latently persist in various anatomical reservoirs. Targeting these reservoirs by any immune surveillance mechanism is difficult due to the lack of viral antigen expression. There are several strategies that aim to induce latent viral expression to allow immune targeting and elimination. One such strategy, known as the "kick and kill" approach, seeks to induce the virus out of latency to allow immune-mediated killing. The current paradigm for "kick and kill" strategies to eliminate the HIV reservoir involves the transcriptional reactivation of the integrated provirus in latently infected cells and allow the viral antigen to be presented to

immune surveillance in ART-treated individuals. CAR T cells can be an optimal "kill" response in the "kick and kill" strategy (**Figure 2**). However, this will depend on robust HIV expression from latently infected cells; therefore, a combinatorial therapy with potent latency reversing agents (LRAs) will be necessary to effectively eradicate the reservoir (Bashiri et al., 2018). LRAs have been tested in animal models and clinical trials and shown to induce HIV expression and is well tolerated *in vivo* (Marsden et al., 2017; Thorlund et al., 2017). However, clinical studies have not shown that LRAs alone can significantly decrease the viral reservoir. A likely explanation for this is that the host immune response present in these sites is dysfunctional and incapable of effectively clearing the virus (Collins et al., 1998; Appay et al., 2000; Day et al., 2006; Trautmann et al., 2006; D'Souza et al., 2007; Buggert et al., 2014; Huang et al., 2018). CAR-mediated redirection of T cells and other immune cells could provide greater numbers of HIV-specific cells with a greater functional capacity to eliminate virus-reactivated cells. Another possible reason CTLs at sites of virus reactivation are not effective in killing HIV infected cells could include Nef mediated immune evasion by downregulation of HLA class I molecules. CAR T cells have the advantage of overcoming this type of immune evasion because of its non-dependence in the use of HLA molecules to recognize HIV envelope. Another potential advantage of CAR T cell is that they will more likely target latently infected cells that express provirus with intact envelope rather than defective provirus with envelope deletions, which natural CTLs will target (Bruner et al., 2016; Pollack et al., 2017; Huang et al., 2018). A key question will pertain to the optimal combination of types of LRAs that will induce the minimal levels of HIV expression required for CAR T cell recognition and killing. Ideally, the best LRAs to aid CAR T cell therapy to target the reservoir will be those that will potently induce HIV expression and innate immune response that can provide cytokine support for CAR T cells without major side effects *in vivo*. However, CAR-expressing cells offer a potentially more effective "Kill" component in this overall approach.

A key issue in the "kick and kill" strategy is finding an optimal LRA that allows effective virus reactivation in the absence of gross immune activation and side effects such as cytokine storms. Many different LRAs are currently under investigation and could complement the use of CAR-based approaches in

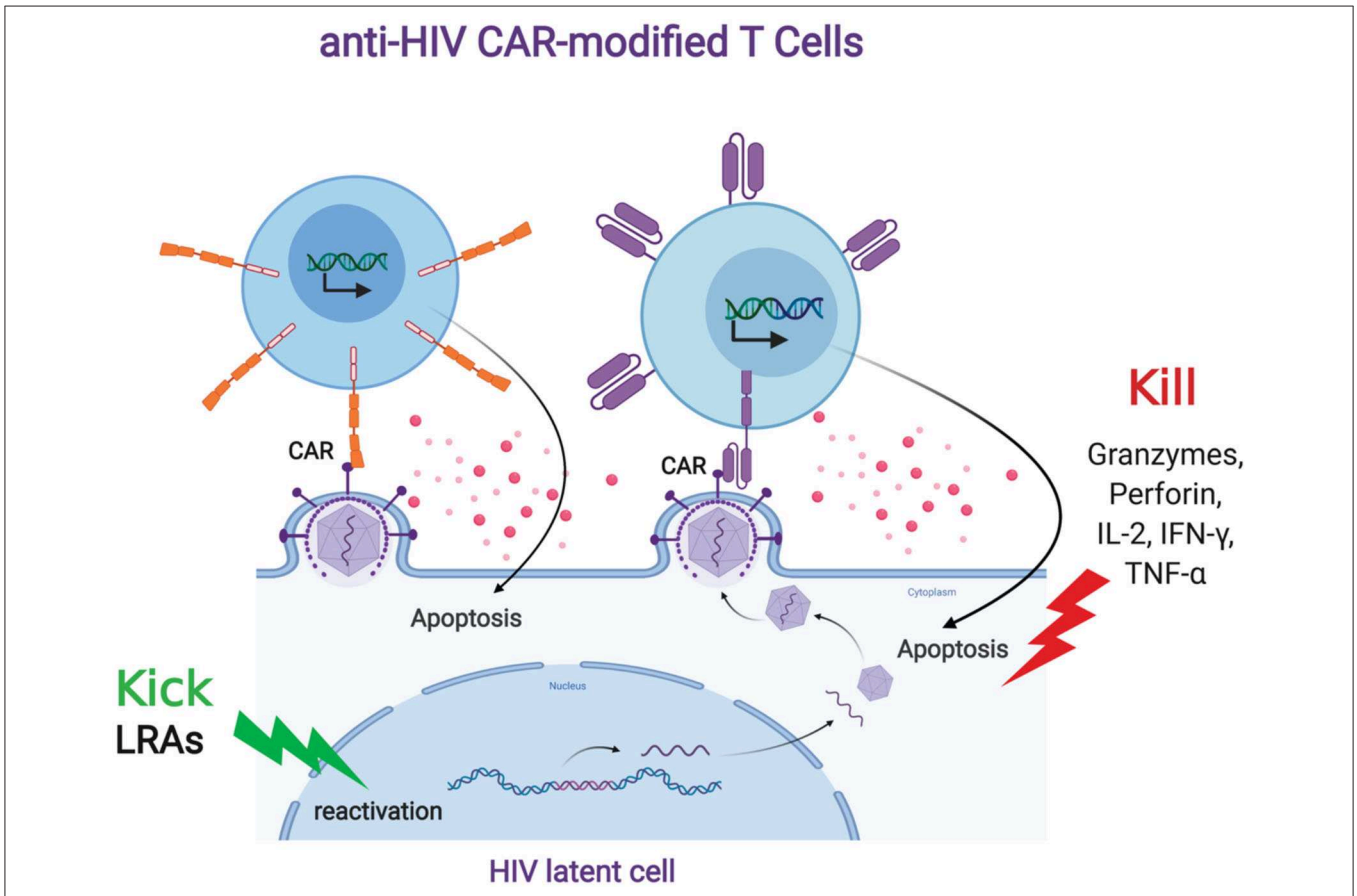


FIGURE 2 | Anti-HIV CAR modified T cells and the "Kick and Kill" strategy to eliminate HIV latently infected cells. The "kick" strategy uses LRAs to induce HIV transcription, protein expression, and virion production. The CD4 (top left, orange) or antibody (top right, purple) based CAR engineered T cell targets an HIV binding site on a viral particle on the cell surface of a reactivated reservoir cell (bottom). Upon binding, the CAR modified T cell will release granzymes and cytokines to "Kill" the HIV infected cells.

targeting the HIV reservoir. LRAs that are classified as histone deacetylase (HDAC) inhibitors (HDACi) have been the most studied in clinical trials (Rasmussen and Lewin, 2016). Although HDAC inhibitors had minimal impact on reducing the size of the viral reservoir in these trials, the drugs Vorinostat, Panobinostat, and Romidepsin did result in measurable increases in the cell-associated HIV RNA levels in CD4+ T cells (Archin et al., 2012, 2017; Elliott et al., 2014; Rasmussen et al., 2014) and plasma viremia in patients (Rasmussen et al., 2014; Sogaard et al., 2015). Thus, because of their safety and induction of latency reversal in several clinical trials, these drugs may be prime candidates to combine with CAR T cell therapy and study the effects on the viral reservoir. Another class of small molecules that have been effective in potently inducing HIV expression are protein kinase C agonists (PKCa) (Williams et al., 2004; Bullen et al., 2014; Jiang and Dandekar, 2015; Laird et al., 2015; Brogdon et al., 2016). The PKCa bryostatin-1 has been tested in phase I clinical trial in ART suppressed patients (Gutierrez et al., 2016). The results of this study showed no impact on the induction of HIV expression, most likely due to a single round of low doses that was well tolerated. However, a study using a humanized mouse model testing synthetic bryostatin analogs showed promising "kick and kill" results (Marsden et al., 2017). The bryostatin analog induced T cell activation in blood and spleen. ART-treated mice that received a single dose of bryostatin analog showed a significant increase in virus expression in blood and spleen and an increase in cell death compared to the control group. The synergistic effect of combining PKCa ingenol-B with HDACi vorinostat was seen in 1 out of 2 ART-treated SIV infected animals that resulted in detectable viral load in plasma and cerebrospinal fluid, which continued to increase several days after LRA interruption (Gama et al., 2017). Other studies using a combination of PKCa and HDAC inhibitors have found similar synergetic results and latency reversal in different memory CD4+ T cell subset populations (Darcis et al., 2015; Laird et al., 2015; Pardons et al., 2019). The combination of HDAC inhibitors and PKCas may provide a more robust "kick" in latency reversal that, in combination with CAR T cells, can potentially lead to a greater killing of the latent reservoir.

Another class of LRAs that have shown to potently induce latency reversal are Toll-like receptor (TLR) agonists (Macedo et al., 2018). In particular, TLR-7 and TLR-9 agonists have shown to be effective HIV latency inducers (Offersen et al., 2016; Tsai et al., 2017). A clinical trial has tested a TLR-9 agonist MGN1703 in virus suppressed individuals (Vibholm et al., 2017). The TLR-9 agonist was well tolerated and led to activation of plasmacytoid dendritic cells, increased activation of NK cells and CD8 T cells. Increased plasma HIV RNA levels were seen only in some of the participants; however, no effect on viral reservoir size was observed. Likewise, with TLR-9 agonists, TLR-7 agonists have also been found to induce viremia, induce activation of CD8+ T cells and NK cells, and target the reservoir in NHPs (Lim et al., 2018). Remarkably, 2 of 9 animals treated with the TLR-7 agonist remained aviremic for over 2 years after ART interruption. In a different study, TLR-7 agonist GS-986 treatment alone led to activation of CD8+ and CD4 T+ cells along with innate immune stimulation (Borducchi et al., 2016). However, a combination treatment using GS-986 (TLR7 agonist) and an Ad26/MVA vaccine led to a significant delay in viral rebound after ART interruption. TLR agonists can potentially be used in combination with CAR T cells to stimulate innate immune cells and support CAR T cells with stimulating cytokines that may lead to increased persistence and expansion, which can result in a synergistic eradication effect.

Cytokines such as IL-2 and IL-7 were initially investigated as potential LRAs to induce HIV expression and target the reservoir (Chun et al., 1999; Stellbrink et al., 2002; Wang et al., 2005; Levy et al., 2012). Recently, Interleukin-15 (IL-15) has also been investigated as a promising target to reactivate the HIV virus and control viral replication (Jones et al., 2016; Ellis-Connell et al., 2018). An IL-15 superagonist ALT-803 was found to induce HIV RNA production in PBMCs obtained from ART-treated participants and enhanced killing of HIV infected cells induced by the superagonist (Jones et al., 2016). More recently, in a NHP model, superagonist IL-15 S-803 induced reactivation of SIV virus but only after depletion of CD8+ T cells, thus revealing a role of CD8+ T cells blocking latency reversing effects of N-803 (McBrien et al., 2020). In addition to the LRA effects of IL-15 superagonist, another study using SIV infected NHP animals has shown that IL-15 ALT 803 can direct SIV specific CD8+ T cells into B cell follicles, which led to decreased SIV RNA or SIV DNA harboring cells in lymph nodes after treatment (Webb et al., 2018). Whether the IL-15 superagonists have the same effects in humans remains to be seen. However, a report from a phase I clinical trial using IL-15 ALT-803 on HIV infected ART-treated participants so far has shown that the drug is well-tolerated, increased NK, CD4+, and CD8+ activation in lymph nodes, and increased HIV transcription following initial treatment was observed (Zachary et al., 2018). Overall, IL-15 superagonists can be used as LRAs with the additional beneficial immune activation properties that, if combined with CAR T cells therapy, can lead to improved trafficking of CAR T cells to reservoir sites *in vivo*.

Although the strategy of combining LRAs that target multiple pathways to induce HIV expression with CAR T cell therapy to purge the viral reservoir can be promising, a current major limitation of LRAs is the inability to reactivate a significant portion of the latent reservoir. To overcome this limitation, other strategies are needed to eliminate unresponsive HIV harboring cells to LRAs. Strategies that target the provirus and attempt to specifically excise it from these cells are under development. Genome editing using CRISPR-Cas9 has been tested *in vivo* using animal models to permanently remove HIV provirus from the genome and shown promising results (Kaminski et al., 2016; Yin et al., 2017; Wang G. et al., 2018; Dash et al., 2019). However, there is a current limitation using CRISPR-Cas9 as HIV can escape through mutations targeted by the single guide RNA (sgRNA) (Wang G. et al., 2016; Wang Z. et al., 2016, 2018). Alternatively, CRISPR-Cas9 system can be used to induce HIV expression by using a catalytically inactive Cas9 fused with a transcriptional activator (Limsirichai et al., 2016). Using this CRISPR-Cas9 transcription activation system, the authors found that combining it with the HDACi suberoylanilide hydroxamic acid (SAHA, also known as Vorinostat) and a prostratin molecule

can synergistically increase HIV expression using J-lat cell lines *in vitro*. It is yet to be seen whether this approach can reactive HIV *in vivo* without any toxic side effects. In summary, CAR T cell therapy can provide the immune surveillance to "kill" latently infected cells in response to agents that can induce HIV expression and innate and cellular immune responses that can support CAR T cell cytotoxicity and trafficking to tissues that harbor viral reservoir.

A major challenge for LRAs to target and reactivate all latent cells is the heterogeneity of the reservoir. The diverse response of latent cells to LRAs have been shown to be based on several factors including cell type, silencing mechanisms inhibiting HIV, tissue reservoir, and patient gender (reviewed in Ait-Ammar et al., 2019). Treatment of CD4+ T cells from ART-treated patients with PKC agonists bryostatin and ingenol revealed that bryostatin was effective in reactivating T effector memory (T_{EM}) cells whereas ingenol was more effective in reactivating HIV in both T central/transitional memory ($T_{CM/TM}$) and T_{EM}, suggesting that T cell memory subsets harboring HIV may not be equally susceptible to the same class of LRAs (Baxter et al., 2016). More recent studies are showing similar results with other classes of LRAs and highlighting the heterogenous responses to several LRA families among different CD4+ T cell subsets; thus, implicating a rationale for the use of combined LRAs to overcome the heterogeneous reservoir (Grau-Expósito et al., 2019; Kulpa et al., 2019). Determining the right combination of LRAs to reactivate these reservoirs will be key to study the effectiveness of anti-HIV CARs, whether it be CD4-based, bi-specific, or tri-specific CARs to kill the reactivated reservoirs. It is likely that cells expressing these different types of CAR molecules would have different effects on clearing out reactivated HIV envelope expressing cells, based on their antigen recognition coverage and the certain limitations that are highlighted above. A CD4-based CAR may provide the widest range of reactivated envelope recognition, but antibody-based CARs, either using multiple single CARs or CARs with multiple specificities could provide sufficient coverage and prevent immune escape. However, it remains to be seen whether anti-HIV CAR expressing cells will be able to target not just CD4+ T cell reservoirs but other cell types such as hematopoietic stem cells and cells from the myeloid lineage that can migrate to anatomical sanctuaries such as the central nervous system (CNS) (Gras and Kaul, 2010; Williams et al., 2014; Gianella et al., 2016; Zaikos et al., 2018). The CNS is considered a sanctuary site that harbors HIV infected cells that can be latently infected, including astrocytes, microglial cells, and perivascular macrophages (Thompson et al., 2011; Wallet et al., 2019). Therefore, it will be important to develop LRAs that can reactivate HIV from CNS cell types. The PKC agonist bryostatin-1 is a promising LRA to target microglial cells and astrocytes (Darcis et al., 2015; Díaz et al., 2015). Information on whether CAR-modified T cells can traffic to the CNS has recently come from clinical studies of B cell malignancies being treated with CD19CAR-modified T cells. These studies show the presence of CD19 CAR-modified T cells in the cerebral spinal fluid (CSF), suggesting these CAR cells may cross the blood brain barrier (Kochenderfer et al., 2017; Santomasso et al., 2018). In addition, a gliospecifc CAR was also found to traffic and target brain tumor in patients (O'Rourke et al., 2017). It has yet to be determined whether anti-HIV CAR T cells will be able to cross the blood brain barrier and target reactivated CNS reservoir cells. However, in our previous study using a stem cell based CD4-based CAR approach in a NHP model of SHIV infection, we observed CD4CAR gene marking in the brain tissue and, compared to control animals, CD4CAR animals showed lower SHIV RNA levels in this compartment (Zhen et al., 2017b). This suggests that, at least in a stem cell CAR model, stem cell derived CD4CAR+ cells can monitor and reduce viral burden in the brain.

Careful consideration should be given about using certain LRAs with CAR-T or CAR-NK cells, as the lack of specificity by LRAs may negatively impact T cell or NK function (Jones et al., 2014; Clutton et al., 2016; Garrido et al., 2016; Pace et al., 2016; Walker-Sperling et al., 2016; Clutton and Jones, 2018; Desimio et al., 2018). In particular, *in vitro* studies show that HDACi panobinostat is found to be toxic to NK cells, decreases their cytotoxicity, antiviral activity, cytokine production, and viability (Garrido et al., 2016). The PKC agonist bryostatin-1 was found to impair NK mediated cytotoxicity, ADCC activity, and clearance of reactivated latently infected CD4+ T cells *in vitro* (Desimio et al., 2018). In T cells, the HDACi romidepsin, panobinosat, and SAHA all found to impair cytokine production and killing of HIV infected CD4+ T cells in CTLs *in vitro*, with the greatest impairment seen with romidepsin (Jones et al., 2014). Similarly, in another study, panobinosat impaired CTL cytotoxicity, whereas romidepsin reduced viability and both impaired proliferation responses in CTLs isolated from HIV infected individuals, although the effects were dependent on exposure time (Clutton et al., 2016). The combination of romidepsin and bryostatin-1 significantly diminished the ability of HIV-specific CD8+ T cells isolated from elite controllers to suppress HIV replication (Walker-Sperling et al., 2016). Thus, some LRAs can have a negative impact on immune function of T and NK cells which may potentially impact CAR-modified T cells and NK cells. More studies are needed to evaluate the optimal doses and combination of LRAs that will reverse latency without toxicity to effector cells that directly kill HIV infected cells. Additionally, a more optimal approach can be to use a cocktail of LRAs that includes immunomodulatory LRAs such as IL-15 agonists and TLR agonists that can boost the immune response and counteract the immunosuppressive side effects of other LRA compounds.

CONCLUSION

There is a considerable amount of effort underway to develop new and novel strategies to eradicate persistent HIV infection. CAR-based approaches represent a promising strategy to enhance the antiviral cellular immune response against HIV in hopes of eradicating the virus. A combination of approaches will likely be necessary to readily facilitate the successful use of anti-HIV CAR therapy to help establish

long-term immune surveillance and kill the reactivated HIV infected cell. However, several issues remain to be answered for further advancement in this field: (1) Whether reactivated antigen expression induced by LRAs is strong enough to be recognized by CAR-modified immune cells? (2) Whether CAR therapy and LRAs could have effects in different anatomical tissue reservoirs, including the gut and the brain? And (3) Whether repeated CAR-modified immune cell infusion and/or repeated rounds of LRA reactivation is needed? There is a high level of optimism that the next generation CAR T cell therapy as part of the "kick and kill" regimen, in combination with other therapies such as LRAs or bNAbs, could eradicate the persistent HIV reservoir by enhancing the immune surveillance and maintaining a long-lasting viral suppression after ART interruption.

