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HIV PATHOGENESIS AND TREATMENT (AL LANDAY AND NS UTAY, SECTION EDITORS)

Tyrosine Kinase Inhibition: a New Perspective in the Fight against HIV

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Abstract

Purpose of Review HIV-1 infection is incurable due to the existence of latent reservoirs that persist in the face of cART. In this review, we describe the existence of multiple HIV-1 reservoirs, the mechanisms that support their persistence, and the potential use of tyrosine kinase inhibitors (TKIs) to block several pathogenic processes secondary to HIV-1 infection.

Recent Findings Dasatinib interferes in vitro with HIV-1 persistence by two independent mechanisms. First, dasatinib blocks infection and potential expansion of the latent reservoir by interfering with the inactivating phosphorylation of SAMHD1. Secondly, dasatinib inhibits the homeostatic proliferation induced by γc-cytokines. Since homeostatic proliferation is thought to be the main mechanism behind the maintenance of the latent reservoir, we propose that blocking this process will gradually reduce the size of the reservoir.

Summary TKIs together with cART will interfere with HIV-1 latent reservoir persistence, favoring the prospect for viral eradication.

Keywords HIV-1 reservoir · Homeostatic cytokines · SAMHD1 · Tyrosine kinase inhibitors · Immune activation

Introduction

CD4+ T lymphocytes and myeloid cells are the main targets for HIV-1 infection. In both cell types, HIV-1 can establish a long-term persistent infection even in the presence of effective combined antiretroviral therapy (cART), leading to the formation of viral reservoirs that persist for the life of the patient [1, 2]. These reservoirs are generated very early, during the first days after infection [3–5], which represents a formidable challenge toward the eradication of the virus. Overall, reservoir size and timing of cART initiation impact the ability of individuals to control the virus and are, therefore, critical determinants of viral load and disease progression [6–8]. The existence of a very small number of infected cells, often undetectable with standard techniques, is sufficient to lead to viral rebound and disease recrudescence after treatment interruption [4, 9, 10••].

Importantly, viral reservoirs in people living with HIV on cART do not appear to be homogeneous. Distinct reservoirs have been observed in vivo, which differ in anatomic location, cell type, and activation/differentiation state of the infected cells (reviewed in [11]). While the existence of a truly latent reservoir in the T cell compartment is universally accepted, the state of the virus in myeloid cells, whether latent/inducible versus undergoing chronic, low level replication, remains unclear. Nevertheless, several recent reports have argued that macrophages and macrophage-like cells in various anatomical locations constitute a bona fide viral reservoir that is sufficient, in several animal models of HIV infection, to give rise to viral recrudescence after cART discontinuation [12••, 13–15]. Understanding the origin and nature of the different viral reservoirs is critical toward designing therapeutic strategies that will be broadly effective across multiple cell types.

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The T Cell Reservoir

Productive HIV-1 infection of CD4+ T lymphocyte cells requires cell activation [16]. Conversely, resting lymphocytes are highly resistant to HIV-1 infection. Therefore, it has been proposed that initial infection of T cells occurs while cells are activated, whereas establishment of latency occurs at or near the time when infected cells transition from an activated state into quiescence. However, the establishment of latency by viruses directly infecting quiescent cells has not been ruled out and, in fact, has been demonstrated in in vitro systems [17, 18].

Several mechanisms contribute to the low infectivity of quiescent T cells. First, resting T cells express low levels of CCR5, a major HIV-1 co-receptor. Secondly, non-cycling cells contain extremely low levels of dNTPs, which are not sufficient to support efficient reverse transcription [19–21]. Third, cellular factors required for HIV-1 transcription, such as cyclin T1 and phospho-CDK9, are also low in resting lymphocytes [22, 23]. Finally, SAMHD1 is a nuclear triphosphohydrolase that controls dNTP levels during cell cycle progression, cleaving dNTPs to deoxynucleosides and inorganic triphosphates [21, 24–30]. Quiescent lymphocytes express the antiviral protein SAMHD1 in its active, non-phosphorylated form, whereas in activated cells, SAMHD1 is predominantly in a phosphorylated, inactive form. In contrast, activated lymphocytes are highly susceptible to HIV-1 infection and full viral replication because in this environment HIV-1 co-receptors are fully expressed, dNTP levels are high to allow cell division, and cellular transcription factors required for HIV-1 gene expression are naturally activated to allow for abundant cellular transcription. Furthermore, concomitant with cellular activation and proliferation, SAMHD1 is inactivated via phosphorylation of threonine 592 (T592) by cyclin A2/Cdk1 and cyclin D2/CDK4 complexes, contributing to a cellular state of permissiveness to HIV-1 infection [24–26, 30].