AUTHOR CONTRIBUTIONS

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

FUNDING

This work was funded by NIH Grants P30AI28697, 1U19AI117941, 1R33AI122390, P01 AI131294, 1R21AI122390, R01CA239261, 1R01AI140775, CIRM DISC2-10748, UPLIFT: UCLA Postdocs' Longitudinal Investment in Faculty (Award # K12 GM106996).

ACKNOWLEDGMENTS

All figures were created with BioRender.com.

REFERENCES

Adachi, K., Kano, Y., Nagai, T., Okuyama, N., Sakoda, Y., and Tamada, K. (2018). IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat. Biotechnol.* 36, 346–351. doi: 10.1038/nbt.4086

Ait-Ammar, A., Kula, A., Darcis, G., Verdikt, R., De Wit, S., Gautier, V., et al. (2019). Current status of latency reversing agents facing the heterogeneity of HIV-1 cellular and tissue reservoirs. *Front. Microbiol.* 10:3060. doi: 10.3389/fmicb.2019.03060

Ali, A., Kitchen, S. G., Chen, I. S. Y., Ng, H. L., Zack, J. A., and Yang, O. O. (2016). HIV-1-specific chimeric antigen receptors based on broadly neutralizing antibodies. *J. Virol.* 90, 6999–7006. doi: 10.1128/JVI.00805-16

Anthony-Gonda, K., Bardhi, A., Ray, A., Flerin, N., Li, M., Chen, W., et al. (2019). Multispecific anti-HIV duoCAR-T cells display broad *in vitro* antiviral activity and potent *in vivo* elimination of HIV-infected cells in a humanized mouse model. *Sci. Transl. Med.* 11:504. doi: 10.1126/scitranslmed.aav5685

Appay, V., Nixon, D. F., Donahoe, S. M., Gillespie, G. M. A., Dong, T., King, A., et al. (2000). HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. *J. Exp. Med.* 192, 63–75. doi: 10.1084/jem.192.1.63

Archin, N. M., Kirchherr, J. L., Sung, J. A., Clutton, G., Sholtis, K., Xu, Y., et al. (2017). Interval dosing with the HDAC inhibitor vorinostat effectively reverses HIV latency. *J. Clin. Invest.* 127, 3126–3135. doi: 10.1172/JCI92684

Archin, N. M., Liberty, A. L., Kashuba, A. D., Choudhary, S. K., Kuruc, J. D., Crooks, A. M., et al. (2012). Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 487, 482–485. doi: 10.1038/nature11286

Banga, R., Procopio, F. A., Noto, A., Pollakis, G., Cavassini, M., Ohmiti, K., et al. (2016). PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat. Med.* 22, 754–761. doi: 10.1038/nm.4113

Bar, K. J., Sneller, M. C., Harrison, L. J., Justement, J. S., Overton, E. T., Petrone, M. E., et al. (2016). Effect of HIV antibody VRC01 on viral rebound after treatment interruption. *N. Engl. J. Med.* 375, 2037–2050. doi: 10.1056/NEJMoa1608243

Bar-On, Y., Gruell, H., Schoofs, T., Pai, J. A., Nogueira, L., Butler, A. L., et al. (2018). Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. *Nat. Med.* 24, 1701–1707. doi: 10.1038/s41591-018-0186-4

Bashiri, K., Rezaei, N., Nasi, M., and Cossarizza, A. (2018). The role of latency reversal agents in the cure of HIV: a review of current data. *Immunol. Lett.* 196, 135–139. doi: 10.1016/j.imlet.2018.02.004

Batra, S. A., Rathi, P., Guo, L., Courtney, A. N., Fleurence, J., Balzeau, J., et al. (2020). Glypican-3-specific CAR T cells coexpressing IL15 and IL21 have superior expansion and antitumor activity against hepatocellular carcinoma. *Cancer Immunol. Res.* 8, 309–320. doi: 10.1158/2326-6066.CIR-19-0293

Baxter, A. E., Niessl, J., Fromentin, R., Richard, J., Porichis, F., Charlebois, R., et al. (2016). Single-cell characterization of viral translation-competent reservoirs in HIV-infected individuals. *Cell Host Microbe* 20, 368–380. doi: 10.1016/j.chom.2016.07.015

Bonaparte, M. I., and Barker, E. (2004). Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. *Blood* 104, 2087–2094. doi: 10.1182/blood-2004-02-0696

Borducchi, E. N., Cabral, C., Stephenson, K. E., Liu, J. Y., Abbink, P., Ng'ang'a, D., et al. (2016). Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature* 540:284. doi: 10.1038/nature20583

Brogdon, J., Ziani, W., Wang, X., Veazey, R. S., and Xu, H. (2016). *In vitro* effects of the small-molecule protein kinase C agonists on HIV latency reactivation. *Sci. Rep.* 6:39032. doi: 10.1038/srep39032

Bruner, K. M., Murray, A. J., Pollack, R. A., Soliman, M. G., and Laskey, S. B., Capoferri, A. A., et al. (2016). Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat. Med.* 22:1043. doi: 10.1038/nm.4156

Bryceson, Y. T., and Ljunggren, H. G. (2008). Tumor cell recognition by the NK cell activating receptor NKG2D. *Eur. J. Immunol.* 38, 2957–2961. doi: 10.1002/eji.200838833

Buggert, M., Tauriainen, J., Yamamoto, T., Frederiksen, J., Ivarsson, M. A., Michaelsson, J., et al. (2014). T-bet and Eomes are differentially linked to the exhausted phenotype of CD8+ T cells in HIV infection. *PLoS Pathog.* 10:e1004251. doi: 10.1371/journal.ppat.1004251

Bullen, C. K., Laird, G. M., Durand, C. M., Siliciano, J. D., and Siliciano, R. F. (2014). New *ex vivo* approaches distinguish effective and ineffective single agents for reversing HIV-1 latency *in vivo*. *Nat. Med.* 20, 425–429. doi: 10.1038/nm.3489

Burton, D. R., Pyati, J., Koduri, R., Sharp, S. J., Thornton, G. B., Parren, P. W., et al. (1994). Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* 266, 1024–1027. doi: 10.1126/science.7973652

Carrillo, M. A., Zhen, A., Zack, J. A., and Kitchen, S. G. (2017). New approaches for the enhancement of chimeric antigen receptors for the treatment of HIV. *Transl. Res.* 187:83–92. doi: 10.1016/j.trsl.2017.07.002

Cherkassky, L., Morello, A., Villena-Vargas, J., Feng, Y., Dimitrov, D. S., Jones, D. R., et al. (2016). Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J. Clin. Invest.* 126, 3130–3144. doi: 10.1172/JCI83092

Chinnasamy, D., Yu, Z., Kerkar, S. P., Zhang, L., Morgan, R. A., Restifo, N. P., et al. (2012). Local delivery of interleukin-12 using T cells targeting VEGF receptor-2 eradicates multiple vascularized tumors in mice. *Clin. Cancer Res.* 18, 1672–1683. doi: 10.1158/1078-0432.CCR-11-3050

Chmielewski, M., and Abken, H. (2015). TRUCKs: the fourth generation of CARs. *Expert Opin. Biol. Ther.* 15, 1145–1154. doi: 10.1517/14712598.2015.1046430

Chmielewski, M., Hombach, A. A., and Abken, H. (2014). Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol. Rev.* 257, 83–90. doi: 10.1111/imr.12125

Chun, T. W., Engel, D., Mizell, S. B., Hallahan, C. W., Fischette, M., Park, S., et al. (1999). Effect of interleukin-2 on the pool of latently infected, resting CD4(+) T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. *Nature Med.* 5, 651–655. doi: 10.1038/9498

Chung, A., Rollman, E., Johansson, S., Kent, S. J., and Stratov, I. (2008). The utility of ADCC responses in HIV infection. *Curr. HIV Res.* 6, 515–519. doi: 10.2174/157016208786501472

Chung, A. W., Navis, M., Isitman, G., Wren, L., Silvers, J., Amin, J., et al. (2011). Activation of NK cells by ADCC antibodies and HIV disease progression. *J. Acquir. Immune Defic. Syndr.* 58, 127–131. doi: 10.1097/QAI.0b013e31822c62b9

Churchill, M. J., Deeks, S. G., Margolis, D. M., Siliciano, R. F., and Swanstrom, R. (2016). HIV reservoirs: what, where and how to target them. *Nat. Rev. Microbiol.* 14, 55–60. doi: 10.1038/nrmicro.2015.5

Clutton, G., Xu, Y., Baldoni, P. L., Mollan, K. R., Kirchherr, J., Newhard, W., et al. (2016). The differential short- and long-term effects of HIV-1 latency-reversing agents on T cell function. *Sci. Rep*. 6:30749. doi: 10.1038/srep30749

Clutton, G. T., and Jones, R. B. (2018). Diverse Impacts of HIV Latency-Reversing Agents on CD8+ T-Cell Function: Implications for HIV Cure. *Front. Immunol.* 9:1452. doi: 10.3389/fimmu.2018.01452

Coiras, M., Bermejo, M., Descours, B., Mateos, E., Garcia-Perez, J., Lopez-Huertas, M. R., et al. (2016). IL-7 induces SAMHD1 phosphorylation in CD4+ T Lymphocytes, improving early steps of HIV-1 life cycle. *Cell Rep.* 14, 2100–2107. doi: 10.1016/j.celrep.2016.02.022

Collins, K. L., Chen, B. K., Kalams, S. A., Walker, B. D., and Baltimore, D. (1998). HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* 391, 397–401. doi: 10.1038/34929

Darcis, G., Kula, A., Bouchat, S., Fujinaga, K., Corazza, F., Ait-Ammar, A., et al. (2015). An in-depth comparison of latency-reversing agent combinations in various *in vitro* and *ex vivo* HIV-1 latency models identified bryostatin-1+JQ1 and ingenol-B+JQ1 to potently reactivate viral gene expression. *PLoS Pathog.* 11:e1005063. doi: 10.1371/journal.ppat.1005063

Dash, P. K., Kaminski, R., Bella, R., Su, H., Mathews, S., Ahooyi, T. M., et al. (2019). Sequential LASER ART and CRISPR treatments eliminate HIV-1 in a subset of infected humanized mice. *Nat. Commun.* 10:2753. doi: 10.1038/s41467-019-10366-y

Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., et al. (2006). PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443, 350–354. doi: 10.1038/nature05115

Deeks, S. G., Wagner, B., Anton, P. A., Mitsuyasu, R. T., Scadden, D. T., Huang, C., et al. (2002). A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy. *Mol. Ther.* 5, 788–797. doi: 10.1006/mthe.2002.0611

Desimio, M. G., Giuliani, E., Ferraro, A. S., Adorno, G., and Doria, M. (2018). In vitro exposure to prostratin but not bryostatin-1 improves natural killer cell functions including killing of CD4+ T cells harboring reactivated human immunodeficiency virus. *Front. Immunol.* 9:514. doi: 10.3389/fimmu.2018.01514

Díaz, L., Martínez-Bonet, M., Sánchez, J., Fernández-Pineda, A., Jiménez, J. L., Muñoz, E., Moreno, S., Álvarez, S., and MÁ Muñoz-Fernández (2015). Bryostatin activates HIV-1 latent expression in human astrocytes through a PKC and NF-κB-dependent mechanism. *Sci. Rep*. doi: 10.1038/srep12442

Doria-Rose, N. A., Schramm, C. A., Gorman, J., Moore, P. L., Bhiman, J. N., DeKosky, B. J., et al. (2014). Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature* 509:55. doi: 10.1038/nature13036

D'Souza, M., Fontenot, A. P., Mack, D. G., Lozupone, C., Dillon, S., Meditz, A., et al. (2007). Programmed death 1 expression on HIV-specific CD4(+) T cells is driven by viral replication and associated with T cell dysfunction. *J. Immunol.* 179, 1979–1987. doi: 10.4049/jimmunol.179.3.1979

Elliott, J. H., Wightman, F., Solomon, A., Ghneim, K., Ahlers, J., Cameron, M. J., et al. (2014). Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. *PLoS Pathog* 10:e1004473. doi: 10.1371/journal.ppat.1004473

Ellis-Connell, A. L., Balgeman, A. J., Zarbock, K. R., Barry, G., Weiler, A., Egan, J. O., et al. (2018). ALT-803 transiently reduces simian immunodeficiency virus replication in the absence of antiretroviral treatment. *J. Virol.* 92:17. doi: 10.1128/JVI.01748-17

Eshhar, Z., Waks, T., Gross, G., and Schindler, D. G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc. Natl. Acad. Sci. U.S.A.* 90, 720–724. doi: 10.1073/pnas.90.2.720

Feinberg, H., Mitchell, D. A., Drickamer, K., and Weis, W. I. (2001). Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* 294, 2163–2166. doi: 10.1126/science.1066371

Fenwick, C., Joo, V., Jacquier, P., Noto, A., Banga, R., Perreau, M., et al. (2019). T-cell exhaustion in HIV infection. *Immunol. Rev.* 292, 149–163. doi: 10.1111/imr.12823

Fesnak, A. D., June, C. H., and Levine, B. L. (2016). Engineered T cells: the promise and challenges of cancer immunotherapy. *Nat. Rev. Cancer* 16, 566–581. doi: 10.1038/nrc.2016.97

Fry, T. J., Shah, N. N., Orentas, R. J., Stetler-Stevenson, M., Yuan, C. M., Ramakrishna, S., et al. (2018). CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat. Med.* 24, 20–28. doi: 10.1038/nm.4441

Gama, L., Abreu, C. M., Shirk, E. N., Price, S. L., Li, M., Laird, G. M., et al. (2017). Reactivation of simian immunodeficiency virus reservoirs in the brain of virally suppressed macaques. *AIDS* 31, 5–14. doi: 10.1097/QAD.0000000000001267

Garrido, C., Spivak, A. M., Soriano-Sarabia, N., Checkley, M. A., Barker, E., Karn, J., et al. (2016). HIV latency-reversing agents have diverse effects on natural killer cell function. *Front. Immunol.* 7:356. doi: 10.3389/fimmu.2016.00356

Ghanem, M. H., Bolivar-Wagers, S., Dey, B., Hajduczki, A., Vargas-Inchaustegui, D. A., Danielson, D. T., et al. (2018). Bispecific chimeric antigen receptors targeting the CD4 binding site and high-mannose Glycans of gp120 optimized for anti-human immunodeficiency virus potency and breadth with minimal immunogenicity. *Cytotherapy* 20, 407–419. doi: 10.1016/j.jcyt.2017.11.001

Gianella, S., Kosakovsky Pond, S. L., Oliveira, M. F., Scheffler, K., Strain, M. C. A., De la Torre Letendre, S., et al. (2016). Compartmentalized HIV rebound in the central nervous system after interruption of antiretroviral therapy. *Virus Evol.* 2. doi: 10.1093/ve/vew020

Goulder, P. J., and Walker, B. D. (1999). The great escape - AIDS viruses and immune control. *Nat. Med.* 5, 1233–1235. doi: 10.1038/15184

Gras, G., and Kaul, M. (2010). Molecular mechanisms of neuroinvasion by monocytes-macrophages in HIV-1 infection. *Retrovirology* 7, 30. doi: 10.1186/1742-4690-7-30

Grau-Expósito, J., Luque-Ballesteros, L., Navarro, J., Curran, A., Burgos, J., Ribera, E., et al. (2019). Latency reversal agents affect differently the latent reservoir present in distinct CD4+ T subpopulations. *PLoS Pathog.* 15:e1007991. doi: 10.1371/journal.ppat.1007991

Grupp, S. A., Kalos, M., Barrett, D., Aplenc, R., Porter, D. L., Rheingold, S. R., et al. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* 368, 1509–1518. doi: 10.1056/NEJMoa1215134

Gschweng, E., De Oliveira, S., and Kohn, D. B. (2014). Hematopoietic stem cells for cancer immunotherapy. *Immunol. Rev.* 257, 237–249. doi: 10.1111/imr.12128

Guedan, S., Calderon, H., Posey, A. D. Jr., and Maus, M. V. (2019). Engineering and design of chimeric antigen receptors. *Mol. Ther. Methods Clin. Dev.* 12, 145–156. doi: 10.1016/j.omtm.2018.12.009

Gutierrez, C., Serrano-Villar, S., Madrid-Elena, N., Perez-Elias, M. J., Martin, M. E., Barbas, C., et al. (2016). Bryostatin-1 for latent virus reactivation in HIV-infected patients on antiretroviral therapy. *AIDS* 30, 1385–1392. doi: 10.1097/QAD.0000000000001064

Hale, M., Mesojednik, T., Romano Ibarra, G. S., Sahni, J., Bernard, A., Sommer, K., et al. (2017). Engineering HIV-resistant, anti-HIV chimeric antigen receptor T cells. *Mol. Ther.* 25, 570–579. doi: 10.1016/j.ymthe.2016.12.023

Haran, K. P., Hajduczki, A., Pampusch, M. S., Mwakalundwa, G., Vargas-Inchaustegui, D. A., Rakasz, E. G., et al. (2018). Simian immunodeficiency virus (SIV)-specific chimeric antigen receptor-T cells engineered to target B cell follicles and suppress SIV replication. *Front. Immunol.* 9:492. doi: 10.3389/fimmu.2018.00492

Herzig, E., Kim, K. C., Packard, T. A., Vardi, N., Schwarzer, R., Gramatica, A., et al. (2019). Attacking latent HIV with convertibleCAR-T cells, a highly adaptable killing platform. *Cell* 179, 880–894. doi: 10.1016/j.cell.2019.10.002

Heuser, C., Hombach, A., Losch, C., Manista, K., and Abken, H. (2003). T-cell activation by recombinant immunoreceptors: impact of the intracellular signalling domain on the stability of receptor expression and antigen-specific activation of grafted T cells. *Gene Ther.* 10, 1408–1419. doi: 10.1038/sj.gt.3302023

Hoyos, V., Savoldo, B., Quintarelli, C., Mahendravada, A., Zhang, M., Vera, J., et al. (2010). Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. *Leukemia* 24, 1160–1170. doi: 10.1038/leu.2010.75

Hu, B., Ren, J., Luo, Y., Keith, B., Young, R. M., Scholler, J., et al. (2017). Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18. *Cell Rep.* 20, 3025–3033. doi: 10.1016/j.celrep.2017.09.002

Hu, B., Zou, Y., Zhang, L., Tang, J., Niedermann, G., Firat, E., et al. (2019a). Nucleofection with plasmid DNA for CRISPR/Cas9-mediated inactivation of programmed cell death protein 1 in CD133-specific CAR T cells. *Hum. Gene Ther.* 30, 446–458. doi: 10.1089/hum.2017.234

Hu, W., Zi, Z., Jin, Y., Li, G., Shao, K., Cai, Q., et al. (2019b). CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions. *Cancer Immunol. Immunother.* 68, 365–377. doi: 10.1007/s00262-018-2281-2

Huang, J., Ofek, G., Laub, L., Louder, M. K., Doria-Rose, N. A., Longo, N. S., et al. (2012). Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature* 491, 406–412. doi: 10.1038/nature11544

Huang, J. H., Kang, B. H., Ishida, E., Zhou, T. Q., Griesman, T., Sheng, Z. Z., et al. (2016). Identification of a CD4-binding-site antibody to HIV that evolved near-pan neutralization breadth. *Immunity* 45, 1108–1121. doi: 10.1016/j.immuni.2016.10.027

Huang, S. H., Ren, Y. Q., Thomas, A. S., Chan, D., Mueller, S., Ward, A. R., et al. (2018). Latent HIV reservoirs exhibit inherent resistance to elimination by CD8(+) T cells. *J. Clin. Invest.* 28, 876–889. doi: 10.1172/JCI97555

Hurton, L. V., Singh, H., Najjar, A. M., Switzer, K. C., Mi, T., Maiti, S., et al. (2016). Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells. *Proc. Natl. Acad. Sci. U.S.A.* 113, E7788–E7797. doi: 10.1073/pnas.1610544113

International et al. (2010). The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. *Science* 330, 1551–1557. doi: 10.1126/science.1195271

Jiang, G. C., and Dandekar, S. (2015). Targeting NF-kappa B signaling with protein kinase C agonists as an emerging strategy for combating HIV latency. *Aids Res. Human Retrov.* 1, 4–12. doi: 10.1089/aid.2014.0199

Jones, R. B., Mueller, S., O'Connor, R., Rimpel, K., Sloan, D. D., Karel, D., et al. (2016). A subset of latency-reversing agents expose HIV-infected resting CD4(+) T-cells to recognition by cytotoxic T-lymphocytes. *PLoS Pathogens.* 12:545. doi: 10.1371/journal.ppat.1005545

Jones, R. B., O'Connor, R., Mueller, S., Foley, M., Szeto, G. L., Karel, D., et al. (2014). Histone deacetylase inhibitors impair the elimination of HIV-infected cells by cytotoxic T-lymphocytes. *PLoS Pathog.* 10:e1004287. doi: 10.1371/journal.ppat.1004287

Jones, R. B., and Walker, B. D. (2016). HIV-specific CD8(+) T cells and HIV eradication. *J. Clin. Invest.* 126, 455–463. doi: 10.1172/JCI80566

June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S., and Milone, M. C. (2018). CAR T cell immunotherapy for human cancer. *Science* 359, 361–1365. doi: 10.1126/science.aar6711

Kalos, M., Levine, B. L., Porter, D. L., Katz, S., Grupp, S. A., Bagg, A., et al. (2011). T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci. Transl. Med.* 3:95ra73. doi: 10.1126/scitranslmed.3002842

Kamata, M., Kim, P. Y., Ng, H. L., Ringpis, G. E., Kranz, E., Chan, J., et al. (2015). Ectopic expression of anti-HIV-1 shRNAs protects CD8(+) T cells modified with CD4zeta CAR from HIV-1 infection and alleviates impairment of cell proliferation. *Biochem. Biophys. Res. Commun.* 463, 216–221. doi: 10.1016/j.bbrc.2015.05.026

Kaminski, R., Chen, Y., Fischer, T., Tedaldi, E., Napoli, A., Zhang, Y., et al. (2016). Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. *Sci. Rep.* 6:22555. doi: 10.1038/srep28213

Kawalekar, O. U., O' Connor, R. S., Fraietta, J. A., Guo, L., McGettigan, S. E., Posey, A. D. Jr., et al. (2016). Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T Cells. *Immunity* 44:712. doi: 10.1016/j.immuni.2016.02.023

Kim, Y., Anderson, J. L., and Lewin, S. R. (2018). Getting the "Kill" into "Shock and Kill": strategies to eliminate latent HIV. *Cell Host Microbe* 23, 14–26. doi: 10.1016/j.chom.2017.12.004

Kitchen, S. G., Levin, B. R., Bristol, G., Rezek, V., Kim, S., Aguilera-Sandoval, C., et al. (2012). In vivo suppression of HIV by antigen specific T cells derived from engineered hematopoietic stem cells. *PLoS Pathog.* 8:e1002649. doi: 10.1371/journal.ppat.1002649

Kochenderfer, J. N., Dudley, M. E., Feldman, S. A., Wilson, W. H., Spaner, D. E., Maric, I., et al. (2012). B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 119, 2709–2720. doi: 10.1182/blood-2011-10-384388

Kochenderfer, J. N., Somerville, R. P., Lu, T., Shi, V., Bot, A., Rossi, J., et al. (2017). Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum interleukin-15 levels. *In. J. Clin. Oncol.* 35, 1803–1813. doi: 10.1200/JCO.2016.71.3024

Kuhlmann, A. S., Peterson, C. W., and Kiem, H. P. (2018). Chimeric antigen receptor T-cell approaches to HIV cure. *Curr. Opin. HIV AIDS* 13, 446–453. doi: 10.1097/COH.0000000000000485

Kulpa, D. A., Talla, A., Brehm, J. H., Ribeiro, S. P., Yuan, S., Bebin-Blackwell, A. G., et al. (2019). Differentiation into an effector memory phenotype potentiates HIV-1 latency reversal in CD4(+) T cells. *J. Virol.* 93:19. doi: 10.1128/JVI.00969-19

Labanieh, L., Majzner, R. G., and Mackall, C. L. (2018). Programming CAR-T cells to kill cancer. *Nat. Biomed. Eng.* 2, 377–391. doi: 10.1038/s41551-018-0235-9

Laird, G. M., Bullen, C. K., Rosenbloom, D. I., Martin, A. R., Hill, A. L., Durand, C. M., et al. (2015). *Ex vivo* analysis identifies effective HIV-1 latency-reversing drug combinations. *J. Clin. Invest.* 125, 1901–1912. doi: 10.1172/JCI80142

Le Bert, N., and Gasser, S. (2014). Advances in NKG2D ligand recognition and responses by NK cells. *Immunol. Cell. Biol.* 92, 230–236. doi: 10.1038/icb.2013.111

Leibman, R. S., Richardson, M. W., Ellebrecht, C. T., Maldini, C. R., Glover, J. A., Secreto, A. J., et al. (2017). Supraphysiologic control over HIV-1 replication mediated by CD8 T cells expressing a re-engineered CD4-based chimeric antigen receptor. *PLoS Pathog.* 13:e1006613. doi: 10.1371/journal.ppat.1006613

Leslie, G. J., Wang, J., Richardson, M. W., Haggarty, B. S., Hua, K. L., Duong, J., et al. (2016). Potent and broad inhibition of HIV-1 by a peptide from the gp41 heptad repeat-2 domain conjugated to the CXCR4 amino terminus. *PLoS Pathog.* 12:e1005983. doi: 10.1371/journal.ppat.1005983

Levy, Y., Sereti, I., Tambussi, G., Routy, J. P., Lelievre, J. D., Delfraissy, J. F., et al. (2012). Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo-controlled, multicenter study. *Clin. Infectious Diseases* 55, 291–300. doi: 10.1093/cid/cis383

Lim, S. Y., Osuna, C. E., Hraber, P. T., Hesselgesser, J., Gerold, J. M., Barnes, T. L., et al. (2018). TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci. Trans. Med.* 10:439. doi: 10.1126/scitranslmed.aao4521

Limsirichai, P., Gaj, T., and Schaffer, D. V. (2016). CRISPR-mediated activation of latent HIV-1 expression. *Mol. Ther.* 24, 499–507. doi: 10.1038/mt.2015.213

Liu, B., Zou, F., Lu, L., Chen, C., He, D., Zhang, X., et al. (2016). Chimeric antigen receptor T cells guided by the single-chain Fv of a broadly neutralizing antibody specifically and effectively eradicate virus reactivated from latency in CD4+ T lymphocytes isolated from HIV-1-infected individuals receiving suppressive combined antiretroviral therapy. *J. Virol.* 90, 9712–9724. doi: 10.1128/JVI.00852-16

Liu, D., Tian, S., Zhang, K., Xiong, W., Lubaki, N. M., Chen, Z., et al. (2017). Chimeric antigen receptor (CAR)-modified natural killer cell-based immunotherapy and immunological synapse formation in cancer and HIV. *Protein Cell* 8, 861–877. doi: 10.1007/s13238-017-0415-5

Liu, E., Marin, D., Banerjee, P., Macapinlac, H. A., Thompson, P., Basar, R., et al. (2020). Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *N. Engl. J. Med.* 382, 545–553. doi: 10.1056/NEJMoa 1910607

Macedo, A. B., Novis, C. L., De Assis, C. M., Sorensen, E. S., Moszczynski, P., Huang, S. H., et al. (2018). Dual TLR2 and TLR7 agonists as HIV latency-reversing agents. *Jci. Insight* 3:673. doi: 10.1172/jci.insight.122673

Managlia, E. Z., Landay, A., and Al-Harthi L. (2006). Interleukin-7 induces HIV replication in primary naive T cells through a nuclear factor of activated T cell (NFAT)-dependent pathway. *Virology* 350, 443–452. doi: 10.1016/j.virol.2006.02.019

Manganaro, L., Hong, P., Hernandez, M. M., Argyle, D., Mulder, L. C. F., Potla, U., et al. (2018). IL-15 regulates susceptibility of CD4(+) T cells to HIV infection. *Proc. Natl. Acad. Sci. U.S.A.* 115, E9659–E9667. doi: 10.1073/pnas.1806695115

Marban, C., Forouzanfar, F., Ait-Ammar, A., Fahmi, F., El Mekdad, H., Daouad, F., et al. (2016). Targeting the brain reservoirs: toward an HIV Cure. *Front. Immunol.* 7:397. doi: 10.3389/fimmu.2016.00397

Marsden, M. D., Loy, B. A., Wu, X. M., Ramirez, C. M., Schrier, A. J., Murray, D., et al. (2017). *In vivo* activation of latent HIV with a synthetic bryostatin analog effects both latent cell "kick" and "kill" in strategy for virus eradication. *PLoS Pathogens* 13:575. doi: 10.1371/journal.ppat.1006575

Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* 371, 1507–1517. doi: 10.1056/NEJMoa1407222

McBrien, J. B., Mavigner, M., Franchitti, L., Smith, S. A., White, E., Tharp, G. K., et al. (2020). Robust and persistent reactivation of SIV and HIV by N-803 and depletion of CD8(+) cells. *Nature.* 578, 154–159. doi: 10.1038/s41586-020-1946-0

Mehta, R. S., and Rezvani, K. (2018). Chimeric antigen receptor expressing natural killer cells for the immunotherapy of cancer. *Front. Immunol.* 9:283. doi: 10.3389/fimmu.2018.00283

Mendoza, P., Gruell, H., Nogueira, L., Pai, J. A., Butler, A. L., Millard, K., et al. (2018). Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature*, 479–484. doi: 10.1038/s41586-018-0531-2

Mitsuyasu, R. T., Anton, P. A., Deeks, S. G., Scadden, D. T., Connick, E., Downs, M. T., et al. (2000). Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. *Blood* 96, 785–793. doi: 10.1182/blood.V96.3.785

Mueller, K. T., Maude, S. L., Porter, D. L., Frey, N., Wood, P., Han, X., et al. (2017). Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia. *Blood* 130, 2317–2325. doi: 10.1182/blood-2017-06-786129

Mueller, Y. M., Do, D. H., Altork, S. R., Artlett, C. M., Gracely, E. J., Katsetos, C. D., et al. (2008). IL-15 treatment during acute simian immunodeficiency virus (SIV) infection increases viral set point and accelerates disease progression despite the induction of stronger SIV-specific CD8+ T cell responses. *J. Immunol.* 180, 350–360. doi: 10.4049/jimmunol.180.1.350

Mylvaganam, G. H., Rios, D., Abdelaal, H. M., Iyer, S., Tharp, G., Mavigner, M., et al. (2017). Dynamics of SIV-specific CXCR5+ CD8 T cells during chronic SIV infection. *Proc. Natl. Acad. Sci. U.S.A.* 114, 1976–1981. doi: 10.1073/pnas.1621418114

Offersen, R., Nissen, S. K., Rasmussen, T. A., Ostergaard, L., Denton, P. W., Sogaard, O. S., et al. (2016). A novel toll-like receptor 9 agonist, MGN1703, enhances HIV-1 transcription and NK cell-mediated inhibition of HIV-1-infected autologous CD4(+) T Cells. *J. Virol.* 90, 4441–4453. doi: 10.1128/JVI.00222-16

Ogasawara, K., and Lanier, L. L. (2005). NKG2D in NK and T cell-mediated immunity. *J. Clin. Immunol.* 25, 534–540. doi: 10.1007/s10875-005-8786-4

O'Rourke, D. M., Nasrallah, M. P., Desai, A., Melenhorst, J. J., Mansfield, K., Morrissette, J. J. D., et al. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci. Transl. Med.* 9:399. doi: 10.1126/scitranslmed.aaa0984

Pace, M., Williams, J., Kurioka, A., Gerry, A. B., Jakobsen, B., Klenerman, P., et al. (2016). Histone deacetylase inhibitors enhance CD4 T cell susceptibility to NK cell killing but reduce NK cell function. *PLoS Pathog.* 12:e1005782. doi: 10.1371/journal.ppat.1005782

Palmer, B. E., Neff, C. P., Lecureux, J., Ehler, A., Dsouza, M., Remling-Mulder, L., et al. (2013). *In vivo* blockade of the PD-1 receptor suppresses HIV-1 viral loads and improves CD4+ T cell levels in humanized mice. *J. Immunol.* 190, 211–219. doi: 10.4049/jimmunol.1201108

Pardons, M., Fromentin, R., Pagliuzza, A., Routy, J. P., and Chomont, N. (2019). Latency-reversing agents induce differential responses in distinct memory CD4 T cell subsets in individuals on antiretroviral therapy. *Cell Rep.* 29, 2783–2795. doi: 10.1016/j.celrep.2019.10.101

Pegram, H. J., Purdon, T. J., van Leeuwen, D. G., Curran, K. J., Giralt, S. A., Barker, J. N., et al. (2015). IL-12-secreting CD19-targeted cord blood-derived T cells for the immunotherapy of B-cell acute lymphoblastic leukemia. *Leukemia* 29, 415–422. doi: 10.1038/leu.2014.215

Perreau, M., Savoye, A. L., De Crignis, E., Corpataux, J. M., Cubas, R., Haddad, E. K., et al. (2013). Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J. Exp. Med.* 210, 143–156. doi: 10.1084/jem.20121932

Pollack, R. A., Jones, R. B., Pertea, M., Bruner, K. M., Martin, A. R., Thomas, A. S., et al. (2017). Defective HIV-1 proviruses are expressed and can be recognized by cytotoxic T lymphocytes, which shape the proviral landscape. *Cell Host Microbe* 21, 494–506. doi: 10.1016/j.chom.2017.03.008

Rafiq, S., Yeku, O. O., Jackson, H. J., Purdon, T. J., van Leeuwen, D. G., Drakes, D. J., et al. (2018). Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy *in vivo*. *Nat. Biotechnol.* 36, 847–856. doi: 10.1038/nbt.4195

Ramos, C. A., Heslop, H. E., and Brenner, M. K. (2016). CAR-T Cell Therapy for Lymphoma. *Annu. Rev. Med.* 67, 165–183. doi: 10.1146/annurev-med-051914-021702

Rasmussen, T. A., and Lewin, S. R. (2016). Shocking HIV out of hiding: where are we with clinical trials of latency reversing agents? *Curr. Opin. HIV AIDS* 11, 394–401. doi: 10.1097/COH.0000000000000279

Rasmussen, T. A., Tolstrup, M., Brinkmann, C. R., Olesen, R., Erikstrup, C., Solomon, A., et al. (2014). Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV* 1, e13–e21. doi: 10.1016/S2352-3018(14)70014-1

Reuter, M. A. P. M., Del Rio, E.strada, Buggert, M., Petrovas, C., Ferrando-Martinez, S., Nguyen, S., Sada Japp, A., et al. (2017). HIV-specific CD8(+) T cells exhibit reduced and differentially regulated cytolytic activity in lymphoid tissue. *Cell Rep.* 21, 3458–3470. doi: 10.1016/j.celrep.2017.11.075

Richard, J., Sindhu, S., Pham, T. N., Belzile, J. P., and Cohen, E. A. (2010). HIV-1 Vpr up-regulates expression of ligands for the activating NKG2D receptor and promotes NK cell-mediated killing. *Blood* 115, 1354–1363. doi: 10.1182/blood-2009-08-237370

Ringpis, G. E., Shimizu, S., Arokium, H., Camba-Colon, J., Carroll, M. V., Cortado, R., et al. (2012). Engineering HIV-1-resistant T-cells from short-hairpin RNA-expressing hematopoietic stem/progenitor cells in humanized BLT mice. *PLoS ONE* 7:e53492. doi: 10.1371/journal.pone.0053492

Ruella, M., Barrett, D. M., Kenderian, S. S., Shestova, O., Hofmann, T. J., Perazzelli, J., et al. (2016). Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. *J. Clin. Invest.* 126, 3814–3826. doi: 10.1172/JCI87366

Rupp, L. J., Schumann, K., Roybal, K. T., Gate, R. E., Ye, C. J., Lim, W. A., et al. (2017). CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. *Sci. Rep.* 7:737. doi: 10.1038/s41598-017-00462-8

Rydyznski, C., Daniels, K. A., Karmele, E. P., Brooks, T. R., Mahl, S. E., Moran, M. T., et al. (2015). Generation of cellular immune memory and B-cell immunity is impaired by natural killer cells. *Nat. Commun.* 6:6375. doi: 10.1038/ncomms7375

Santomasso, B. D., Park, J. H., Salloum, D., Riviere, I., Flynn, J., Mead, E., et al. (2018). Clinical and Biologic Correlates of Neurotoxicity Associated with CAR T Cell Therapy in Patients with B-cell Acute Lymphoblastic Leukemia (B-ALL). *Cancer Discov.* 8, 958–971. doi: 10.1158/2159-8290.CD-17-1319

Savoldo, B., Ramos, C. A., Liu, E., Mims, M. P., Keating, M. J., Carrum, G., et al. (2011). CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J. Clin. Invest.* 121, 1822–1826. doi: 10.1172/JCI46110

Scheid, J. F., Horwitz, J. A., Bar-On, Y., Kreider, E. F., Lu, C. L., Lorenzi, J. C., et al. (2016). HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 535, 556–560. doi: 10.1038/nature18929

Scheid, J. F., Mouquet, H., Ueberheide, B., Diskin, R., Klein, F., Oliveira, T. Y. K., et al. (2011). Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* 333, 1633–1637. doi: 10.1126/science.1207227

Scholler, J., Brady, T. L., Binder-Scholl, G., Hwang, W. T., Plesa, G., Hege, K. M., et al. (2012). Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Sci. Transl. Med.* 4:132ra53. doi: 10.1126/scitranslmed.3003761

Seung, E., Dudek, T. E., Allen, T. M., Freeman, G. J., Luster, A. D., and Tager, A. M. (2013). PD-1 blockade in chronically HIV-1-infected humanized mice suppresses viral loads. *PLoS ONE* 8:e77780. doi: 10.1371/journal.pone.0077780

Shimizu, S., Hong, P., Arumugam, B., Pokomo, L., Boyer, J., Koizumi, N., et al. (2010). A highly efficient short hairpin RNA potently down-regulates CCR5 expression in systemic lymphoid organs in the hu-BLT mouse model. *Blood* 115, 1534–1544. doi: 10.1182/blood-2009-04-215855

Sogaard, O. S., Graversen, M. E., Leth, S., Olesen, R., Brinkmann, C. R., Nissen, S. K., et al. (2015). The depsipeptide romidepsin reverses HIV-1 latency *in vivo*. *PLoS Pathog.* 11:e1005142. doi: 10.1371/journal.ppat.1005142

Sok, D., and Burton, D. R. (2018). Recent progress in broadly neutralizing antibodies to HIV. *Nature Immunol.* 19, 1179–1188. doi: 10.1038/s41590-018-0235-7

Sommermeyer, D., Hill, T., Shamah, S. M., Salter, A. I., Chen, Y., Mohler, K. M., et al. (2017). Fully human CD19-specific chimeric antigen receptors for T-cell therapy. *Leukemia* 31, 2191–2199. doi: 10.1038/leu.2017.57

Spivak, A. M., Andrade, A., Eisele, E., Hoh, R., Bacchetti, P., Bumpus, N. N., et al. (2014). A pilot study assessing the safety and latency-reversing activity of disulfiram in HIV-1-infected adults on antiretroviral therapy. *Clin. Infect. Dis.* 58, 883–890. doi: 10.1093/cid/cit813

Stellbrink, H. J., van Lunzen, J., Westby, M., O'Sullivan, E., Schneider, C., Adam, A., et al. (2002). Effects of interleukin-2 plus highly active antiretroviral therapy on HIV-1 replication and proviral DNA (COSMIC trial) (vol 16, pg 1479, 2002). *Aids* 16, 2103–2103. doi: 10.1097/00002030-200207260-00004

Stojanovic, A., Correia, M. P., and Cerwenka, A. (2018). The NKG2D/NKG2DL axis in the crosstalk between lymphoid and myeloid cells in health and disease. *Front. Immunol.* 9:827. doi: 10.3389/fimmu.2018.00827

Thompson, K. A., Cherry, C. L., Bell, J. E., and McLean, C. A. (2011). Brain cell reservoirs of latent virus in presymptomatic HIV-infected individuals. *Am. J. Pathol.* 179, 1623–1629. doi: 10.1016/j.ajpath.2011.06.039

Thorlund, K., Horwitz, M. S., Fife, B. T., Lester, R., and Cameron, D. W. (2017). Landscape review of current HIV 'kick and kill' cure research - some kicking, not enough killing. *BMC Infectious Diseases* 17:833. doi: 10.1186/s12879-017-2683-3

Tran, A. C., Zhang, D., Byrn, R., and Roberts, M. R. (1995). Chimeric zeta-receptors direct human natural killer (NK) effector function to permit killing of NK-resistant tumor cells and HIV-infected T lymphocytes. *J. Immunol.* 155, 1000–1009.

Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., et al. (2006). Upregulation of PD-1 expression on HIV-specific CD8(+) T cells leads to reversible immune dysfunction (vol 12, 1198, 2006). *Nat. Med.* 12, 1329–1329. doi: 10.1038/nm1106-1329b

Tsai, A., Irrinki, A., Kaur, J., Cihlar, T., Kukolj, G., Sloan, D. D., et al. (2017). Toll-like receptor 7 agonist GS-9620 induces HIV expression and HIV-specific immunity in cells from HIV-infected individuals on suppressive antiretroviral therapy. *J. Virol.* 91:8. doi: 10.1128/JVI.02166-16

Vassena, L., Miao, H., Cimbro, R., Malnati, M. S., Cassina, G., Proschan, M. A., et al. (2012). Treatment with IL-7 prevents the decline of circulating CD4+ T cells during the acute phase of SIV infection in rhesus macaques. *PLoS Pathog* 8:e1002636. doi: 10.1371/journal.ppat.1002636

Vibholm, L., Schleimann, M. H., Hojen, J. F., Benfield, T., Offersen, R., Rasmussen, K., et al. (2017). Short-course toll-like receptor 9 agonist treatment impacts innate immunity and plasma viremia in individuals with human immunodeficiency virus infection. *Clin. Infect. Diseases* 64, 1686–1695. doi: 10.1093/cid/cix201

Walker, B. D., and Yu, X. G. (2013). Unravelling the mechanisms of durable control of HIV-1. *Nat Rev Immunol* 13, 487–498. doi: 10.1038/nri3478

Walker, L. M., Huber, M., Doores, K. J., Falkowska, E., Pejchal, R., Julien, J. P., et al. (2011). Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* 477, 466–U117. doi: 10.1038/nature 10373

Walker, R. E., Bechtel, C. M., Natarajan, V., Baseler, M., Hege, K. M., Metcalf, J. A., et al. (2000). Long-term *in vivo* survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. *Blood* 96, 467–474.

Walker-Sperling, V. E., Pohlmeyer, C. W., Tarwater, P. M., and Blankson, J. N. (2016). The effect of latency reversal agents on primary CD8 + T cells: implications for shock and kill strategies for human immunodeficiency virus eradication. *EBio.Med.* 8, 217–229. doi: 10.1016/j.ebiom.2016.04.019

Wallet, C., De Rovere, M., Van Assche, J., Daouad, F., De Wit, S., Gautier, V., et al. (2019). Microglial cells: the main HIV-1 reservoir in the brain. *Front. Cell Infect. Microbiol.* 9:362. doi: 10.3389/fcimb.2019.00362

Wang, C. Y., Wong, W. W., Tsai, H. C., Chen, Y. H., Kuo, B. S., Lynn, S., et al. (2019). Effect of anti-CD4 antibody UB-421 on HIV-1 rebound after treatment interruption. *N. Engl. J. Med.* 380, 1535–1545. doi: 10.1056/NEJMoa 1802264

Wang, F. X., Xu, Y., Sullivan, J., Souder, E., Argyris, E. G., Acheampong, E. A., et al. (2005). IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J. Clin. Invest.* 115, 128–137. doi: 10.1172/JCI200522574

Wang, G., Zhao, N., Berkhout, B., and Das, A. T. (2016a). CRISPR-Cas9 can inhibit HIV-1 replication but NHEJ repair facilitates virus escape. *Mol. Ther.* 24, 522–526. doi: 10.1038/mt.2016.24

Wang, G., Zhao, N., Berkhout, B., and Das, A. T. (2018). CRISPR-Cas based antiviral strategies against HIV-1. *Virus Res.* 244, 321–332. doi: 10.1016/j.virusres.2017.07.020

Wang, Z., Pan, Q., Gendron, P., Zhu, W., Guo, F., Cen, S., et al. (2016b). CRISPR/Cas9-derived mutations both inhibit HIV-1 replication and accelerate viral escape. *Cell. Rep.* 15, 481–489. doi: 10.1016/j.celrep.2016.03.042

Wang, Z., Wang, W., Cui, Y. C., Pan, Q., Zhu, W., Gendron, P., et al. (2018). HIV-1 employs multiple mechanisms to resist Cas9/single guide RNA targeting the viral primer binding site. *J. Virol.* 92:20. doi: 10.1128/JVI.01135-18

Webb, L., Perry-Parrish, C., Ellen, J., and Sibinga, E. (2018). Mindfulness instruction for HIV-infected youth: a randomized controlled trial. *AIDS Care* 30, 688–695. doi: 10.1080/09540121.2017.1394434

Williams, D. W., Veenstra, M., Gaskill, P. J., Morgello, S., Calderon, T. M., and Berman, J. W. (2014). Monocytes mediate HIV neuropathogenesis: mechanisms that contribute to HIV associated neurocognitive disorders. *Curr. HIV Res.* 12, 85–96. doi: 10.2174/1570162X12666140526114526

Williams, S. A., Chen, L. F., Kwon, H., Fenard, D., Bisgrove, D., Verdin, E., et al. (2004). Prostratin antagonizes HIV latency by activating NF-kappaB. *J. Biol. Chem.* 279, 42008–42017. doi: 10.1074/jbc.M402124200

Wonderlich, E. R., Leonard, J. A., and Collins, K. L. (2011). HIV immune evasion disruption of antigen presentation by the HIV Nef protein. *Adv. Virus Res.* 80, 103–127. doi: 10.1016/B978-0-12-385987-7.00005-1

Xu, Y., Zhang, M., Ramos, C. A., Durett, A., Liu, E., Dakhova, O., et al. (2014). Closely related T-memory stem cells correlate with *in vivo* expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. *Blood* 123, 3750–3759. doi: 10.1182/blood-2014-01-552174

Yin, C., Zhang, T., Qu, X., Zhang, Y., Putatunda, R., Xiao, X., et al. (2017). *In vivo* excision of HIV-1 provirus by saCas9 and multiplex single-guide RNAs in animal models. *Mol. Ther.* 25, 1168–1186. doi: 10.1016/j.ymthe.2017.03.012

Younan, P. M., Polacino, P., Kowalski, J. P., Peterson, C. W., Maurice, N. J., Williams, N. P., et al. (2013). Positive selection of mC46-expressing CD4+ T cells and maintenance of virus specific immunity in a primate AIDS model. *Blood* 122, 179–187. doi: 10.1182/blood-2013-01-482224

Zachary, D., Thorkelson, A., Anderson, J., Wong, H. C., Karn, J., Dobrowlski, C., et al. (2018). *A Phase 1 Study of ALT-803 (IL-15 Superagonist) to Clear Latent HIV Reservoirs.* Conference On Retrovirus and Opportunistic Infections 356.

Zaikos, T. D., Terry, V. H., Sebastian Kettinger, N. T., Lubow, J., Painter, M. M., Virgilio, M. C., et al. (2018). Hematopoietic stem and progenitor cells are a distinct HIV reservoir that contributes to persistent viremia in suppressed patients. *Cell Rep.* 25, 3759–3773. doi: 10.1016/j.celrep.2018.11.104

Zhang, H., Snyder, K. M., Suhoski, M. M., Maus, M. V., Kapoor, V., June, C. H., et al. (2007). 4-1BB is superior to CD28 costimulation for generating

CD8+ cytotoxic lymphocytes for adoptive immunotherapy. *J. Immunol.* 179, 4910–4918. doi: 10.4049/jimmunol.179.7.4910

Zhao, Y., Wang, Q. J., Yang, S., Kochenderfer, J. N., Zheng, Z., Zhong, X., et al. (2009). A herceptin-based chimeric antigen receptor with modified signaling domains leads to enhanced survival of transduced T lymphocytes and antitumor activity. *J. Immunol.* 183, 5563–5574. doi: 10.4049/jimmunol.0900447

Zhen, A., Carrillo, M. A., and Kitchen, S. G. (2017a). Chimeric antigen receptor engineered stem cells: a novel HIV therapy. *Immunotherapy* 9, 401–410. doi: 10.2217/imt-2016-0121

Zhen, A., Kamata, M., Rezek, V., Rick, J., Levin, B., and Kasparian, S. (2015). HIV-specific immunity derived from chimeric antigen receptor-engineered stem cells. *Mol. Ther.* 23, 1358–1367. doi: 10.1038/mt.2015.102

Zhen, A., and Kitchen, S. (2013). Stem-cell-based gene therapy for HIV infection. *Viruses* 6, 1–12. doi: 10.3390/v6010001

Zhen, A., Peterson, C. W., Carrillo, M. A., Reddy, S. S., Youn, C. S., Lam, B. B., et al. (2017b). Long-term persistence and function of hematopoietic stem cell-derived chimeric antigen receptor T cells in a nonhuman primate model of HIV/AIDS. *PLoS Pathog* 14:e1006891: doi: 10.1371/journal.ppat.1006891

Zhou, J., Jin, L., Wang, F., Zhang, Y., Liu, B., and Zhao, T. (2019). Chimeric antigen receptor T (CAR-T) cells expanded with IL-7/IL-15 mediate superior antitumor effects. *Protein Cell.* 10, 764–769. doi: 10.1007/s13238-019-0643-y

Conflict of Interest: SK is a founder of CDR3 Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 14 August 2020
doi: 10.3389/fcimb.2020.00424

Block and Lock HIV Cure Strategies to Control the Latent Reservoir

Chantelle L. Ahlenstiel[1], Geoff Symonds[2], Stephen J. Kent[3,4,5] and Anthony D. Kelleher[1]*

[1] Kirby Institute, UNSW, Sydney, NSW, Australia, [2] CSL Australia Ltd, Sydney, NSW, Australia, [3] Department of Microbiology and Immunology, Peter Doherty Institute, The University of Melbourne, Melbourne, VIC, Australia, [4] Melbourne Sexual Health Centre and Department of Infectious Diseases, Alfred Hospital and Central Clinical School, Monash University, Melbourne, VIC, Australia, [5] ARC Centre for Excellence in Convergent Bio-Nano Science and Technology, The University of Melbourne, Parkville, VIC, Australia

The HIV latent reservoir represents the major challenge to cure development. Residing in resting CD4+ T cells and myeloid cells at multiple locations in the body, including sanctuary sites such as the brain, the latent reservoir is not eliminated by ART and has the ability to reactivate virus replication to pre-therapy levels when ART is ceased. There are four broad areas of HIV cure research. The only successful cure strategy, thus far, is stem cell transplantation using naturally HIV resistant CCR5Δ32 stem cells. A second potential cure approach uses gene editing technology, such as zinc-finger nucleases and CRISPR/Cas9. Another two cure strategies aim to control the HIV reservoir, with polar opposite concepts; The "shock and kill" approach, which aims to "shock" or reactivate the latent virus and then "kill" infected cells via targeted immune responses. Lastly, the "block and lock" approach, which aims to enhance the latent virus state by "blocking" HIV transcription and "locking" the HIV promoter in a deep latent state via epigenetic modifications. "Shock and kill" approaches are a major focus of cure studies, however we predict that the increased specificity of "block and lock" approaches will be required for the successful development of a sustained HIV clinical remission in the absence of ART. This review focuses on the current research of novel "block and lock" approaches being explored to generate an HIV cure via induction of epigenetic silencing. We will also discuss potential future therapeutic delivery and the challenges associated with progressing "block and lock" cure approaches as these move toward clinical trials.

Keywords: HIV-1, block and lock, cure strategies, epigenetic silencing, latent reservoir

OPEN ACCESS

Edited by:
Renee Marije Van Der Sluis, Aarhus Institute of Advanced Studies, Denmark

Reviewed by:
Estanislao Nistal-Villan, San Pablo CEU University, Spain
Zeger Debyser, KU Leuven, Belgium

****Correspondence:***
Chantelle L. Ahlenstiel
cahlenstiel@kirby.unsw.edu.au

Specialty section:
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

Received: *26 February 2020*
Accepted: *10 July 2020*
Published: *14 August 2020*

Citation:
Ahlenstiel CL, Symonds G, Kent SJ and Kelleher AD (2020) Block and Lock HIV Cure Strategies to Control the Latent Reservoir. Front. Cell. Infect. Microbiol. 10:424. doi: 10.3389/fcimb.2020.00424

INTRODUCTION

Persistence of the HIV-1 latent reservoir is the major barrier to an HIV cure. Combined antiretroviral therapy (ART) is now such a highly efficacious treatment, that once a person living with HIV (PLWH) has commenced therapy and has an undetectable viral load they are then unable to transmit the virus (U=U) (Cohen et al., 2016; Bavinton et al., 2018; Rodger et al., 2019). However, ART interruption or cessation leads to a rapid rebound in viral load and significant morbidity. Therefore, treatment must be life-long. Hence, the aim of an HIV cure is to either (i) eradicate the latent reservoir from the body or (ii) have life-long remission of virus without the need for ART. Long-lived resting memory CD4+ T cells contribute a major component of the latent reservoir, followed by dendritic cells, macrophages, and microglial cells (Kumar et al., 2014; Kandathil et al., 2016; Honeycutt et al., 2017; Wallet et al., 2019). These cells types have a wide

range of anatomical locations, including lymph nodes, gut-associated lymph tissue (GALT) (Yukl et al., 2013), liver (Penton and Blackard, 2014), genital tract (Cantero-Perez et al., 2019), and brain (Wallet et al., 2019). Some of these sites are further termed sanctuary sites, which are protected from ART penetration (i.e., the brain, testis, and lymph node B cell germinal centers) and pose additional challenges for HIV cure treatments (Eisele and Siliciano, 2012; Fletcher et al., 2014). Persistence of the latent reservoir occurs due to clonal expansion of infected cells and/or infection of long-lived reservoir cells (Chomont et al., 2009; Hiener et al., 2017; Lee et al., 2019). Many studies are attempting to characterize the HIV reservoir in order to understand the unique cell types and subsets involved (Hiener et al., 2017; Lee et al., 2019; Pardons et al., 2019; Horsburgh et al., 2020), and the provirus state; whether intact or defective (Bruner et al., 2016, 2019; Hiener et al., 2017), reactivatable, or non-reactivatable (Battivelli et al., 2018). Studies investigating the fundamentals of HIV persistence are vital to developing cure strategies. The current focus of HIV cure strategies can be broadly segmented into four main areas: 1. Cell/Gene therapy using stem cell transplantation, 2. Gene therapy via gene editing, 3. Shock and kill approaches, and 4. Block and lock approaches (**Figure 1**).

There are so far two examples of PLWH being successfully cured of HIV, with both cases utilizing cell therapy with stem cell transplantation, i.e., the Berlin and London patients (Hutter et al., 2009; Gupta et al., 2019). This approach employed naturally HIV-resistant donor CCR5delta32 stem cells for transplantation in patients being treated for associated malignancies i.e., undergoing myeloablative/chemotherapy treatments (Hutter et al., 2009; Gupta et al., 2019). While studies are ongoing to attempt to recapitulate this cure approach in other patients who also require stem cell transplants due to associated malignancies, this approach is not currently scalable nor feasible or desirable in the general population of PLWH who do not require such heroic treatment for malignant disease. This is due to the high risk involved in undertaking an allogeneic stem cell transplantation.

Gene therapy cure approaches also aim to eradicate the integrated latent reservoir and use a number of nuclease-mediated gene editing tools, i.e., molecular scissors, that cut genomic DNA in a highly specific manner. Some examples include clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (CRISPR/Cas9) technologies (Kaminski et al., 2016a,b; Wang et al., 2016a; Miller et al., 2017; Dash et al., 2019), zinc finger nuclease (ZFN) (DiGiusto et al., 2016; Ji et al., 2018), and the transcription-activator-like effector nucleases (TALEN) (Shi et al., 2017) gene editing to excise the HIV-1 genome from the host genome. A benefit of these gene editing sequence approaches is the high specificity required to match the target sequence. However, this also means that due to the extreme sequence diversity present in the HIV-1 genome, a combination of multiple sequences will be required in order to ensure sequence diversity and the potential for future virus mutations in the target site is addressed. Additionally, off-target effects and virus escape have been reported (Wang et al., 2016b).