The latent HIV-1 reservoir in quiescent T cells is extremely stable over time. Longitudinal measurements of the T cell latent reservoir in patients on cART over multiple years revealed an estimated half-life of about 44 months and a predicted time to eradication of 73 years on average [31]. This observation has dramatic clinical implications, as drug-resistant variants can persist long-term in the presence of adequate cART. Close examination of viral sequences in the reservoir as well as viral integration sites have revealed that the latent reservoir exists in a dynamic state in which certain proviruses can be greatly amplified through clonal expansion of T cells whereas others may remain in low numbers [32]. The main factor influencing this dynamic equilibrium is thought to be the rate of cell proliferation, as influenced by exposure to cytokines, antigen recognition by the cells, and viral integration at or near gene regulating cell division [33–35].

The Myeloid Cell Reservoir

Myeloid cells such as macrophages and dendritic cells (DCs) display HIV-1 receptors and co-receptors, and are therefore potential targets for infection in vivo [36]. HIV-1-infected macrophages have been found in many tissues, including the central nervous system (CNS), lymph nodes, lungs, liver, kidneys, and gastrointestinal system, and are thought to contribute to inflammation and tissue damage despite immune reconstitution achieved by cART-induced viral suppression [37–42]. It has been proposed that macrophages can support HIV-1 infection and harbor virus over prolonged periods of time independently of T cells, even in the presence of cART [38–40]. This notion has recently been strengthened by experiments conducted in humanized myeloid-only mice [12••, 15] and by infection of rhesus macaques with neurotropic SIV strains [13, 14]. Therefore, strategies aimed at preventing virus spread to tissue macrophages, either independently or in the context of latency reversal, will be important components of ongoing HIV-1 cure efforts.

Whereas well-characterized mechanisms of viral persistence in latently infected CD4+ T cells are documented, including transcriptional silencing of proviral DNA and a profoundly quiescent metabolic phenotype, similar mechanisms have not been described in persistently infected macrophages. Important questions regarding the nature of HIV-1 persistence in tissue macrophages in vivo remain unanswered, including their half-life, and the antiretroviral drug accessibility and efficacy in these cells. Several studies described a gene transcription profile in HIV-1 infected macrophages that, unlike that of CD4+ T cells, favors apoptosis resistance and prolonged survival despite infection [43, 44]. Therefore, the development of pharmacological strategies to suppress macrophage infection remains essential and it will likely require different strategies that those intended for suppressing HIV-1 in CD4+ T cells.

DCs are less permissive to HIV-1 infection as compared with macrophages, and their contribution to the viral reservoirs in vivo is still debated. DCs are thought to participate in HIV-1 infection in two different modes. First, DCs can capture HIV-1 at or near sites of transmission and transfer it to secondary lymph nodes where resident CD4+ T cells can be productively infected [45, 46]. Second, dissemination of HIV-1 through the body can also be accomplished by DCs after they become infected (reviewed in [47]). The possible contribution of DCs to the latent reservoir is not known.

Dynamics of the Latent Reservoir

Once the viral latent reservoir is established, the proliferation of memory CD4+ T cells can be sustained by antigenic stimulation (antigen-driven proliferation) or by γc-cytokines

(homeostatic proliferation), rendering the reservoir very stable over time [31, 32, 48, 49]. Although CD4+ T cells will proliferate upon activation by either of the above forms of stimulation, the cellular activation states resulting from these stimuli are completely different. Antigen-driven proliferation leads to multiple rounds of cell division and simultaneous proviral reactivation and virion production. In contrast, homeostatic proliferation is highly inefficient in reactivating proviruses, when compared with antigen-driven proliferation [49]. Therefore, homeostatic proliferation induced by γc-cytokines favors the mitotic spread of proviruses and is likely a major component behind the longevity of the CD4+ T cell latent reservoir [49–51, 52•, 53].