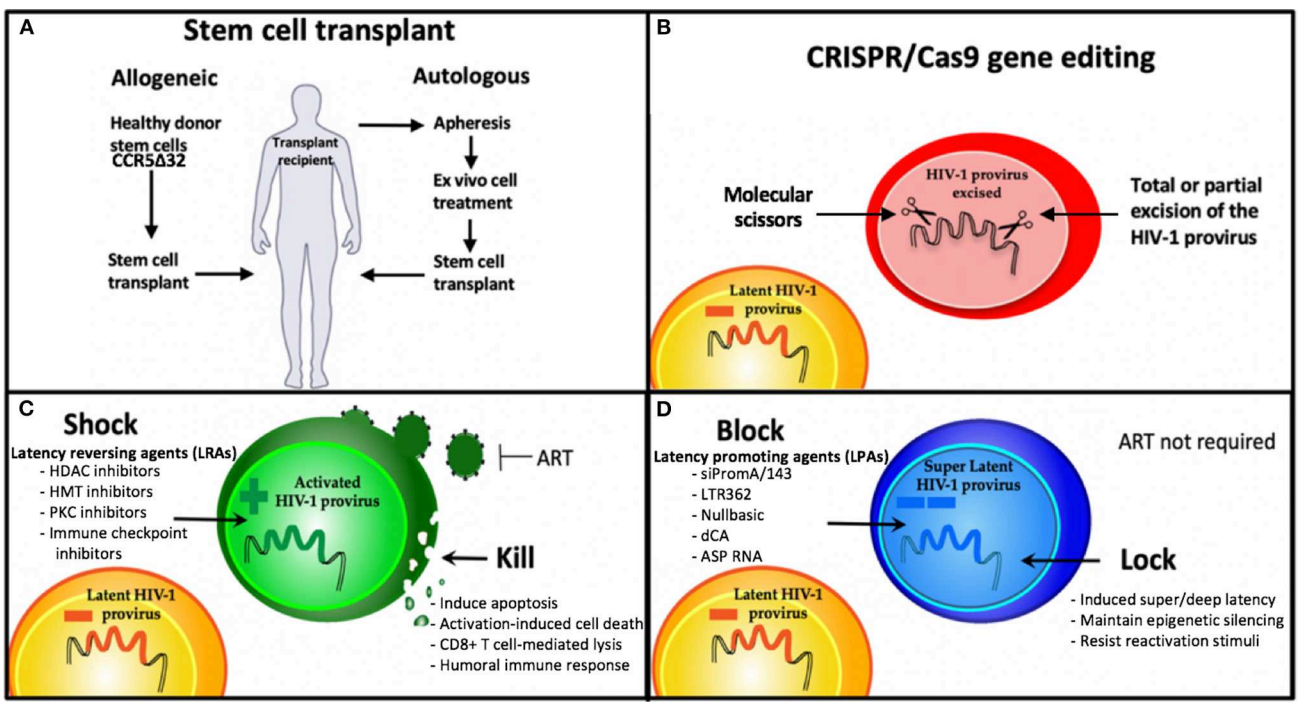


FIGURE 1 | Strategies being developed for an HIV cure. **(A)** Stem cell therapies and **(B)** CRISPR gene therapy use modified cell therapies to target the latent reservoir. **(C)** Shock and Kill approach using latency reversing agents (LRAs) to eradicate the latent reservoir, and **(D)** Block and Lock approach using latency promoting agents (LPAs) to induce silencing of the latent reservoir to achieve sustained HIV remission, that is refractory to reactivation. HDAC, Histone deacetylase; HMT, histone methyl transferase; PKC, protein kinase C; dCA, didehydro-cortistatin A; ART, antiretroviral therapy.

Delivery of gene editing therapies to the target site are also a major challenge.

A further eradication cure approach is the commonly known "shock and kill" strategy, recently reviewed by Ait-Ammar et al. (2019). This approach aims to "shock" latently-infected cells into a reactivated state, using latency-reversing agents (LRAs) to activate virus transcription and then "kill" these reservoir cells via cytopathic effects, host immune responses or other targeted mechanisms. Several classes of LRAs have been investigated (**Figure 1**). One class of LRAs are epigenetic modifiers, such as histone deacetylase inhibitors (HDACi), histone methyltransferase inhibitors (HMTi), and DNA methyltransferase inhibitors (DNMTi), which all act on reversing the repressive epigenetic marks present in the HIV-1 promoter during latency. These LRAs result in global activation, relaxing epigenetic marks in not just the HIV-1 promoter, but any promoter that is epigenetically silenced by these mechanisms. Another challenge of LRAs is the variability of effect depending on the specific cell model (Spina et al., 2013). Successful reactivation by most LRAs *in vitro* have failed to induce sufficient reactivation to make a detectable impact on the HIV reservoir *in vivo* or *ex vivo* in patient latently-infected cells (Spina et al., 2013). Additional improvements in the ability to kill reactivated cells are also likely to be needed, such as a broadly neutralizing antibody (bNAb) PGT121 and a Toll-like receptor 7 (TLR7) agonist (Borducchi et al., 2018). Moreover, the shock and kill approach is not suitable for all cell types harboring latent virus, such as microglial cells in the brain, reviewed in Wallet et al. (2019). This is due to reactivation of microglial cell reservoirs resulting in neuroinflammation, a key component of HIV-associated neurocognitive disorders (HAND) (Wallet et al., 2019). As demonstrated by several groups, it is unlikely that targeting a single mechanism of HIV-1 latency will be sufficient to reactivate the majority of the virus reservoir (Jiang et al., 2015; Rochat et al., 2017; Das et al., 2018; Ait-Ammar et al., 2019). Instead, a combination of LRAs, targeting multiple mechanisms of HIV-1 latency is likely to be required for an effective sterilizing cure without ART.

A fundamentally different and potentially more realistic approach to reservoir control is known as "block and lock." This functional cure strategy aims to permanently silence the latent reservoir using latency promoting agents (LPAs) to "block" virus transcription and "lock" the virus promoter in a latent state via repressive epigenetic modifications. Permanent control of the HIV-1 promoter means ART is no longer required. The block and lock approach mimics natural virus latency by inducing a state of latency, described recently by the terms "super latency" or "deep latency." The features of HIV-1 transcription and the latency are described below, including the specific aspects mimicked by the block and lock approach. A precedent for forcing HIV-1 into a permanently silenced state via the block and lock epigenetic silencing approach has been set by the many ancient, epigenetically silenced human endogenous retroviruses (HERVs) that comprise ∼8% of the human genome (Lander et al., 2001). This supports the feasibility and potential longevity of the block and lock approach. A major benefit of this cure approach, when mediated by RNA therapeutics, is that highly specific sequence targeting is required. Similar to CRISPR gene editing approaches, multiplexing of several RNA therapeutics targeting different sites in the virus genome will be necessary to address the global sequence diversity of HIV-1 (Ahlenstiel et al., 2015; Pang et al., 2018).

Understanding the process of HIV-1 transcription and the molecular mechanisms involved in regulating HIV-1 latency is important for developing targeted therapies (**Figure 2**). A trademark feature of all retroviruses, including HIV-1, is integration of the viral genome into the host genome. Integration site selection is not random and can affect the transcriptional status depending on whether integration occurs in an active or silent gene. The process requires the HIV-1 protein integrase (IN) and the host protein Lens Epithelium-Derived Growth Factor (LEDGF/p75) [reviewed in Symons et al. (2018)]. Establishment and maintenance of integrated HIV-1 provirus in a range of latent reservoir cell types likely requires different latency molecular mechanisms. Transcriptional activity of the HIV-1 promoter, the 5′LTR, is regulated by multiple different factors. Autoregulation by the HIV-1 transactivator Tat protein is a major factor and results from Tat binding to the transactivation-responsive region (TAR), an RNA loop element located downstream of the transcription initiation start site located at nucleotides +1 to +59 (**Figure 2A**). Following Tat binding to TAR, the positive elongation factor, P-TEFb, is recruited to form a transcription complex, which results in Tat-mediated transactivation of transcription initiation and elongation via RNAPol II.

Host cellular transcription factors also play role in regulating HIV-1 transcription, such as NF-κB and Sp1, which are located between nucleosome (nuc)-0 and nuc-1 in the 5′LTR (**Figure 2**). NF-κB in particular is a major transactivator of virus transcription and has been specifically targeted by several RNA block and lock cure approaches. The chromatin environment is also a factor in HIV transcription regulation. Epigenetic silencing can reduce levels of NF-κB, which then change the efficiency of initiation and reduces Tat protein levels to result in transcription inhibition and the onset of HIV-1 latency. NF-κB is then required for virus reactivation by re-stimulating Tat production to restore transcription efficiency. Epigenetic silencing of the latent provirus can include histone post-translation modifications, such as histone methylation (Marban et al., 2007; Imai et al., 2010; Zhang et al., 2017), histone deacetylation (Verdin et al., 1993; Van Lint et al., 1996; Lusic et al., 2003), and crotonylation (Jiang et al., 2018) (**Figure 2**). Another characteristic of repressive epigenetic silencing in latent HIV-1 is DNA methylation of CpG islands (Kauder et al., 2009; Chavez et al., 2011).

BLOCK AND LOCK STRATEGIES

Epigenetic Silencing/Transcriptional Gene Silencing (TGS)

HIV cure strategies that follow the block and lock approach all have a common feature, which is the induction of epigenetic silencing or transcriptional gene silencing (TGS) in the HIV-1 promoter to suppress virus replication. The term epigenetics refers to heritable changes in gene expression that

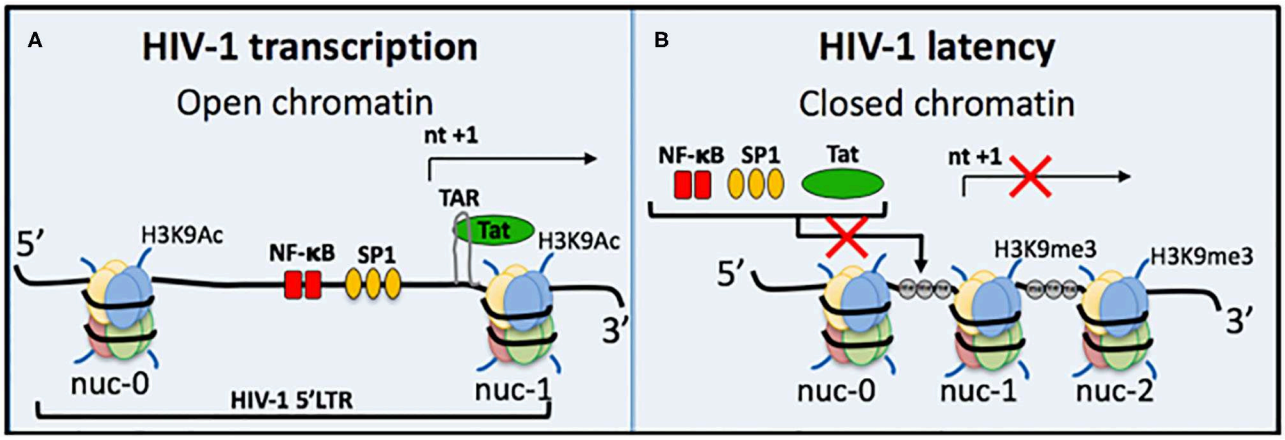


FIGURE 2 | Regulation of HIV-1 transcriptional activity. **(A)** Active HIV-1 transcription occurs when chromatin is in an open structure, enabling important transcription factors to bind and activate virus transcription. These include NF-κB (red bars) and Sp1 (orange ovals). The TAR loop is also accessible for HIV-1 Tat protein to bind and further activate transcription. Active epigenetic marks, e.g., Histone 3 Lysine 9 Acetylation (H3K9Ac), are also present. **(B)** During HIV-1 latency several mechanisms can prevent virus transcription; (i) repositioning of nucleosomes causes chromatin compaction to form heterochromatin, (ii) heterochromatin occludes important transcription factors and Tat from binding, (iii) DNA methylation (gray circle, me) of CpG islands also prevents transcription, and (iv) histone post-translational modifications include increased repressive epigenetic marks, e.g., Histone 3 Lysine 9 trimethylation, and a decrease in active epigenetic marks, e.g., H3K9Ac. Some block and lock cure approaches mimic all four of these traits of HIV-1 latency, e.g., siRNA PromA.

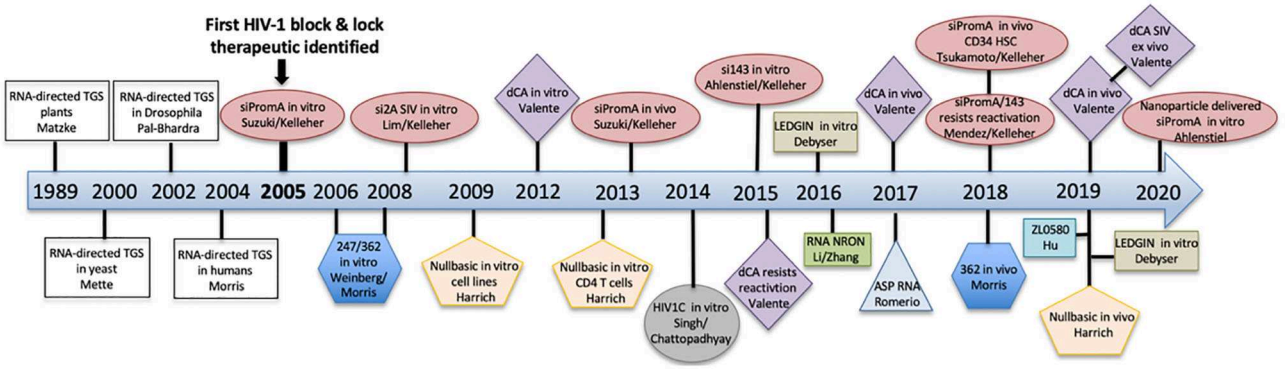


FIGURE 3 | A timeline summarizing the discovery of transcriptional gene silencing and the development of block and lock HIV-1 therapeutics.

are independent of DNA sequence (Eccleston et al., 2007). Epigenetic silencing, or TGS, is a highly conserved process that was first discovered in plants, followed by studies showing existence of the pathway in *Caenorhabditis elegans*, Drosophila, yeast and finally in humans in 2004 pioneered by Morris et al. (2004) (**Figure 3**). Although the term block and lock has only been adopted in the last 4–5 years, studies extensively developing, identifying and characterizing block and lock HIV-1 therapeutics have been ongoing since the mid-2000s. Closely following on the discovery of transcriptional gene silencing in mammalian cells in 2004, was the first published "block and lock" HIV-1 study in 2005, which identified an HIV-1 promoter-targeted siRNA, known as siPromA (**Figure 3**). A comprehensive summary of block and lock HIV-1 cure/therapeutic development is described in **Table 1**. These block and lock therapeutics, while all inducing various degrees of epigenetic silencing, can be distinguished by effect longevity, i.e., whether the antiviral agent can only block HIV transcription while the treatment is given or if the treatment can maintain HIV latency following ART interruption.

RNA-Directed Epigenetic Silencing

Epigenetic silencing can be induced by a range of RNA molecules, such as short interfering (si)RNA, short hairpin (sh)RNA, and long non-coding (lnc)RNA (**Figure 4**). In the case of siRNA, *in vitro* delivery is achieved via transfection reagents, e.g., lipofectamine, nucleofection, calcium phosphate; or cell penetrating nanoparticles that are loaded with siRNA. Once siRNA has been successfully delivered across the plasma membrane into the cytoplasm, it is then loaded onto the Argonaute 1 (Ago1) protein, with the 5′ siRNA region binding to the Ago1 PAZ domain and the 3′ siRNA end binding to the Ago1 MID domain. Subsequent transport of the siRNA/Ago1 complex into the nucleus is highly sequence dependent, as we have

TABLE 1 | Summary of block and lock HIV-1 cure/therapeutic development.

Block and lock therapeutic	Class	HIV-1 target	Stage	Sponsor/ collaborator	References
PromA	si/shRNA	Promoter, NF-kB sites	*In vivo*	Calimmune Inc.	Suzuki et al., 2005, 2008, 2011, 2013; Yamagishi et al., 2009; Ahlenstiel et al., 2012, 2015; Méndez et al., 2018; Tsukamoto et al., 2018a,b
143	si/shRNA	Promoter AP-1/COUP-TF Nuc-0	*In vitro*	University of New South Wales	Ahlenstiel et al., 2015; Méndez et al., 2018
LTR362as	si/shRNA	Promoter, NF-kB sites	*In vivo*	City of Hope	Weinberg et al., 2006; Turner et al., 2009, 2012; Zhou et al., 2018
ASP	HIV RNA	Promoter	*In vitro*	University of Maryland	Romerio et al., 2016; Zapata et al., 2017; Affram et al., 2019
LncRNA	lncRNA	Promoter	*In vitro*	City of Hope	Saayman et al., 2014
NRON	lncRNA	Tat	*In vitro* *Ex vivo*	Sun Yat-sen University	Li et al., 2016
Nullbasic	Small molecule inhibitor	Tat	*In vivo*	QIMR Berghofer Medical Research Institute	Meredith et al., 2009; Lin et al., 2012, 2014, 2015; Apolloni et al., 2013; Jin et al., 2016, 2019; Rustanti et al., 2017, 2018
dCA	Small molecule inhibitor	Tat	*In vivo*	The Scripps Research Institute	Mousseau et al., 2012, 2015, 2019; Kessing et al., 2017; Li et al., 2019; Mediouni et al., 2019a,b
LEDGIN	Small molecule inhibitor	Integrase	*In vitro*	Katholieke Universiteit, Leuven	Vranckx et al., 2016; Lampi et al., 2019; Vansant et al., 2019
BRD4-inhibitor ZL0580	Small molecule inhibitor	Tat	*In vitro* *Ex vivo*	University of Texas Medical Branch	Niu et al., 2019
Torin1, pp242	Small molecule inhibitor	mTor	*In vitro* *Ex vivo*		Besnard et al., 2016

shown that only siRNA sequences that target complementary sites present in the host genome are able to be trafficked in the nucleus (Ahlenstiel et al., 2015). Once the siRNA/Ago1 complex has entered the nucleus, further proteins are recruited to form the RNA-Induced Transcriptional Silencing Complex (RITS), which results in repressive epigenetic marks being deposited on the promoter, such as increased histone and CpG methylation and decreased histone acetylation. Viral delivery of shRNA involves a cell being transduced with a viral vector expressing the shRNA of interest and entering the cell using a viral envelope (e.g., VSV-G). Nuclear delivery of shRNA then occurs, followed by export and processing into siRNA, where they follow the path outlined above (**Figure 4**). Novel RNAs that have been shown to induce the block and lock phenomena in HIV-1 are summarized below.

HIV-1 Provirus-Targeted RNA: PromA/143

The first anti-HIV-1 therapeutic to induce epigenetic silencing via siRNA, consistent with the term "block and lock" was identified by the Kelleher laboratory in 2005, termed siPromA (**Figure 3**, **Table 1**). This specific RNA sequence targets the unique tandem NF-κB sites in the HIV-1 promoter to induce potent transcriptional gene silencing mediated by repressive epigenetic marks (Suzuki et al., 2005, 2008, 2011, 2013, 2015; Yamagishi et al., 2009; Ahlenstiel et al., 2012, 2015; Méndez et al., 2015, 2018). Due to the conserved sequence of the NF-κB transcription factor site, extensive studies have been performed to investigate the potential of this siRNA to induce off-target effects (Suzuki et al., 2011). However, since the sequence of the HIV encoded NF- κB binding sites is substantially different from those encoded by the host genome and as the 19 bp sequence of siPromA includes portions of both the tandem NF-κB sites and their linking sequence, a 19 bp sequence which is unique to the HIV-1 promoter and not found in the human genome sequence can be targeted with no identified off-target effects (Suzuki et al., 2011; Ahlenstiel et al., 2015). To demonstrate longevity of the silencing effect, *in vitro* studies in cell lines have shown a single siRNA dose was sufficient to suppress virus replication 1,000-fold for up to 15 days and for >1 year in cells stably expressing lentiviral shPromA (Suzuki et al., 2008; Ahlenstiel et al., 2015). The virus silencing effect induced by siPromA has also been reported in *in vitro* in PBMCs and monocyte-derived macrophages (Suzuki et al., 2008, 2013; Ahlenstiel et al., 2015).

A recent study has demonstrated that cells expressing promoter-targeted siPromA and/or si143 are robustly resistant to reactivation stimuli, with each siRNA sequence inducing a unique repressive epigenetic profile (Méndez et al., 2018). This supports the approach of multiplexing RNA sequences to both achieve enhanced virus latency and address the global diversity of HIV sequences. Characterization of siPromA has progressed into *in vivo* humanized mouse models, with a study in 2013 using PBMCs transduced with lentiviral vector expressing shPromA (Suzuki et al., 2013) and more recently a study using CD34+ HSCs transduced with lentiviral vector expressing

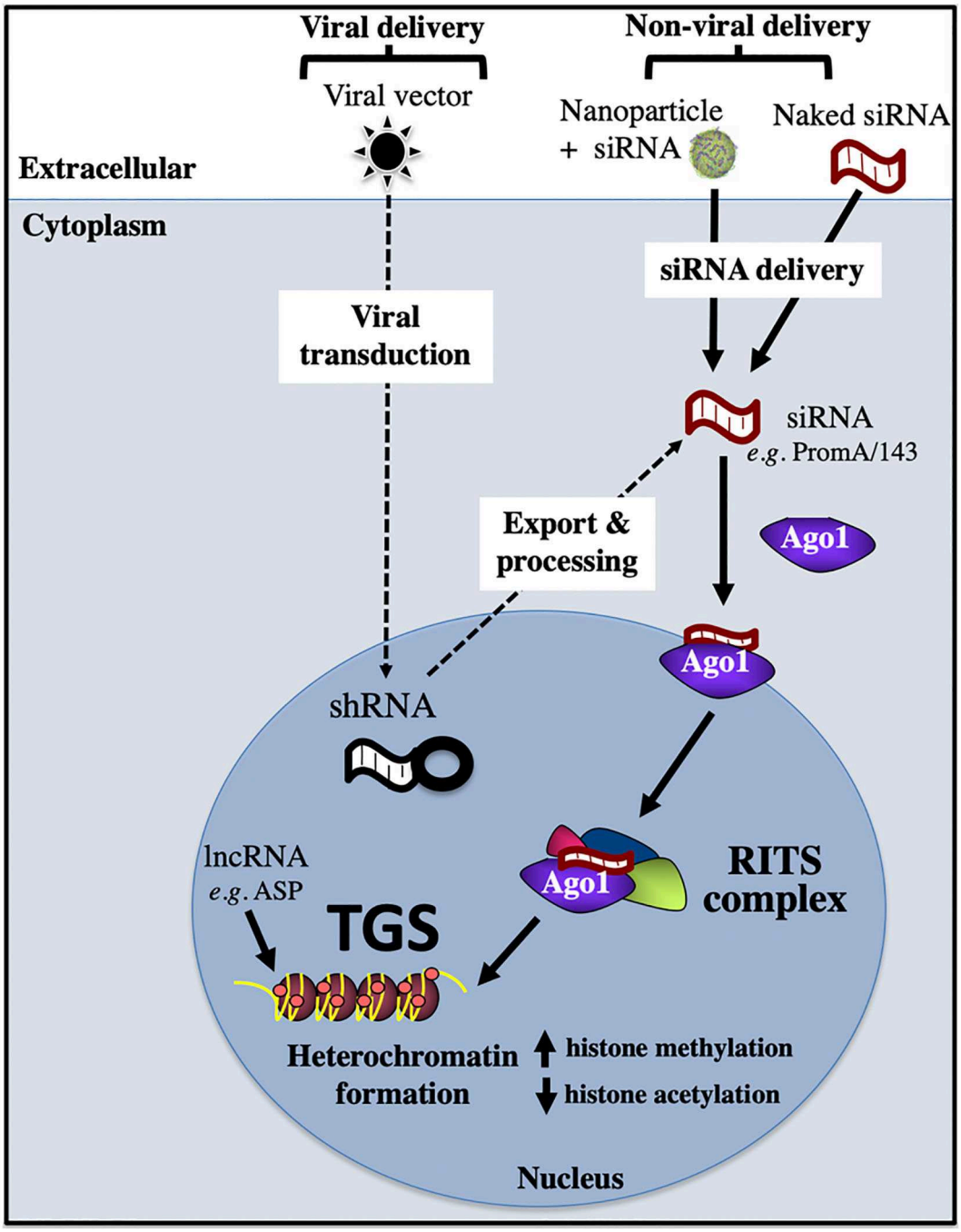


FIGURE 4 | Transcriptional gene silencing (epigenetic silencing) pathway. Transcriptional gene silencing (TGS) can be mediated by viral or non-viral delivery of RNA sequences, which associate with the Argonaute protein, Ago1, then enter the nucleus to form the RITS complex, which recruits repressive epigenetic marks to induce chromatin compaction and silence gene expression. Ago1, Argonaute 1; shRNA, short hairpin RNA; RISC, RNA induced silencing complex; RITS, RNA induced transcriptional silencing complex; ASP, HIV-1 encoded antisense protein.

shPromA (Tsukamoto et al., 2018a,b). Both studies showed that siPromA-induced silencing could provide protection against HIV-1 infection. These studies demonstrated significantly lower HIV-1 cell-associated RNA levels in siPromA-expressing CD4+ T cells isolated from blood and tissue (lymph nodes/spleen) and normal CD4:CD8 ratios when compared to controls. Current studies are investigating the viral rebound effect following withdrawal of ART treatment in humanized mouse models. Promising results indicate siPromA induces a delay in virus rebound post-ART interruption. A short, animated infographic

describing the HIV-1 block and lock approach and the potential therapeutic use of siPromA is described in the following URL (https://kirby.unsw.edu.au/news/block-lock-pathway-hiv-remission).

HIV-1 Provirus-Targeted RNA: LTR362

The next block and lock HIV-1 therapeutic identified was LTR362 in the Morris laboratory (Weinberg et al., 2006). This RNA sequence also targets the tandem NF-κB sites in the HIV-1 promoter and overlaps with 8 bp of the 19 bp siPromA sequence. A recent *in vivo* study in a humanized mouse model of HIV-1 infection investigated combining this RNA sequence with the gp120 A-1 aptamer and multiplexing with two post-transcriptional gene silencing (PTGS) RNAs targeting Tat and Rev mRNA (Zhou et al., 2018). The study demonstrated virus suppression and increased CD4+ T cells levels compared to controls. However, although LTR362 has been reported to induce epigenetic silencing through CpG methylation in the 5′LTR *in vitro* (Weinberg et al., 2006), this did not translate in the *in vivo* model, with protection against virus infection being attributed to the post-transcriptional control of Tat and Rev (Zhou et al., 2018). Further studies will be required *in vivo* to investigate the contribution of histone methylation in TGS-induced by LTR362. This study highlights the potential therapeutic advantage of multiplexing both TGS and PTGS RNA sequences.

SIV Provirus-Targeted RNA: si2A

Investigations by the Kelleher laboratory targeting the SIV provirus identified the third block and lock RNA sequence, termed si2A (Lim et al., 2008). This study was motivated to enable confirmation of the TGS-inducing effect of siPromA in a non-human primate model. Given the substantial difference in the SIV and HIV-1 promoter sequences, a novel RNA target in the SIV promoter needed to be identified to enable future non-human primate studies. The RNA sequence si2A targets ∼51 bp upstream of the $SIV_{mac}251$ NF-κB site. Additional RNA sequences targeting the $SIV_{mac}251$ promoter were also identified, termed SIV-S4a and SIV-S12, which are directly adjacent to the NF-kB site and transcriptional start site (TSS), respectively (Lim et al., 2008). The SIV-S4a and SIV-S12 RNA sequences also induced TGS of SIV, but not to the same degree as si2A (Lim et al., 2008).

HIV-1 Provirus-Targeted RNA: S4

A further TGS-inducing siRNA sequence that also targets the NF-κB sequence in HIV-1 subtype C was identified in 2014, termed S4-siRNA (Singh et al., 2014). The Chattopadhyay laboratory designed S4-siRNA to specifically target subtype C, which is prevalent in nearly 50% of PLWH worldwide and commonly in countries with a high HIV prevalence, e.g., Southern Africa and India. This study demonstrated significantly decreased vRNA levels in TZM-bl cells transfected with S4-siRNA *in vitro* and *ex vivo* in human PBMCs transfected with S4-siRNA (Singh et al., 2014). TGS induction was confirmed using chromatin immunoprecipitation (ChIP) assay to demonstrate recruitment of repressive epigenetic marks, H3K27me3 and H3K9me2 (Singh et al., 2014). While this RNA has therapeutic potential specifically for HIV-1 subtype C, no other subtypes are targeted, as HIV subtype C has three NF-κB sites and the linking sequence is substantially different from other subtypes.

HIV-1 Encoded Antisense Protein *ASP*

An HIV-1 encoded antisense protein that functions to promote virus latency was identified by the Romerio laboratory in 2016 and is termed *ASP* (Romerio et al., 2016). ASP RNA is derived from the 3′LTR and has been shown to recruit the polycomb group repressive complex 2 (PRC2) to the HIV-1 5′LTR promoter, resulting in repressive epigenetic modifications, i.e., increased H3K27me3 and reduced RNAPII occupancy at the promoter (Zapata et al., 2017). A recent study by Affram et al. demonstrated that ASP is located in the nucleus of latent myeloid and lymphoid cell lines but translocates to the cytoplasm and cell surface upon stimulation with PMA (Affram et al., 2019). Further, ASP co-localized with gp120 on the cell surface and was observed in cell-free HIV-1 particles. This study reported that ASP is an accessory protein incorporated into the surface of HIV-1 virions and has potential as a therapeutic target (Affram et al., 2019).

HIV-1 Encoded Antisense Long Non-coding RNA

The first HIV-1 encoded long non-coding (lnc)RNA was identified in 2014 by the Morris laboratory (Saayman et al., 2014). The lncRNA was shown to induce HIV-1 transcriptional silencing *in vitro* via recruitment of a chromatin-remodeling complex, involving DNMT3a, EZH2, and HDAC-1, to the virus promoter (Saayman et al., 2014).

Long Non-coding RNA NRON

In 2016 the Zhang laboratory identified a lncRNA, termed NRON, which was observed to be highly expressed in resting CD4+ T cells (Li et al., 2016). NRON suppresses virus transcription by inducing degradation of the transactivator protein Tat. This process occurs via NRON binding to Tat and then associating with the ubiquitin proteasome components CUL4B and PSMD11, resulting in Tat degradation. These *in vitro* studies indicated NRON plays a role in HIV-1 latency. Further *ex vivo* studies in resting CD4+ T cells isolated from patients on suppressive ART demonstrated that NRON used in combination with the LRA SAHA was able to reactivate the latent provirus and has potential as a new target for latency reversal (Li et al., 2016). In pursuit of latency reversal, two lncRNAs have also been identified to activate HIV-1 replication, such as lncRNA HEAL (Chao et al., 2019) and lncRNA MALAT1 (Qu et al., 2019).

Inhibitors Targeting Tat

Tat is 14 kDa protein that is a potent activator of HIV gene expression and essential for RNA polymerase II (RNAPII) synthesis of full-length transcripts of integrated provirus (Sodroski et al., 1985). Transcriptional elongation from the HIV-1 promoter is dependent on the Tat-mediated association of the pTEFb (positive transcription elongation factor) complex and TAR (trans-activation response element) of the nascent viral RNA (Laspia et al., 1989). Due to the critical requirement of Tat

for robust viral gene expression, it is a promising target in the development of HIV cure therapeutics.

NullBasic

The Harrich laboratory identified NullBasic in 2009, which was the first Tat inhibitor to induce a block and lock HIV-1 silencing effect (Meredith et al., 2009). This 101 amino acid transdominant Tat mutant has an altered basic domain (amino acids 49–57) and replaces wild-type Tat with the amino acid sequence GGGGAGGG. Thus, the basic domain has been mutated, including the TAR-binding region, hence the name NullBasic. HIV-1 transcription is inhibited by NullBasic through competition with endogenous Tat, but also via inhibition of Rev-mediated transport of virus mRNA (Meredith et al., 2009). *In vitro* studies have reported that CD4+ T cells transduced with a retroviral vector expressing NullBasic showed suppression of virus transcription and replication (Jin et al., 2016). The mechanism was confirmed to be via TGS using ChIP assay to detect repressive epigenetic marks, i.e., reduced RNAPII occupancy at the promoter and decreased H3K9 acetylation (Jin et al., 2016). A recent *in vivo* study using retroviral vector delivery of NullBasic to primary human CD4+ T cells and engraftment in a NSG mouse model demonstrated undetectable viral RNA in plasma samples up to day 14 post-infection and significantly reduced viral RNA levels in tissue-derived CD4+ T cells (Jin et al., 2019). Although there was no difference in viral mRNA levels at later time points, there were increased levels of CD4+ T cells in NullBasic treated mice, suggesting a survival advantage (Jin et al., 2019). NullBasic shows potential as a gene therapy candidate and warrants further investigation to optimize the permanence of silencing.

Didehydro-Cortistatin A (dCA)

Another small molecule inhibitor targeting Tat is the Cortistatin A (CA) analog, didehydro-Cortistatin A (dCA), identified by the Valente laboratory in 2012 (Mousseau et al., 2012). This molecule induces a block and lock HIV-1 silencing effect via Tat inhibition, specifically by binding to the RNA hairpin TAR-binding/basic domain of Tat (Mousseau et al., 2012). CA is a natural steroidal alkaloid derived from the marine sponge *Corticium simplex* (Aoki et al., 2006), however the chemical synthesis of dCA is more sustainable and cost-effective and is generated from the steroid prednisone (Shi et al., 2011). Initial studies in cell lines and primary cells confirmed a significant decrease in viral mRNA and capsid p24 antigen following dCA treatment. Treatment with dCA was demonstrated to block transcription initiation/elongation with a decrease in RNAPII associated with the HIV promoter in dCA treated HeLa-CD4 cells compared to controls (Mousseau et al., 2012). A further study showed dCA treatment is resistant to reactivation by various LRAs in both cell lines and primary CD4+ T cells (Mousseau et al., 2015). This small molecule inhibitor progressed to *in vivo* humanized BLT mouse studies in 2017, which demonstrated that dCA treatment delayed virus rebound until day 19 compared to day 10 in control mice (Kessing et al., 2017). The silencing mechanism was recently reported to include formation of heterochromatin in the 5′LTR, with the presence repressive epigenetic marks, including decreased H3 acetylation and reduced RNAPII occupancy at the promoter (Li et al., 2019). dCA is the only block and lock therapeutic, thus far, to progress to *ex vivo* non-human primate studies. A study in 2019 reported the dCA treatment inhibits reactivation of SIV in latently infected Hut78 cells, as well as *ex vivo* isolated primary CD4+ T cell isolated from $SIV_{mac}239$-infected rhesus macaques (Mediouni et al., 2019b). This is encouraging data and, similar to NullBasic, dCA warrants further investigation in order to enhance the permanency of silencing. Another important avenue of investigation is whether the dCA resistant strains isolated *in vitro* (Mediouni et al., 2019b) are also present in PLWA globally (Rice, 2019).

Small Molecule Inhibitors Targeting Integration

LEDGINs

First identified in 2010 by the Debyser laboratory, LEDGINs inhibit HIV-1 integration (Christ et al., 2010). The rational design of these small molecule inhibitors specifically targeted the interaction between LEDGF/p75 and HIV integrase, which is essential for virus integration via tethering of the pre-integration complex to chromatin (Christ et al., 2010). Interestingly, *in vitro* studies in cell lines and primary cells treated with LEDGINs during infection demonstrated provirus integrated into silent genes, which were subsequently resistant to reactivation by various LRAs (Vranckx et al., 2016; Vansant et al., 2019). This approach has potential for treatment during acute infection to reduce integration events and potentially during shock and kill reactivation via LRAs to prevent reseeding of the reservoir.

Small Molecule Inhibitors Targeting Epigenetic Readers

ZL0580 Targeting BRD4

The epigenetic reader bromodomain and extraterminal (BET) family protein BRD4 is involved in regulation of HIV-1 transcription. Previous studies by the Verdin laboratory showed BRD4 can suppress HIV-1 transcription elongation specifically via competition with Tat for binding to pTERb/CDK9 (Bisgrove et al., 2007). Structure-guided drug design in the Hu laboratory recently identified the small molecule inhibitor ZL0580, which induces Tat inhibition via selective binding to BRD4 (Niu et al., 2019). Additionally, suppression of transcription elongation was reported and shown to be due to repressive epigenetic marks in the HIV-1 5′LTR (Niu et al., 2019). Importantly, combined treatment with ZL0580 and ART in *ex vivo* CD4+ T cells isolated from three ART suppressed HIV-infected participants reported accelerated HIV-1 suppression and delayed virus rebound by 8, 9, and 15 days, respectively, compared to ART alone (Niu et al., 2019). Similar to other small molecule inhibitors, further investigations are needed, with possible combination of molecules, to ensure permanent silencing.

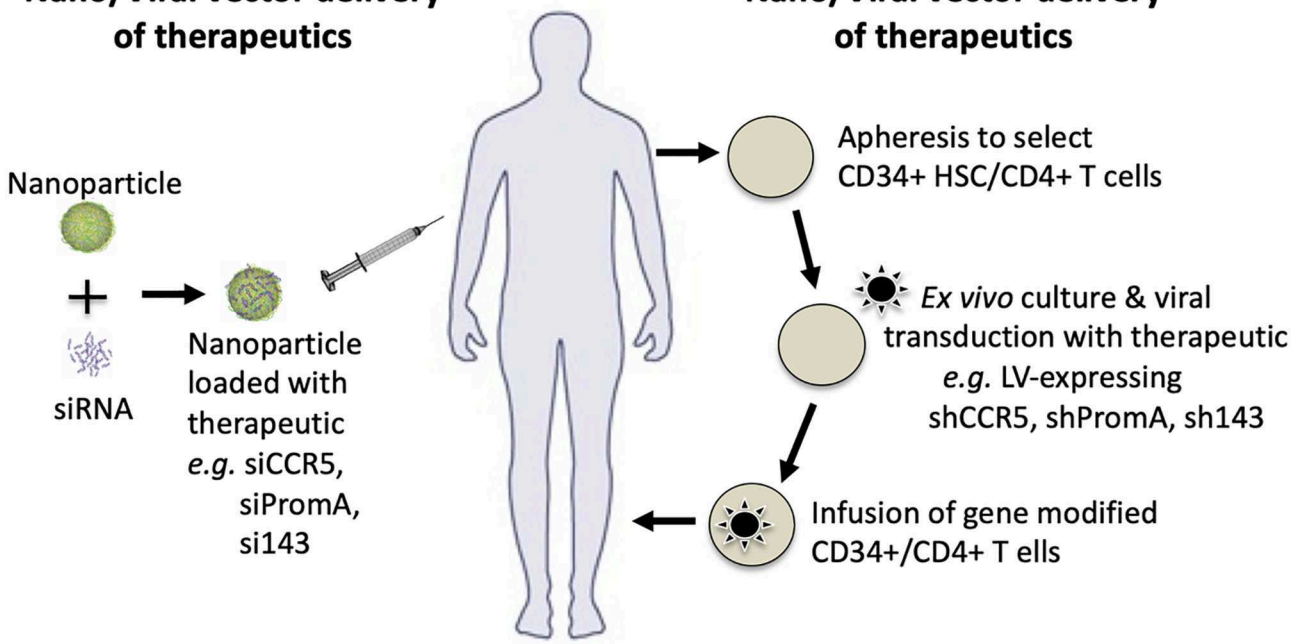


FIGURE 5 | Gene therapy strategies for *in vivo* and *ex vivo* therapeutic delivery.