Homeostatic proliferation is essential for the immune system in order to maintain normal T cell counts and to correct for deviations due to expansion or depletion of the memory cell pool [54–56]. Homeostatic proliferation of CD4+ T cells is driven by extrinsic signals, typically γc-cytokines IL-2, IL-7, and IL-15 [54, 57, 58]. In clinical trials in which HIV-infected patients have been treated with IL-7 for lymphopenia, an increase in the reservoir size along with poor viral activation has been consistently detected, supporting the role for IL-7-dependent proliferation in reservoir expansion [59, 60]. Furthermore, T cell proliferation, whether induced by γc-cytokines or by antigen recognition, can result in enhancement of HIV-1 spread due to the inactivation of SAMHD1 by phosphorylation [53]. This key finding means that episodic instances of viral reactivation due, for example, to antigen recognition by T cells can lead to reinfection of neighboring cells, especially in anatomic locations with poor cART penetration.

Successful cART treatment stops ongoing replication following antigenic stimulation of infected cells, but has no effect on homeostatic proliferation of the reservoir, which explains the long-term stability of the viral reservoir despite years or even decades of viral suppression on cART [31]. Accordingly, we hypothesize that new therapeutic strategies able to hinder T cell activation and homeostatic proliferation, combined with effective cART, may lead to the eventual elimination of the latent reservoir. In this regard, certain tyrosine kinase inhibitors (TKIs) such as dasatinib may interfere with γc-cytokine-induced homeostatic proliferation [53]. Additionally, TKIs can block T cell activation via inhibition of kinases such as $p56^{lck}$ [61] and downstream PKCθ [62], which leads to dramatic decreases in viral replication levels.

Vpx and HIV-2

HIV-2 is a less common type of the human immunodeficiency virus, and it is known to be associated with lesser mortality and morbidity than HIV-1, and unlike HIV-1, HIV-2 is not considered a pandemic virus. An important difference between both types of viruses is that the non-structural, accessory gene *vpr* underwent a duplication giving rise to the paralog *vpx* in HIV-2. Thus, while HIV-1 encodes *vpr*, HIV-2 and certain SIV lineages encode both *vpr* and *vpx* [63]. Vpx specifically recruits SAMHD1 to the Cul4-DDB1-DCAF1 E3 ubiquitin ligase, leading to SAMHD1 ubiquitination and proteolytic destruction in the proteasome [64]. HIV-1 is not thought to counteract SAMHD1 whereas HIV-2 does so efficiently through Vpx. Accordingly, HIV-2 Vpx induces rapid destruction of SAMHD1, regardless of its state of phosphorylation, thus allowing macrophages to be efficiently infected by HIV-2 [25]. Based on the above observations, we predict that the use of dasatinib to maintain SAMHD1 in a dephosphorylated/active state in the context of HIV-2 will be less effective, or perhaps not at all, in protecting macrophages and quiescent T lymphocytes from infection by HIV-2.

Tyrosine Kinase Inhibitors in Cancer Treatment

TKIs are currently used in the clinic for long-term treatment of chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and other blood cancers due to their ability to inhibit the aberrant activity of the tyrosine kinase BCR-ABL, which results in unrestrained cell proliferation [65–67]. In CML and ALL a reciprocal translocation between chromosomes 9 and 22 in hematopoietic stem cells produces a shortened chromosome 22 known as the Philadelphia chromosome [68, 69]. This translocation event results in the production of BCR-ABL, a fusion gene encoding a chimeric protein kinase with constitutive activity. Initially, the small-molecule TKI imatinib was developed to target this translocation product. After being approved by the FDA in 2001 to be used in patients with CML, imatinib revolutionized the treatment of these patients, achieving high remission rates and greatly improving patient's survival [70]. Due to the generation of resistance to imatinib by mutations in BCR-ABL, a second generation of TKIs was developed, including nilotinib, dasatinib, and bosutinib. These TKIs showed higher potency than imatinib against BCR-ABL (> 20–300-fold, depending on the inhibitor) [71, 72], although resistance development was not completely eliminated. Dasatinib is administered indefinitely in these patients to prevent relapses, which may occur due to lack of adherence to the treatment or to the emergence of resistance mutations in the BCR-ABL open reading frame [73]. More recently, a third generation of Src-TKIs, represented by ponatinib, has been approved [74].

CML is not commonly associated with HIV-1 infection. However, several cases have been published of HIV-infected patients on cART who developed CML [75–77]. The outcome

of concurrent CML and HIV-1 infection was generally poor prior to the introduction of imatinib and cART [78]. Presently, however, patients with concurrent CML and HIV infection can be treated simultaneously with cART and one TKI, more commonly imatinib, demonstrating good tolerance to TKI and excellent clinical evolution, with complete hematological, cytological, and molecular responses to the leukemia as well as cART-induced HIV-1 control [75–78]. However, as both imatinib and dasatinib are primarily metabolized by CYP3A4 [79, 80], it is important to check for potential interactions with CYP3A4 inducers among the cART drugs, such as nevirapine and efavirenz (non-nucleoside reverse transcriptase inhibitors), or with CYP3A4 inhibitors such as ritonavir (protease inhibitor) [81, 82], adjusting dosage as necessary.

Effect of TKIs on HIV-1 Replication

Control of SAMHD1 Activity by TKIs

We previously demonstrated that CD4+ T cells isolated from peripheral blood of patients with CML on treatment with TKIs such as dasatinib and ponatinib showed resistance to HIV-1 infection and, consequently, a reduced rate of proviral integrants downstream [61]. This interference with HIV-1 replication was mostly due to the induction of a cytostatic state by TKIs that maintained SAMHD1 in a dephosphorylated/active state, enforcing low levels of dNTPs, and also restricting the activation of cellular transcription factors that are essential for HIV-1 replication, such as NF-κB [82]. Therefore, we hypothesize that treatment of HIV-1-infected patients with TKIs and cART may circumvent reservoir replenishment or maintenance by interfering with the homeostatic proliferation of latently infected cells. A potential, additional advantage of certain TKI (see the "TKIs and Cellular Senescence" section below) resides in their recently described ability to inhibit the chronic inflammation and immune senescence that are characteristic of HIV-infected patients [83].

As discussed above, SAMHD1, in its active form, imposes a potent blockade against HIV-1 infection. Therefore, it would be ideal if the activity of SAMHD1 could be controlled pharmacologically. In this regard, most TKIs led to HIV-1 restriction, with dasatinib and ponatinib being the most potent ones in T cells [61, 84] and dasatinib and crenolanib in macrophages [85]. HIV-1 infectivity inversely correlated with the proportion of SAMHD1 present in its active, dephosphorylated state as manipulated by TKIs. We also confirmed the central role of SAMHD1 in the antiviral effect of dasatinib using Vpx(+) VLPs, which specifically induced SAMHD1 degradation and overcame the TKI-imposed restriction on the virus [53, 85]. Therefore, SAMHD1 activity can be manipulated with TKIs to render CD4+ T cells and macrophages refractory to HIV-1 infection.

Interferons and Dasatinib Modulate SAMHD1 Activity

Interferons (IFNs) constitute a family of critical cytokines involved in first-line defense mechanisms against several infections, including HIV [86]. Human monocyte-derived macrophages (MDM) are less permissive to HIV-1 infection than activated CD4+ T cells mostly due to the presence of active host restriction factors such as SAMHD1 that control the establishment and spread of viral infection. In this regard, dephosphorylation of SAMHD1 is a conserved effector mechanism that results from stimulation of MDM by IFNs type I and II and, to a lesser extent, type III [85], apparently acting in a similar way as dasatinib. Based on the structure of their receptors on the cell surface, IFNs are classified into type I (α, β, ε, ω, and others), type II (γ), and type III (λ) [87, 88]. The various IFN species display vastly different HIV-1-specific antiviral efficiencies, as evidenced by the higher potency of IFN-α, IFN-ε, and IFN-γ, in contrast with the limited potency of IFN-λ [85]. Enhanced dNTPase activity appears to be the functional outcome of SAMHD1 activation, as supported by several lines of evidence, including the complete reversal of IFN-induced restriction by addition of exogenous dNTPs and the degradation of SAMHD1 by the HIV-2/SIV protein Vpx [26, 89]. The finding that different IFNs are able to modulate SAMHD1 phosphorylation and activation without changing the total levels of this protein [85] is an important step in understanding innate immune responses in macrophages and, possibly, other immune cell subsets. Despite similarities, the signaling pathway through which dasatinib prevents SAMHD1 phosphorylation and enhances its antiviral activity is likely different from that involved in stimulation by IFN types I and II because dasatinib, unlike IFNs, does not induce the expression of IFN-stimulated genes (ISGs) [85]. Additionally, TKIs target a variety of cellular pathways that control cell activation, including not only ABL- and Src-family kinases, but also type III receptors of tyrosine kinases (RTK) such as c-KIT and FLT3, and cyclin-dependent kinases involved in progression through the cell cycle such as CDK1, CDK2, CDK4, and CDK6 [61, 90]. Type I and II IFNs regulate dephosphorylation of SAMHD1 at least in part through the downregulation of CDK1. Thus, siRNAs against CDK1 phenocopied the effects of type I and II IFNs on SAMHD1 and viral infection in macrophages [85]. Dasatinib dramatically downregulated phosphorylation of SAMHD1, without affecting total SAMHD1 protein levels, and also downregulated the levels of CDK1 in macrophages [85] and T cells [61].

TKIs and Cellular Senescence

Senescent cells are major drivers of aging-related diseases [91]. Frailty, an integrative measure identifying patients at high risk of adverse clinical outcomes from aging-related

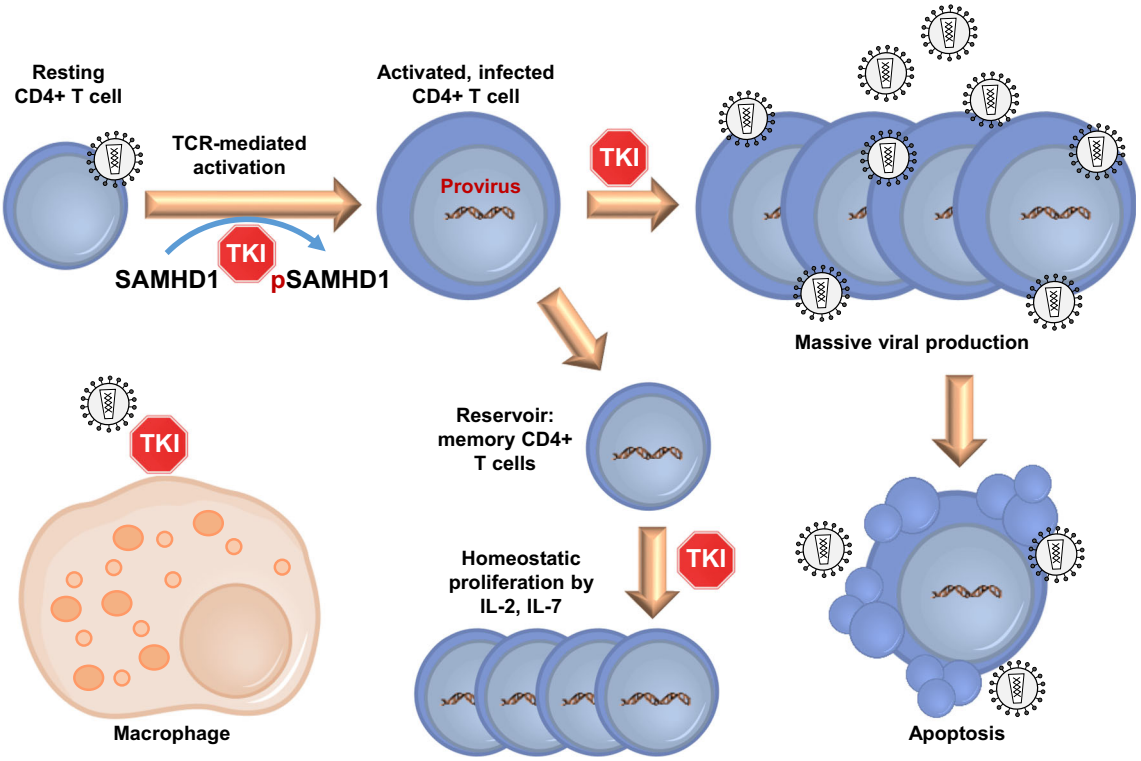

Fig. 1 Different mechanisms by which TKIs are thought to interfere with HIV-1 reservoir formation and replenishment. TKIs such as dasatinib may interfere with HIV-1 reservoir at several levels: (a) blocking SAMHD1 phosphorylation to preserve its antiviral activity and avoid the infection of new target cells; (b) impeding the proliferation of clonally expanded, infected CD4+ T cells during activation of the immune response; (c) interfering with the γC-cytokine-induced homeostatic proliferation of infected memory CD4+ T cells that contribute to the continuous expansion and maintenance of the latent reservoir; (d) impeding the infection of long-lived cells such as macrophages that may reside in tissues with limited access to drug exposure such as CNS

conditions, is common among people living with treated HIV-1 infection [92]. In fact, frailty is increasingly being considered an important HIV-associated, non-AIDS complication in this population [93, 94]. Cellular senescence is characterized by secretion of pro-inflammatory, pro-apoptotic, pro-fibrotic products (collectively known as the senescence-associated secretory phenotype or SASP) and resistance to apoptosis [95]. Senescent cells incite chronic immune activation and cell death among bystander cells, which in turn results in local or systemic dysfunction. A murine aging model has demonstrated that direct targeting of senescent cells results in increased health- and life-span [96]. This discovery has given rise to the identification and characterization of compounds that can either specifically kill senescent cells (senolytics) or modify their phenotype (senomorphics) [91]. The first human trial of senolytics tested a combination of dasatinib and quercetin (a natural product that targets BCL2, insulin-like growth factor 1, and hypoxia-inducible factor 1-alpha) in patients with idiopathic pulmonary fibrosis, and demonstrated that these compounds were safe, well-tolerated, and were associated with improved physical function [97]. In keeping with its role as a senolytic, dasatinib is known to inhibit pro-inflammatory functions of neutrophils as well as T cell activation and proliferation [98–101]. Dasatinib may therefore directly address both HIV persistence through its antiproliferative effects and the pro-inflammatory consequences of chronic viral infection in its role as a senolytic.

Conclusion

Eradication of HIV-1 infection will require comprehensive approaches that focus on all possible viral reservoirs, including persistently infected macrophages that localize to sanctuary sites like the CNS, to which therapeutic drugs and immune interventions have poor access. As a very small number of infected cells are sufficient to re-seed the reservoir, future therapeutic approaches will have to eradicate virtually all latently infected cells in order to be effective. Dasatinib and other TKIs may be valuable therapeutics that, by blocking homeostatic proliferation of T cells, are predicted to contribute to a quantitative and, hopefully, progressive decline of the reservoir size and its settlement in sanctuaries such as CNS (Fig. 1). The additional ability of dasatinib to preserve SAMHD1 antiviral function in T cells and macrophages would provide added value to the activities of conventional cART. Since dasatinib and other TKIs have been extensively tested in humans with success in certain cancers and are

generally well-tolerated, the prospect for advancing these drugs through clinical trials is highly favorable [102]. The available information from clinical trials and beyond will be an asset that will allow for rapid translation of initial in vitro findings into in vivo mouse and non-human primate studies, followed by pilot human clinical trials in the context of HIV-1 eradication. Last, but not least, dasatinib has demonstrated positive immunomodulatory properties [103–106] that can have an adjuvant effect additional to the primary effects on homeostatic proliferation and viral inhibition. The available information from years of clinical use of these drugs is an asset that will allow for rapid translation of our initial in vitro findings into in vivo mouse and non-human primate studies, followed by pilot clinical trials in the context of HIV-1 eradication.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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