Small Molecule Inhibitors Targeting mTor

Torin1 and pp242

In 2016, the Verdin laboratory identified the mammalian target of rapamycin (mTOR) signaling pathway as an important modulator of HIV-1 latency (Besnard et al., 2016). Inhibition of mTOR by small molecules Torin1 and pp242 suppressed the reactivation of provirus in the Bcl-2 HIV latency primary cell model and *ex vivo* in CD4+ T cells isolated from ART suppressed HIV-1 infected participants (Besnard et al., 2016).

DELIVERY OF BLOCK AND LOCK THERAPEUTICS

As described in **Table 1**, the development of several block and lock therapeutics has progressed to *in vivo* studies, primarily using humanized mouse models (Suzuki et al., 2013; Kessing et al., 2017; Tsukamoto et al., 2018a,b; Zhou et al., 2018; Jin et al., 2019) and one *ex vivo* study in non-human primates (Mediouni et al., 2019a). Delivery in these models is entirely dependent on the therapeutic class. RNA therapeutics have been delivered *ex vivo* to human CD34+ stem cells (**Figure 5**) or PBMCs using viral transduction of vectors expressing shRNA prior to infusion of modified stem or peripheral blood cells into the mouse. These studies have used a range of different humanized mouse models. The Tat small molecule inhibitor, dCA, has been orally delivered *in vivo* in a humanized mouse model. For cost effective and ease of treatment, direct *in vivo* delivery is preferable. An example of *in vivo* gene therapy currently being developed is nanoparticle delivery of the siPromA RNA therapeutic (**Figure 5**). Two of the biggest challenges for *in vivo* gene therapy to cure HIV, is (i) the widely distributed anatomical locations of the latent reservoir and (ii) the range of latently-infected cell types. Addressing both of these challenges will require *in vivo* gene therapies to be able to specifically target latently-infected cells, which is difficult as investigations to identify definitive latent reservoir specific biomarkers are ongoing (Fromentin et al., 2016; Descours et al., 2017; Sivro et al., 2018; Darcis et al., 2019; Pardons et al., 2019). An *in vivo* gene therapy that may inform HIV studies is being pioneered in the Peter-Kiem laboratory using foamy virus vector delivery of gene therapy for human X-linked severe combined immunodeficiency (SCID-X1) (Humbert et al., 2018). Foamy virus vectors have the specific advantage over VSV-G pseudotyped lentiviral vectors of being resistant to human serum inactivation, which is beneficial during *in vivo* delivery.

Although *in vivo* gene therapy is preferable, delivery of modified stem cells via viral vectors and *ex vivo* gene therapy has progressed into Phase I/II clinical trials. Targeting HIV-1 infection is the Cal-1 therapeutic lentiviral vector, which expresses shCCR5 and the fusion inhibitor C46, and has consistently demonstrated therapeutic benefits both *in vitro* and *in vivo* (Ringpis et al., 2012; Wolstein et al., 2014; Burke et al., 2015; Peterson et al., 2016; Symonds et al., 2016). The current clinical trial will provide data on reduced conditioning

treatments that will guide future studies. Additionally, outside the field of HIV, *ex vivo* gene therapy is steadily improving, with multiple clinical trials studies currently underway in AAV-gene therapy for hemophilia A and B reviewed in Doshi and Arruda (2018).

CONCLUSION

With the identification of the first block and lock HIV-1 therapeutic in 2005, the field has now started to rapidly expand the number of potential anti-HIV-1 therapeutics. These novel block and lock agents include RNA, transdominant protein, and small molecule inhibitors. Unlike a large number of "repurposed" shock and kill therapeutics, block and lock therapeutics are highly novel molecules, with no prior FDA approval and hence clinical trials of block and lock therapies have lagged behind. An advantage of the block and lock approach is the highly specific, HIV-1 targeted treatments in comparison to some non-specific shock and kill treatments e.g., histone deacetylase inhibitors that target global gene expression. A challenge of the block and lock approach, depending on the treatment e.g., siRNA, is the need to develop improved delivery systems that specifically target latent reservoir cells. As highlighted in this review, identification of potential block and lock therapeutics has increased dramatically in the last few years and studies in this area are steadily moving toward the clinical trials. Human trials will be necessary to assess whether this approach will ultimately help achieve a sustained HIV remission. Whether the best aspects of both cure approaches can be combined to potentially shock and kill latent reservoir cells that are able to be reactivated and then block and lock the remaining latent reservoir cells to ensure permanent HIV-1 latency is a combined approach that warrants future investigation.

AUTHOR CONTRIBUTIONS

CA and AK wrote the review. CA designed the figures and wrote the figure legends. GS and SK participated in the writing of the review. All authors contributed to the article and approved the submitted version.

FUNDING

CA and AK were supported by an Australian NHMRC Project grant (no. APP1128012). AK and SK were supported by an Australian NHMRC Program grant (no. APP1149990).

REFERENCES

Affram, Y., Zapata, J. C., Gholizadeh, Z., Tolbert, W. D., Zhou, W., Iglesias-Ussel, M. D., et al. (2019). The HIV-1 antisense protein ASP is a transmembrane protein of the cell surface and an integral protein of the viral envelope. *J. Virol.* 93:e00574-19. doi: 10.1128/JVI.00574-19

Ahlenstiel, C., Mendez, C., Lim, S. T., Marks, K., Turville, S., Cooper, D. A., et al. (2015). Novel RNA duplex locks HIV-1 in a latent state via chromatin-mediated transcriptional silencing. *Mol. Ther. Nucleic Acids* 4:e261. doi: 10.1038/mtna.2015.31

Ahlenstiel, C. L., Lim, H. G., Cooper, D. A., Ishida, T., Kelleher, A. D., and Suzuki, K. (2012). Direct evidence of nuclear Argonaute distribution during transcriptional silencing links the actin cytoskeleton to nuclear RNAi machinery in human cells. *Nucleic Acids Res.* 40, 1579–1595. doi: 10.1093/nar/gkr891

Ait-Ammar, A., Kula, A., Darcis, G., Verdikt, R., De Wit, S., Gautier, V., et al. (2019). Current status of latency reversing agents facing the heterogeneity of HIV-1 cellular and tissue reservoirs. *Front. Microbiol.* 10:3060. doi: 10.3389/fmicb.2019.03060

Aoki, S., Watanabe, Y., Sanagawa, M., Setiawan, A., Kotoku, N., and Kobayashi, M. (2006). Cortistatins A, B, C, and D, anti-angiogenic steroidal alkaloids, from the marine sponge Corticium simplex. *J. Am. Chem. Soc.* 128, 3148–3149. doi: 10.1021/ja057404h

Apolloni, A., Lin, M. H., Sivakumaran, H., Li, D., Kershaw, M. R. H., and Harrich, D. (2013). A mutant Tat protein provides strong protection from HIV-1 infection in human CD4+ T cells. *Hum. Gene Ther.* 24, 270–282. doi: 10.1089/hum.2012.176

Battivelli, E., Dahabieh, M. S., Abdel-Mohsen, M., Svensson, J. P., Tojal Da Silva, I., Cohn, L. B., et al. (2018). Distinct chromatin functional states correlate with HIV latency reactivation in infected primary CD4(+) T cells. *Elife* 7:e34655. doi: 10.7554/eLife.34655.028

Bavinton, B. R., Pinto, A. N., Phanuphak, N., Grinsztejn, B., Prestage, G. P., Zablotska-Manos, I. B., et al. (2018). Viral suppression and HIV transmission in serodiscordant male couples: an international, prospective, observational, cohort study. *Lancet HIV* 5, 438–447. doi: 10.1016/S2352-3018(18)30132-2

Besnard, E., Hakre, S., Kampmann, M., Lim, H. W., Hosmane, N. N., Martin, A., et al. (2016). The mTOR complex controls HIV latency. *Cell Host Microbe* 20, 785–797. doi: 10.1016/j.chom.2016.11.001

Bisgrove, D. A., Mahmoudi, T., Henklein, P., and Verdin, E. (2007). Conserved P-TEFb-interacting domain of BRD4 inhibits HIV transcription. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13690–13695. doi: 10.1073/pnas.0705053104

Borducchi, E. N., Liu, J., Nkolola, J. P., Cadena, A. M., Yu, W.-H., Fischinger, S., et al. (2018). Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* 563, 360–364. doi: 10.1038/s41586-018-0600-6

Bruner, K. M., Murray, A. J., Pollack, R. A., Soliman, M. G., Laskey, S. B., Capoferri, A. A., et al. (2016). Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat. Med.* 22, 1043–1049. doi: 10.1038/nm.4156

Bruner, K. M., Wang, Z., Simonetti, F. R., Bender, A. M., Kwon, K. J., Sengupta, S., et al. (2019). A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566, 120–125. doi: 10.1038/s41586-019-0898-8

Burke, B. P., Levin, B. R., Zhang, J., Sahakyan, A., Boyer, J., Carroll, M. V., et al. (2015). Engineering cellular resistance to HIV-1 infection *in vivo* using a dual therapeutic lentiviral vector. *Mol. Ther. Nucleic Acids* 4:e236. doi: 10.1038/mtna.2015.10

Cantero-Perez, J., Grau-Exposito, J., Serra-Peinado, C., Rosero, D. A., Luque-Ballesteros, L., Astorga-Gamaza, A., et al. (2019). Resident memory T cells are a cellular reservoir for HIV in the cervical mucosa. *Nat. Commun.* 10:4739. doi: 10.1038/s41467-019-12732-2

Chao, T.-C., Zhang, Q., Li, Z., Tiwari, S. K., Qin, Y., Yau, E., et al. (2019). The long noncoding RNA *HEAL* regulates HIV-1 replication through epigenetic regulation of the HIV-1 promoter. *MBio* 10:e02016-19. doi: 10.1128/mBio.02016-19

Chavez, L., Kauder, S., and Verdin, E. (2011). *In vivo*, *in vitro*, and *in silico* analysis of methylation of the HIV-1 provirus. *Methods* 53, 47–53. doi: 10.1016/j.ymeth.2010.05.009

Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F. A., Yassine-Diab, B., et al. (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat. Med.* 15, 893–900. doi: 10.1038/nm.1972

Christ, F., Voet, A., Marchand, A., Nicolet, S., Desimmie, B. A., Marchand, D., et al. (2010). Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. *Nat. Chem. Biol.* 6, 442–448. doi: 10.1038/nchembio.370

Cohen, M. S., Chen, Y. Q., Mc Cauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., et al. (2016). Antiretroviral therapy for the prevention of HIV-1 transmission. *N. Engl. J. Med.* 375, 830–839. doi: 10.1056/NEJMoa1600693

Darcis, G., Berkhout, B., and Pasternak, A. O. (2019). The quest for cellular markers of HIV reservoirs: any color you like. *Front. Immunol.* 10:2251. doi: 10.3389/fimmu.2019.02251

Das, B., Dobrowolski, C., Luttge, B., Valadkhan, S., Chomont, N., Johnston, R., et al. (2018). Estrogen receptor-1 is a key regulator of HIV-1 latency that imparts gender-specific restrictions on the latent reservoir. *Proc. Natl. Acad. Sci. U.S.A.* 115, 7795–7804. doi: 10.1073/pnas.1803468115

Dash, P. K., Kaminski, R., Bella, R., Su, H., Mathews, S., Ahooyi, T. M., et al. (2019). Sequential LASER ART and CRISPR treatments eliminate HIV-1 in a subset of infected humanized mice. *Nat. Commun.* 10:2753. doi: 10.1038/s41467-019-10366-y

Descours, B., Petitjean, G., Lopez-Zaragoza, J. L., Bruel, T., Raffel, R., Psomas, C., et al. (2017). CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature* 543, 564–567. doi: 10.1038/nature21710

DiGiusto, D. L., Cannon, P. M., Holmes, M. C., Li, L., Rao, A., Wang, J., et al. (2016). Preclinical development and qualification of ZFN-mediated CCR5 disruption in human hematopoietic stem/progenitor cells. *Mol. Ther. Methods Clin. Dev.* 3:16067. doi: 10.1038/mtm.2016.67

Doshi, B. S., and Arruda, V. R. (2018). Gene therapy for hemophilia: what does the future hold? *Ther. Adv. Hematol.* 9, 273–293. doi: 10.1177/2040620718791933

Eccleston, A., DeWitt, N., Gunter, C., Marte, B., and Nath, D. (2007). Epigenetics. *Nature* 447, 395–395. doi: 10.1038/447395a

Eisele, E., and Siliciano, R. F. (2012). Redefining the viral reservoirs that prevent HIV-1 eradication. *Immunity* 37, 377–388. doi: 10.1016/j.immuni.2012.08.010

Fletcher, C. V., Staskus, K., Wietgrefe, S. W., Rothenberger, M., Reilly, C., Chipman, J. G., et al. (2014). Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc. Natl. Acad. Sci. U.S.A.* 111, 2307–2312. doi: 10.1073/pnas.1318249111

Fromentin, R., Bakeman, W., Lawani, M. B., Khoury, G., Hartogensis, W., DaFonseca, S., et al. (2016). CD4+ T cells expressing PD-1, TIGIT and LAG-3 contribute to HIV persistence during ART. *PLoS Pathog.* 12:e1005761. doi: 10.1371/journal.ppat.1005761

Gupta, R. K., Abdul-Jawad, S., Mc Coy, L. E., Mok, H. P., Peppa, D., Salgado, M., et al. (2019). HIV-1 remission following CCR5Delta32/Delta32 haematopoietic stem-cell transplantation. *Nature* 568, 244–248. doi: 10.1038/s41586-019-1027-4

Hiener, B., Horsburgh, B. A., Eden, J. S., Barton, K., Schlub, T. E., Lee, E., et al. (2017). Identification of genetically intact HIV-1 proviruses in specific CD4(+) T cells from effectively treated participants. *Cell Rep.* 21, 813–822. doi: 10.1016/j.celrep.2017.09.081

Honeycutt, J. B., Thayer, W. O., Baker, C. E., Ribeiro, R. M., Lada, S. M., Cao, Y., et al. (2017). HIV persistence in tissue macrophages of humanized myeloid-only mice during antiretroviral therapy. *Nat. Med.* 23, 638–643. doi: 10.1038/nm.4319

Horsburgh, B. A., Lee, E., Hiener, B., Eden, J. S., Schlub, T. E., von Stockenstrom, S., et al. (2020). High levels of genetically-intact HIV in HLA-DR+ memory T-cells indicates their value for reservoir studies. *AIDS* 34, 659–668. doi: 10.1097/QAD.0000000000002465

Humbert, O., Chan, F., Rajawat, Y. S., Torgerson, T. R., Burtner, C. R., Hubbard, N. W., et al. (2018). Rapid immune reconstitution of SCID-X1 canines after G-CSF/AMD3100 mobilization and *in vivo* gene therapy. *Blood Adv.* 2, 987–999. doi: 10.1182/bloodadvances.2018016451

Hutter, G., Nowak, D., Mossner, M., Ganepola, S., Mussig, A., Allers, K., et al. (2009). Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N. Engl. J. Med.* 360, 692–698. doi: 10.1056/NEJMoa0802905

Imai, K., Togami, H., and Okamoto, T. (2010). Involvement of histone H3 lysine 9 (H3K9) methyltransferase G9a in the maintenance of HIV-1 latency and its reactivation by BIX01294. *J. Biol. Chem.* 285, 16538–16545. doi: 10.1074/jbc.M110.103531

Ji, H., Lu, P., Liu, B., Qu, X., Wang, Y., Jiang, Z., et al. (2018). Zinc-finger nucleases induced by HIV-1 tat excise HIV-1 from the host genome in infected and latently infected cells. *Mol. Ther. Nucleic Acids* 12, 67–74. doi: 10.1016/j.omtn.2018.04.014

Jiang, G., Mendes, E. A., Kaiser, P., Wong, D. P., Tang, Y., Cai, I., et al. (2015). Synergistic reactivation of latent HIV expression by Ingenol-3-Angelate, PEP005, targeted NF-kB signaling in combination with JQ1 induced p-TEFb activation. *PLoS Pathog.* 11:e1005066. doi: 10.1371/journal.ppat.1005066

Jiang, G., Nguyen, D., Archin, N. M., Yukl, S. A., Mendez-Lagares, G., Tang, Y., et al. (2018). HIV latency is reversed by ACSS2-driven histone crotonylation. *J. Clin. Invest.* 128, 1190–1198. doi: 10.1172/JCI98071

Jin, H., Li, D., Sivakumaran, H., Lor, M., Rustanti, L., Cloonan, N., et al. (2016). Shutdown of HIV-1 transcription in T cells by Nullbasic, a mutant Tat protein. *MBio* 7:e00518-16. doi: 10.1128/mBio.00518-16

Jin, H., Sun, Y., Li, D., Lin, M. H., Lor, M., Rustanti, L., et al. (2019). Strong *in vivo* inhibition of HIV-1 replication by Nullbasic, a Tat mutant. *MBio* 10:e01769-19. doi: 10.1128/mBio.01769-19

Kaminski, R., Bella, R., Yin, C., Otte, J., Ferrante, P., Gendelman, H. E., et al. (2016a). Excision of HIV-1 DNA by gene editing: a proof-of-concept *in vivo* study. *Gene Ther.* 23, 690–695. doi: 10.1038/gt.2016.41

Kaminski, R., Chen, Y., Fischer, T., Tedaldi, E., Napoli, A., Zhang, Y., et al. (2016b). Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. *Sci. Rep.* 6:22555. doi: 10.1038/srep28213

Kandathil, A. J., Sugawara, S., and Balagopal, A. (2016). Are T cells the only HIV-1 reservoir? *Retrovirology* 13:86. doi: 10.1186/s12977-016-0323-4

Kauder, S. E., Bosque, A., Lindqvist, A., Planelles, V., and Verdin, E. (2009). Epigenetic regulation of HIV-1 latency by cytosine methylation. *PLoS Pathog.* 5:e1000495. doi: 10.1371/journal.ppat.1000495

Kessing, C. F., Nixon, C. C., Li, C., Tsai, P., Takata, H., Mousseau, G., et al. (2017). *In Vivo* suppression of HIV rebound by didehydro-cortistatin A, a "Block-and-Lock" strategy for HIV-1 treatment. *Cell Rep.* 21, 600–611. doi: 10.1016/j.celrep.2017.09.080

Kumar, A., Abbas, W., and Herbein, G. (2014). HIV-1 latency in monocytes/macrophages. *Viruses* 6, 1837–1860. doi: 10.3390/v6041837

Lampi, Y., Van Looveren, D., Vranckx, L. S., Thiry, I., Bornschein, S., Debyser, Z., et al. (2019). Targeted editing of the PSIP1 gene encoding LEDGF/p75 protects cells against HIV infection. *Sci. Rep.* 9:2389. doi: 10.1038/s41598-019-38718-0

Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., et al. (2001). Initial sequencing and analysis of the human genome. *Nature* 409, 860–921. doi: 10.1038/35057062

Laspia, M. F., Rice, A. P., and Mathews, M. B. (1989). HIV-1 Tat protein increases transcriptional initiation and stabilizes elongation. *Cell* 59, 283–292. doi: 10.1016/0092-8674(89)90290-0

Lee, E., Bacchetti, P., Milush, J., Shao, W., Boritz, E., Douek, D., et al. (2019). Memory CD4 + T-cells expressing HLA-DR contribute to HIV persistence during prolonged antiretroviral therapy. *Front. Microbiol.* 10:2214. doi: 10.3389/fmicb.2019.02214

Li, C., Mousseau, G., and Valente, S. T. (2019). Tat inhibition by didehydro-Cortistatin A promotes heterochromatin formation at the HIV-1 long terminal repeat. *Epigenetics Chromatin* 12:23. doi: 10.1186/s13072-019-0267-8

Li, J., Chen, C., Ma, X., Geng, G., Liu, B., Zhang, Y., et al. (2016). Long noncoding RNA NRON contributes to HIV-1 latency by specifically inducing Tat protein degradation. *Nat. Commun.* 7:11730. doi: 10.1038/ncomms11730

Lim, H. G., Suzuki, K., Cooper, D. A., and Kelleher, A. D. (2008). Promoter-targeted siRNAs induce gene silencing of simian immunodeficiency virus (SIV) infection *in vitro*. *Mol. Ther.* 16, 565–570. doi: 10.1038/sj.mt.6300380

Lin, M. H., Apolloni, A., Cutillas, V., Sivakumaran, H., Martin, S., Li, D., et al. (2015). A mutant Tat protein inhibits HIV-1 reverse transcription by targeting the reverse transcription complex. *J. Virol.* 89, 4827–4836. doi: 10.1128/JVI.03440-14

Lin, M. H., Sivakumaran, H., Apolloni, A., Wei, T., Jans, D. A., and Harrich, D. (2012). Nullbasic, a potent anti-HIV Tat mutant, induces CRM1-dependent disruption of HIV rev trafficking. *PLoS ONE* 7:e51466. doi: 10.1371/journal.pone.0051466

Lin, M. H., Sivakumaran, H., Jones, A., Li, D., Harper, C., Wei, T., et al. (2014). A HIV-1 Tat mutant protein disrupts HIV-1 Rev function by targeting the DEAD-Box RNA helicase DDX1. *Retrovirology* 11:121. doi: 10.1186/s12977-014-0121-9

Lusic, M., Marcello, A., Cereseto, A., and Giacca, M. (2003). Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter. *EMBO J.* 22, 6550–6561. doi: 10.1093/emboj/cdg631

Marban, C., Suzanne, S., Dequiedt, F., de Walque, S., Redel, L., Van Lint, C., et al. (2007). Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. *EMBO J.* 26, 412–423. doi: 10.1038/sj.emboj.7601516

Mediouni, S., Chinthalapudi, K., Ekka, M. K., Usui, I., Jablonski, J. A., Clementz, M. A., et al. (2019a). Didehydro-Cortistatin A inhibits HIV-1 by specifically binding to the unstructured basic region of Tat. *MBio* 10:e02662-18. doi: 10.1128/mBio.02662-18

Mediouni, S., Kessing, C. F., Jablonski, J. A., Thenin-Houssier, S., Clementz, M., Kovach, M. D., et al. (2019b). The Tat inhibitor didehydro-cortistatin A suppresses SIV replication and reactivation. *Faseb J.* 33, 8280–8293. doi: 10.1096/fj.201801165R

Méndez, C., Ahlenstiel, C. L., and Kelleher, A. D. (2015). Post-transcriptional gene silencing, transcriptional gene silencing and human immunodeficiency virus. *World J. Virol.* 4:219. doi: 10.5501/wjv.v4.i3.219

Méndez, C., Ledger, S., Petoumenos, K., Ahlenstiel, C., and Kelleher, A. D. (2018). RNA-induced epigenetic silencing inhibits HIV-1 reactivation from latency. *Retrovirology* 15:67. doi: 10.1186/s12977-018-0451-0

Meredith, L. W., Sivakumaran, H., Major, L., Suhrbier, A., and Harrich, D. (2009). Potent inhibition of HIV-1 replication by a tat mutant. *PLoS ONE* 4:e7769. doi: 10.1371/journal.pone.0007769

Miller, J. B., Zhang, S., Kos, P., Xiong, H., Zhou, K., Perelman, S. S., et al. (2017). Non-viral CRISPR/Cas gene editing *in vitro* and *in vivo* enabled by synthetic nanoparticle co-delivery of Cas9 mRNA and sgRNA. *Angew. Chem. Int. Ed. Engl.* 56, 1059–1063. doi: 10.1002/anie.201610209

Morris, K. V., Chan, S. W., Jacobsen, S. E., and Looney, D. J. (2004). Small interfering RNA-induced transcriptional gene silencing in human cells. *Science* 305, 1289–1292. doi: 10.1126/science.1101372

Mousseau, G., Aneja, R., Clementz, M. A., Mediouni, S., Lima, N. S., Haregot, A., et al. (2019). Resistance to the Tat inhibitor didehydro-cortistatin A is mediated by heightened basal HIV-1 transcription. *mBio*. 10:e01750–18. doi: 10.1128/mBio.01750-18

Mousseau, G., Clementz, M. A., Bakeman, W. N., Nagarsheth, N., Cameron, M., Shi, J., et al. (2012). An analog of the natural steroidal alkaloid cortistatin A potently suppresses Tat-dependent HIV transcription. *Cell Host Microbe* 12, 97–108. doi: 10.1016/j.chom.2012.05.016

Mousseau, G., Kessing, C. F., Fromentin, R., Trautmann, L., Chomont, N., and Valente, S. T. (2015). The Tat inhibitor didehydro-cortistatin A prevents HIV-1 reactivation from latency. *MBio* 6:e00465–15. doi: 10.1128/mBio.00465-15

Niu, Q. L., Liu, Z. Q., Alamer, E., Fan, X. Z., Chen, H. Y., Endsley, J., et al. (2019). Structure-guided drug design identifies a BRD4-selective small molecule that suppresses HIV. *J. Clin. Invest.* 129, 3361–3373. doi: 10.1172/JCI120633

Pang, K. M., Castanotto, D., Li, H., Scherer, L., and Rossi, J. J. (2018). Incorporation of aptamers in the terminal loop of shRNAs yields an effective and novel combinatorial targeting strategy. *Nucleic Acids Res.* 46:e6. doi: 10.1093/nar/gkx980

Pardons, M., Baxter, A. E., Massanella, M., Pagliuzza, A., Fromentin, R., Dufour, C., Leyre, L., et al. (2019). Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection. *PLoS Pathog.* 15:e1007619. doi: 10.1371/journal.ppat.1007619

Penton, P. K., and Blackard, J. T. (2014). Analysis of HIV quasispecies suggests compartmentalization in the liver. *AIDS Res. Hum. Retroviruses* 30, 394–402. doi: 10.1089/aid.2013.0146

Peterson, C. W., Haworth, K. G., Burke, B. P., Polacino, P., Norman, K. K., Adair, J. E., et al. (2016). Multilineage polyclonal engraftment of Cal-1 gene-modified cells and *in vivo* selection after SHIV infection in a nonhuman primate model of AIDS. *Mol. Ther. Methods Clin. Dev.* 3:16007. doi: 10.1038/mtm.2016.7

Qu, D., Sun, W. W., Li, L., Ma, L., Sun, L., Jin, X., et al. (2019). Long noncoding RNA MALAT1 releases epigenetic silencing of HIV-1 replication by displacing the polycomb repressive complex 2 from binding to the LTR promoter. *Nucleic Acids Res.* 47, 3013–3027. doi: 10.1093/nar/gkz117

Rice, A. P. (2019). Unexpected mutations in HIV-1 that confer resistance to the tat inhibitor didehydro-cortistatin A. *MBio* 10:e01547-19. doi: 10.1128/mBio.01547-19

Ringpis, G. E., Shimizu, S., Arokium, H., Camba-Colon, J., Carroll, M. V., Cortado, R., et al. (2012). Engineering HIV-1-resistant T-cells from short-hairpin RNA-expressing hematopoietic stem/progenitor cells in humanized BLT mice. *PLoS ONE* 7:e53492. doi: 10.1371/journal.pone.0053492

Rochat, M. A., Schlaepfer, E., and Speck, R. F. (2017). Promising role of toll-like receptor 8 agonist in concert with prostratin for activation of silent HIV. *J. Virol.* 91:e02084-16. doi: 10.1128/JVI.02084-16

Rodger, A. J., Cambiano, V., Bruun, T., Vernazza, P., Collins, S., Degen, O., et al. (2019). Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study. *Lancet* 393, 2428–2438. doi: 10.1016/S0140-6736(19)30418-0

Romerio, F., Zapata, J., and Kashanchi, F. (2016). The HIV-1 antisense transcript AST promotes latency by recruiting PRC2 to the 5′ LTR. *J. Acquir. Immune Defic. Syndr.* 71, 45–45. doi: 10.1097/01.qai.0000479747.49476.e5

Rustanti, L., Jin, H., Li, D., Lor, M., Sivakumaran, H., and Harrich, D. (2018). Differential effects of strategies to improve the transduction efficiency of lentiviral vector that conveys an anti-HIV protein, Nullbasic, in human T cells. *Virol. Sin.* 33, 142–152. doi: 10.1007/s12250-018-0004-7

Rustanti, L., Jin, H., Lor, M., Lin, M. H., Rawle, D. J., and Harrich, D. (2017). A mutant Tat protein inhibits infection of human cells by strains from diverse HIV-1 subtypes. *Virol. J.* 14:52. doi: 10.1186/s12985-017-0705-9

Saayman, S., Ackley, A., Turner, A. W., Famiglietti, M., Bosque, A., Clemson, M., et al. (2014). An HIV-encoded antisense long noncoding RNA epigenetically regulates viral transcription. *Mol. Ther.* 22, 1164–1175. doi: 10.1038/mt.2014.29

Shi, B., Li, J., Shi, X., Jia, W., Wen, Y., Hu, X., et al. (2017). TALEN-mediated knockout of CCR5 confers protection against infection of human immunodeficiency virus. *J. Acquir. Immune Defic. Syndr.* 74, 229–241. doi: 10.1097/QAI.0000000000001190

Shi, J., Manolikakes, G., Yeh, C. H., Guerrero, C. A., Shenvi, R. A., Shigehisa, H., et al. (2011). Scalable synthesis of Cortistatin A and related structures. *J. Am. Chem. Soc.* 133, 8014–8027. doi: 10.1021/ja202103e

Singh, A., Palanichamy, J. K., Ramalingam, P., Kassab, M. A., Bhagat, M., Andrabi, R., et al. (2014). Long-term suppression of HIV-1C virus production in human peripheral blood mononuclear cells by LTR heterochromatization with a short double-stranded RNA. *J. Antimicrob. Chemother.* 69, 404–415. doi: 10.1093/jac/dkt348

Sivro, A., Schuetz, A., Sheward, D., Joag, V., Yegorov, S., Liebenberg, L. J., et al. (2018). Integrin alpha4beta7 expression on peripheral blood CD4(+) T cells predicts HIV acquisition and disease progression outcomes. *Sci. Transl. Med.* 10:eaam6354. doi: 10.1126/scitranslmed.aam6354

Sodroski, J., Patarca, R., Rosen, C., Wongstaal, F., and Haseltine, W. (1985). Location of the trans-activating region on the genome of human T-cell lymphotropic virus type-Iii. *Science* 229, 74–77. doi: 10.1126/science.2990041

Spina, C. A., Anderson, J., Archin, N. M., Bosque, A., Chan, J., Famiglietti, M., et al. (2013). An in-depth comparison of latent HIV-1 reactivation in multiple cell model systems and resting CD4+ T cells from aviremic patients. *PLoS Pathog.* 9:e1003834. doi: 10.1371/journal.ppat.1003834

Suzuki, K., Ahlenstiel, C., Marks, K., and Kelleher, A. D. (2015). Promoter targeting RNAs: unexpected contributors to the control of HIV-1 transcription. *Mol. Ther. Nucleic Acids* 4:e222. doi: 10.1038/mtna.2014.67

Suzuki, K., Hattori, S., Marks, K., Ahlenstiel, C., Maeda, Y., Ishida, T., et al. (2013). Promoter targeting shRNA suppresses HIV-1 infection *in vivo* through transcriptional gene silencing. *Mol. Ther. Nucleic Acids* 2:e137. doi: 10.1038/mtna.2013.64

Suzuki, K., Ishida, T., Yamagishi, M., Ahlenstiel, C., Swaminathan, S., Marks, K., et al. (2011). Transcriptional gene silencing of HIV-1 through promoter targeted RNA is highly specific. *RNA Biol.* 8, 1035–1046. doi: 10.4161/rna.8.6.16264

Suzuki, K., Juelich, T., Lim, H., Ishida, T., Watanebe, T., Cooper, D. A., et al. (2008). Closed chromatin architecture is induced by an RNA duplex targeting the HIV-1 promoter region. *J. Biol. Chem.* 283, 23353–23363. doi: 10.1074/jbc.M709651200

Suzuki, K., Shijuuku, T., Fukamachi, T., Zaunders, J., Guillemin, G., Cooper, D., et al. (2005). Prolonged transcriptional silencing and CpG methylation induced by siRNAs targeted to the HIV-1 promoter region. *J. RNAi Gene Silencing* 1, 66–78.

Symonds, G., Bartlett, J. S., Kiem, H. P., Tsie, M., and Breton, L. (2016). Cell-delivered entry inhibitors for HIV-1: CCR5 downregulation and blocking virus/membrane fusion in defending the host cell population. *AIDS Patient Care STDS* 30, 545–550. doi: 10.1089/apc.2016.0245

Symons, J., Cameron, P. U., and Lewin, S. R. (2018). HIV integration sites and implications for maintenance of the reservoir. *Curr. Opin. HIV AIDS* 13, 152–159. doi: 10.1097/COH.0000000000000438

Tsukamoto, T. K. R., Marks, K., Hattori, S., Ahlenstiel, C., Symonds, G., Okada, S., et al. (2018a). Transcriptional gene silencing limits CXCR4-associated depletion of bone marrow CD34+ cells in HIV-1 infection. *AIDS* 32, 1737–1747. doi: 10.1097/QAD.0000000000001882

Tsukamoto, T. K. R., Marks, K., Hattori, S., Ahlenstiel, C., Symonds, G., Okada, S., et al. (2018b). Erratum: transcriptional gene silencing limits CXCR4-associated depletion of bone marrow CD34+ cells in HIV-1 infection. *AIDS* 32, 2857–2858. doi: 10.1097/01.aids.0000547984.16868.93

Turner, A. M., Ackley, A. M., Matrone, M. A., and Morris, K. V. (2012). Characterization of an HIV-targeted transcriptional gene-silencing RNA in primary cells. *Hum. Genet. Ther.* 23, 473–483. doi: 10.1089/hum.2011.165

Turner, A. M., De La Cruz, J., and Morris, K. V. (2009). Mobilization-competent lentiviral vector-mediated sustained transcriptional modulation of HIV-1 expression. *Mol. Ther.* 17, 360–368. doi: 10.1038/mt.2008.268

Van Lint, C., Emiliani, S., Ott, M., and Verdin, E. (1996). Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *EMBO J.* 15, 1112–1120. doi: 10.1002/j.1460-2075.1996.tb00449.x

Vansant, G., Vranckx, L. S., Zurnic, I., Van Looveren, D., Van de Velde, P., Nobles, C., et al. (2019). Impact of LEDGIN treatment during virus production on residual HIV-1 transcription. *Retrovirology* 16:8. doi: 10.1186/s12977-019-0472-3

Verdin, E., Paras, P. Jr., and Van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. *EMBO J.* 12, 3249–3259. doi: 10.1002/j.1460-2075.1993.tb05994.x

Vranckx, L. S., Demeulemeester, J., Saleh, S., Boll, A., Vansant, G., Schrijvers, R., et al. (2016). LEDGIN-mediated inhibition of integrase-LEDGF/p75 interaction reduces reactivation of residual latent HIV. *Ebiomedicine* 8, 248–264. doi: 10.1016/j.ebiom.2016.04.039

Wallet, C., De Rovere, M., Van Assche, J., Daouad, F., De Wit, S., Gautier, V., et al. (2019). Microglial cells: the main HIV-1 reservoir in the brain. *Front. Cell. Infect. Microbiol.* 9:362. doi: 10.3389/fcimb.2019.00362

Wang, G., Zhao, N., Berkhout, B., and Das, A. T. (2016a). A combinatorial CRISPR-Cas9 attack on HIV-1 DNA extinguishes all infectious provirus in infected T cell cultures. *Cell Rep.* 17, 2819–2826. doi: 10.1016/j.celrep.2016.11.057

Wang, G., Zhao, N., Berkhout, B., and Das, A. T. (2016b). CRISPR-Cas9 can inhibit HIV-1 replication but NHEJ repair facilitates virus escape. *Mol. Ther.* 24, 522–526. doi: 10.1038/mt.2016.24

Weinberg, M. S., Villeneuve, L. M., Ehsani, A., Amarzguioui, M., Aagaard, L., Chen, Z. X., et al. (2006). The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells. *RNA* 12, 256–262. doi: 10.1261/rna.2235106

Wolstein, O., Boyd, M., Millington, M., Impey, H., Boyer, J., Howe, A., et al. (2014). Preclinical safety and efficacy of an anti-HIV-1 lentiviral vector containing a short hairpin RNA to CCR5 and the C46 fusion inhibitor. *Mol. Ther. Methods Clin. Dev.* 1:11. doi: 10.1038/mtm.2013.11

Yamagishi, M., Ishida, T., Miyake, A., Cooper, D. A., Kelleher, A. D., Suzuki, K., et al. (2009). Retroviral delivery of promoter-targeted shRNA induces long-term silencing of HIV-1 transcription. *Microbes Infect.* 11, 500–508. doi: 10.1016/j.micinf.2009.02.003

Yukl, S. A., Shergill, A. K., Ho, T., Killian, M., Girling, V., Epling, L., et al. (2013). The distribution of HIV DNA and RNA in cell subsets differs in gut and blood of HIV-positive patients on ART: implications for viral persistence. *J. Infect. Dis.* 208, 1212–1220. doi: 10.1093/infdis/jit308

Zapata, J. C., Campilongo, F., Barclay, R. A., DeMarino, C., Iglesias-Ussel, M. D., Kashanchi, F., et al. (2017). The Human Immunodeficiency Virus 1 ASP RNA promotes viral latency by recruiting the Polycomb Repressor Complex 2 and promoting nucleosome assembly. *Virology* 506, 34–44. doi: 10.1016/j.virol.2017.03.002

Zhang, Z., Nikolai, B. C., Gates, L. A., Jung, S. Y., Siwak, E. B., He, B., et al. (2017). Crosstalk between histone modifications indicates that inhibition of arginine methyltransferase CARM1 activity reverses HIV latency. *Nucleic Acids Res.* 45, 9348–9360. doi: 10.1093/nar/gkx550

Zhou, J., Lazar, D., Li, H., Xia, X., Satheesan, S., Charlins, P., et al. (2018). Receptor-targeted aptamer-siRNA conjugate-directed transcriptional regulation of HIV-1. *Theranostics* 8, 1575–1590. doi: 10.7150/thno.23085

Conflict of Interest: GS was employed by the company CSL Australia, Ltd. CA, GS, and AK have a patent for siRNA sequences.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past co-authorship with one of the authors SK.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership