Tyrosine kinase inhibitors: potential use and safety considerations in HIV-1 infection

Abstract

Introduction: Infection caused by HIV-1 is nowadays a chronic disease due to a highly efficient antiretroviral treatment that is nevertheless, unable to eliminate the virus from the organism. New strategies are necessary in order to impede the formation of the viral reservoirs, responsible for the failure of the antiretroviral treatment to cure the infection.

Areas covered: The purpose of this review is to discuss the possibility of using tyrosine kinase inhibitors (TKIs) for the treatment of HIV-1 infection. These inhibitors are successfully used in patients with distinct cancers such as chronic myeloid leukemia. The most relevant papers have been selected and commented.

Expert opinion: The family of TKIs are directed against the activation of tyrosine kinases from the Src family. Some of these kinases are essential for the activation of CD4+ T cells, the major target of HIV-1. During acute or primary infection the CD4+ T cells are massively activated, which is mostly responsible for the generation of the reservoirs, the spread of the infection and the destruction of activated CD4+ T cells, infected or not. Consequently, we discuss the possibility of using TKIs as adjuvant of the antiretroviral treatment against HIV-1 infection mostly, but not exclusively, during the acute/recent phase.

Keywords: HIV/AIDS; Tyrosine kinase inhibitors; chronic myeloid leukemia; immunomodulation; viral reservoirs; T-cell activation

Article highlights

- HIV-1 infection is currently incurable due to the formation of viral reservoirs.
- Antiretroviral treatment has transformed HIV-1 infection into a chronic disease that needs life-long treatment.
- CD4+ T cell massive activation during HIV-1 acute infection is mostly responsible for viral spread, the formation of the reservoirs and the destruction of CD4+ T cells.
- Control of CD4+ T cell activation could avoid viral replication and spread and, consequently, the formation of the viral reservoirs.
- Src tyrosine kinases are essential for the activation of CD4+ T cells and their inhibition could avoid the formation of HIV-1 reservoirs.
- Tyrosine kinase inhibitors currently used for treating chronic myeloid leukemia could be potential adjuvants of the antiretroviral treatment during HIV-1 infection.

Body of the review paper

1. Lymphocytic activation as an antiviral target

1.1. The role of immune activation in the HIV-1 cycle

HIV-1 may infect both quiescent and activated CD4+ T cells (1), but only activated cells support HIV-1 replication (2). Overall, resting/non-activated T cells are highly restrictive to HIV-1 infection and replication due to several factors (Figure 1). First, CCR5 chemokine receptor, the major HIV-1 co-receptor, is highly expressed only in activated T cells (3). Second, as resting T cells are in G0 phase, DNA synthesis is not required and dNTP levels are low. This prevents an efficient reverse transcription of the viral RNA. Among the checkpoint factors that regulate cell cycle, the phosphohydrolase SAMHD1 (SAM domain and HD domain-containing protein 1) decreases dNTP levels when is active, thereby constituting an essential antiviral factor against HIV-1 infection in resting cells (4). Third, resting, non-cycling T cells have low ATP levels, which impedes an active transport of the viral pre-integration complex to the nucleus (5). Finally, resting T cells do not require the active expression of genes involved in the generation of the immune response. Therefore, the activity of transcriptional factors such as NF-κB, SP1 and NFAT that are necessary for HIV-1 transcription (6, 7) is very low in resting T cells. However, triggering an immune response leads to the activation of these transcription factors that are required not only for the expression of genes encoding receptors, cytokines and chemokines, but also for driving the expression of viral genes involved in the production of new virions.

The first three mechanisms are required for *de novo* HIV-1 infection, viral reverse transcription and integration, while the last mechanism is necessary to trigger an efficient transcription and elongation of the HIV-1 genome from a quiescent, integrated molecular double-stranded DNA form in the host genome called provirus. Therefore,

HIV-1 has successfully adapted to the cellular environment of CD4+ T cells by using a Trojan horse mechanism. The provirus remains in a state of latency in resting CD4+ T cells, allowing viral persistence and establishing viral reservoirs. Due to the lack of transcription and expression of viral mRNA and proteins, these infected resting cells are not recognized by the immune system and escape the immune surveillance. In contrast, when these latently infected lymphocytes become activated in the context of the normal generation of an immune response, the cell provides HIV-1 with the transcription factors required to induce the expression of the viral genome and a massive viral replication occurs in the activated CD4+ T cells (8).

This viral strategy represents a major barrier to HIV-1 cure due to the establishment of undetectable viral reservoirs in quiescent CD4+ T cells (9). Paradoxically, normal lymphocytic activation in response to HIV-1 infection or other pathogens, results in a robust viral replication, a permissive environment to new rounds of HIV-1 infection and the destruction of activated and thereafter infected lymphocytes. Lymphocytic activation is particularly detrimental in early phases of HIV-1 infection and in those lymphoid organs in which a high level of cellular activation occurs due to the encounter with antigens such as the lymph nodes and mostly, the gut-associated lymphoid tissue (GALT). Actually, it has been shown that in acute HIV-1 infection up to 80% of CD4+ T cells from the GALT are destroyed through different cytopathic mechanisms driven by HIV-1, being among them direct cytopathic effect, apoptosis and pyroptosis (10-14). However, a small subset of infected CD4+ T cells will persist and create the reservoir due to not completely known mechanisms such as the expression of the survival protein BCL-2 in long-lived CD4+ T cells (15) or the lingering presence of HIV-1 regulator protein Tat (16). On the other hand, T-cell activation is required to built-up effective immune responses targeting the virus but this sustained activation during the chronic

illness also contributes to the clonal exhaustion of virus-specific CD8+ T cells (17), as well as to the destruction of CD4+ T cells, particularly those committed to the recognition of HIV-1 antigens (18). These specific anti-HIV-1 T-cell cytotoxic and proliferative responses are not restored despite months of effective ART (19). Moreover, persistent replication occurs during the chronic phase of the infection even in the presence of an efficient antiretroviral treatment (ART) (20-23). In consequence, new strategies must be developed in order to avoid massive activation and infection of CD4+ T cells during the acute phase of HIV-1 infection and to interfere with the low-level, persistent viral replication that occurs during the chronic stage.

1.2. Immunomodulatory strategies in HIV-1 infection

Due to the close relationship between immune activation and viral replication, different authors proposed a controlled immune suppression together with ART. Actually, using immunomodulatory therapies in combination with ART could help control the spread of the infection, the formation of the viral reservoir, the development of escape mutant variants and the exhaustion of the immune system due to the sustained activation. In this regard, some clinical trials performed in HIV-infected patients who were treated with immunosuppressive drugs as adjuvant of ART showed contradictory results, such as those using cyclosporine A (CsA). Rizzardi et al. (24) did not report a significant effect in plasma viral load of HIV-infected patients treated with CsA and ART, but they observed a persistent increase in CD4 count that was sustained after withdrawal of CsA. However, Calabrese et al. (25) showed no positive effect on CD4 count and they even reported an increase in the viral load after administration of CsA. Markowitz et al. (26) also described that administering CsA and ART to patients with acute early HIV-1 infection did not provide any immunological or virological benefit. Therefore, while

some studies showed a clinical benefit in using CsA with ART (24, 27, 28), others did not (25, 26, 29, 30).

It has also been described that both mycophenolic acid (MPA) and its ester derivate mycophenolate mofetil (MMF) suppress HIV-1 infection by interfering with the synthesis of guanosine nucleotides (31). Some clinical trials stated that MPA and MMF decreased both titers of infected CD4+ T cells (31) and viral load (32) in ART-treated HIV-infected patients. However, Sankatsing et al. (33) did not find significant changes in the viral RNA between MMF-treated and untreated groups. Besides, although it was determined that the combination of MMF and ART could delay viral rebound and improve control of viral replication during structured treatment interruption (STI), this strongly correlated with the simultaneous inhibition of lymphocyte proliferation (34, 35). This correlation was also observed in HIV-infected patients that were treated with hydroxyurea (HU) and ART before STI (36). Consequently, the interference with lymphocyte proliferation was essential to cause a delay in the viral rebound during STI likely due to a smaller size of the reservoir. However, this does not guarantee that the immunosuppressors could also have a detrimental activity against the viral reservoir that is already formed. Moreover, although no major toxicity was reported in these trials, clinicians were reluctant to use immunosuppressive drugs in patients that could already be immunosuppressed. Therefore, more specific immunosuppressants must be found in order to direct their inhibitory effect specifically to the HIV-1 target cells without causing a general immunosuppression.

1.2.1. Role of tyrosine kinases in lymphocyte activation

Lymphocyte activation by antigens occurs through the interaction of the T-cell receptor (TCR) with cytoplasmic non-receptor tyrosine protein kinases. One of the most important tyrosine kinases for lymphocyte activation is LCK that induces the activation

of downstream kinases such as the protein kinase C theta (PKCθ) (37). PKCθ in turn activates transcription factors such as NF-κB, NFAT and AP-1 (38, 39) that are essential for both T-cell activation and HIV-1 replication. LCK belongs to the SRC-family kinases (SFKs), a family of nine non-receptor tyrosine kinases that perform highly specialized functions within the various lineages (40). B cells primarily express LYN, FYN, and BLK, whereas T cells mostly express LCK and FYN-T, a spliced form of FYN that partially encodes the kinase domain (41). LCK (56 kDa) is expressed constantly through most of the lifespan of a T cell but the expression of FYN (59 kDa) mostly increases in mature cells (42). This implies that LCK has a unique role throughout T-cell development and function whereas FYN appears to be less critical for mature T-cell generation and function (43). Both molecules have several domains in common but LCK has a unique domain that mediates non-covalent interaction with CD4 and CD8 (44), which facilitates the initiation of T-cell receptor (TCR)/CD3 complex signal transduction during antigen recognition (43). Consequently, LCK is mostly found at the plasma membrane, whereas FYN colocalizes with centrosomal and mitotic structures (45, 46).

LCK activation is regulated through phosphorylation of the tyrosine residue Y394 located in the kinase domain. In resting T cells, this site is normally not phosphorylated, rendering LCK inactive. Upon TCR-major histocompatibility complex (MHC)/peptide interaction, LCK is recruited and phosphorylated at the activation loop Y394. Active LCK induces direct or indirect phosphorylation of several substrates such as SAMHD1 (47) and also specific tyrosine residues within the ITAMs (immunoreceptor tyrosine-based activation motifs) of CD3 chains, leading to the recruitment, phosphorylation and activation of SYK-family kinases (ZAP-70 in T cells) to the ITAMs. This triggers a signaling cascade that initiates Ca2+ influx, activates protein kinase C theta (PKCθ) and

mitogen-activated protein kinase (MAPK) cascades, ultimately resulting in NFAT, NF-κB, and AP-1 activation (39, 48), as well as full T-cell activation, characterized by entry into cell cycle, changes in gene expression and T-cell effector function (43). NF-κB is a family of transcriptional regulators considered a central mediator of the immune response as it mostly participates in promoting the expression of cytokines and chemokines, receptors for immune recognition and migration, and proteins for antigen presentation (49). Activation of NF-κB is very fast and strong and some viruses have evolved to modulate NF-κB signaling pathway in order to enhance viral replication and host cell survival, as well as to evade immune response (50). HIV-1 replication succeeds mostly due to the presence of NF-κB consensus binding sites in the enhancer proximal region of the viral long terminal repeat (LTR) promoter. In fact, the interference with NF-κB in HIV-infected cells inhibits viral transcription and replication (51, 52).

Consequently, using immunomodulators able to suppress selectively the activity of SFKs such as LCK would be an advantage over the immunosuppressive strategies that have been tested so far for the treatment of HIV-1 infection as they would be directed specifically against the activation of the main target of the virus but would not cause a general immunosuppression (53, 54). In this regard, tyrosine kinase inhibitors (TKIs) are currently used in clinic for the treatment of chronic myeloid leukemia (CML) and other types of cancer such as lung cancer, thyroid cancer, or renal cell carcinoma.

2. Overview of TKI approved for CML: clinical practice, effects and toxicity

CML is a neoplastic disorder of the hematopoietic stem cell characterized by the uncontrolled growth of myeloid cells in the bone marrow and their accumulation in peripheral blood (55). CML occurs as a result of an acquired reciprocal translocation between chromosomes 9 and 22 in the hematopoietic stem cells, resulting in the

Philadelphia chromosome, whose molecular counterpart is the BCR-ABL oncogene. Thus, the BCR gene, encoding for a protein whose function is not completely understood, is located in the breakpoint of chromosome 22, whereas the ABL proto-oncogene, encoding for a tyrosine kinase protein, is located at the breakpoint of chromosome 9 (56, 57). Therefore, the translocation produces the chimeric fusion protein BCR-ABL, encoded by both *BCR* and *ABL1* genes and that is responsible for the development of CML due to its uncontrolled tyrosine kinase activity (58, 59).

The treatment of CML is currently based on the use of a group of oral small molecules that selectively inhibit the BCR-ABL oncogenic protein (60). These molecules are summarized in Table 1, adapted from Steegmann et al (61). Imatinib, the first TKI against BCR-ABL introduced in clinical practice, revolutionized the therapy of CML due to the achievement of unprecedentedly high rates of deep responses that translate into high progression-free and overall survival similar to that of the control population (62). The so-called second-generation TKIs, including nilotinib, dasatinib, and bosutinib, were introduced later in CML treatment (63) and were initially given to patients resistant or intolerant to imatinib. Nilotinib is at least 20-fold and dasatinib at least 300-fold more potent than imatinib against BCR-ABL (64), whereas bosutinib is 100-200-fold more potent than imatinib (65). More recently, a third-generation TKI, ponatinib, has been approved for the treatment of patients with the T315I mutation of the *BCR-ABL* kinase domain, which is resistant to the other TKIs, and for patients with resistance to the second-generation TKIs (66).

In addition to inhibiting the BCR-ABL protein, the TKIs have other molecular targets, a fact that would explain their different toxicity profile (63). In this regard, imatinib is the TKI with a better toxicity profile since, although it causes frequent, low-grade chronic side effects, no safety issues have been observed with prolonged follow-up. The main

toxicity of nilotinib is the development of ischemic events (67), a complication registered in 12 to 18% of patients at 5 years of treatment. Pleural effusion is the most characteristic adverse effect of dasatinib (68), appearing in 30% of patients at one year, more frequently in patients with previous chronic obstructive pulmonary disease. This complication requires dose reduction or cessation of dasatinib administration. In CML patients, with the usual doses, other adverse reactions that have been reported as very frequent (> 1/10 patients) for dasatinib (69), as is shown in Table 2. Diarrhea and elevation in serum transaminases are the main toxicities of bosutinib (70). Finally, ponatinib is the most toxic TKI, due to its association with the appearance of ischemic events in a substantial proportion of the patients (71). All TKIs toxicity is detailed in Table 2.

A number of immunological effects of TKIs have also been described (72). Imatinib produces lymphopenia and decreased immunoglobulin plasma levels (73), whereas nilotinib inhibits CD8+ T cell function (74). Dasatinib inhibits the proliferation and activation of T cells and suppresses the cytotoxic activity of Natural Killer cells *in vitro* (75). In contrast, 30% of patients receiving dasatinib develop absolute lymphocytosis with clonal expansion of cytotoxic T cells and Natural Killer cells (76-78). However, patients usually show a good therapeutic outcome with deep responses to dasatinib (78) and no increased susceptibility to opportunistic infections (79). This supports the notion that the immunological response triggered by dasatinib and targeting Natural Killer cells and CD8+ T lymphocytes would be a mechanism that contributes to its therapeutic activity. In fact, control of lymphocyte activation has been associated to increased immune responses and disease control in cancer (80, 81).

3. Pre-clinical data on the use of TKIs in HIV-1 infection

Once the provirus is integrated, it remains hidden inside the resting CD4+ T cells in a latent state until the TCR/CD3-mediated activation promotes an active viral transcription and new viral particles are produced. Consequently, the control of T-cell activation might provide a way to control HIV-1 infection and replication. Our group described previously that the selective inhibition of PKCθ activity interfered with HIV-1 transcription because they inhibit the activity of the transcription factors NFAT, NF-κB and AP-1 (53, 82). We also demonstrated that PKCθ selective inhibitors were able to partially interfere with SAMHD1 phosphorylation at T592, preserving the antiviral activity and avoiding HIV-1 proviral integration. One of the main barriers that HIV-1 encounters when it enters the target cell is SAMHD1. As indicated above, SAMHD1 regulates cell cycle progression and HIV-1 reverse transcription by depleting the intracellular pool of dNTPs (83), thereby interfering with the integration of the provirus. SAMHD1 activity is regulated by phosphorylation of threonine residue T592 by Cdk1 and cyclin A2 during T-cell activation. Therefore, when SAMHD1 is phosphorylated at T592 it loses the antiviral activity because T-cell cycle progresses and HIV-1 vital cycle can be completed (84). Therefore, inhibition of PKCθ would inhibit through two different mechanisms: on one hand, a decrease in lymphocytic activation would result in a quiescent state highly restrictive to HIV infection; on the other hand, through inhibition of SAMHD1 phosphorylation, PKCθ inhibitors will directly tackle reverse transcription, integration and the formation of reservoirs. As LCK is upstream PKCθ signaling pathway, we determined that the inhibition of LCK activity with dasatinib was able to inhibit HIV-1 replication (54). Dasatinib inhibited HIV-1 replication of PBMCs isolated from healthy donors after activation with PHA/IL-2 in the presence of this TKI before infecting them in vitro with HIV-1 for 5 days (IC50 = 16 nM; CC50 > 10 μM) (Figure 2A). SAMHD1 phosphorylation at T592 was inhibited at 18.75 nM (Figure 2B),

proving that preservation of SAMHD1 antiviral activity and interference with HIV-1 reverse transcription and subsequent integration were the main mechanisms of action for dasatinib-mediated inhibition of HIV-1 infection in primary T cells (47, 54). It should also be considered that dasatinib has a wide range of activity, being able to inhibit BCR-ABL and all SFKs as Lck, whereas other ITKs such as imatinib are quite selective of BCR-ABL (85). This implies that not all the TKIs might have the same mechanism of action.

Dasatinib is administered chronically to CML patients at a dose of 100 mg dq. Our results proved that concentrations of dasatinib displaying antiviral activity in vitro may be lower than those used in clinical practice for the treatment of CML. Full inhibition of SAMHD1 phosphorylation at 18.75 nM demonstrated that dasatinib exhibits linear pharmacokinetics, thereby suggesting a proportional increase in AUC (area under the concentration-time curve) and linear elimination characteristics over the dose range of 25 mg to 120 mg BID (twice a day). The antiviral activity of dasatinib in vitro was also confirmed ex vivo by isolating PBMCs from five CML patients on chronic treatment with dasatinib for at least 2 years. PBMCs were activated with PHA/IL-2 48 hours before ex vivo infection with HIV-1 and then incubated for 5 days. Similar results to PBMCs treated in vitro with dasatinib were obtained, observing a decrease in HIV-1 proviral integration and transcription, as well as lower phosphorylation of SAMHD1 in CD4+ T cells from CML patients upon activation (54). These results proved not only that the dose of dasatinib used for the treatment of CML was able to interfere with HIV-1 infection, but also that long-lasting intracellular dasatinib levels able to inhibit HIV-1 replication can be achieved with doses currently used in CML treatment.

4-Potential use of TKIs in HIV-1 infection: timing of infection and risk of toxicity

ART is the cornerstone for HIV-1 treatment. Although ART has tremendously evolved since 1995 (start of modern triple ART) and has transformed the lethal HIV-1 infection into a chronic disease, no other adjuvant or alternative strategy has proved to be effective so far (86). We hypothesized that TKIs, particularly dasatinib that inhibits a large spectrum of tyrosine kinases, could reduce the size of the viral reservoirs during acute/recent infection (54), and even reduce replenishment of the reservoirs during chronic infection. Consequently, although its impact may be higher in acute/recent infection, it could also be useful in chronically infected patients. Dasatinib, when administered during short-time periods, might also decrease immune activation, interfering with the characteristic chronic inflammation that leads to non-AIDS comorbidities related to HIV-1 infection. This sustained, low-grade inflammation and increased immune activation are strongly associated with a heightened risk for cardiovascular disease, osteoporosis, cancer, physical function impairments and frailty, among other non-AIDS-defining events and mortality (87, 88).

As described above, a number on immunological approaches have been evaluated trying to improve the clinical outcome of HIV-1 infection. In this regard, TKIs, and particularly dasatinib, may provide an interesting additional antiretroviral tool due to several different mechanisms: first, a direct antiretroviral effect through the inhibition of SAMHD1 phosphorylation and consequently, of proviral integration, which would lead to a reduced viral reservoir; second, an indirect anti-reservoir effect through the inhibition of the clonal expansion of the infected cells due to its cytostatic effect (54); and finally, a putative role of increased Natural Killer or γδ T lymphocytes activity after TKI discontinuation has been proposed in CML patients with treatment-free remission (89) (90, 91). If such mechanisms produced by TKI could also contribute to the immune control of HIV replication remains speculative. All three effects might be useful to

improve viral control at any stage of HIV-1 infection, both in acute/recent and chronically infected patients. Although the third mechanism is completely speculative in the HIV field, a growing body of evidence suggests that the immune system has a major role in determining the therapeutic efficacy of TKIs (92). Figure 3 summarizes the potential mechanisms of TKIs to interfere with HIV-1 replication and spread, as well as their possible effect on the size of the viral reservoir. Besides, TKIs could also be beneficial against other aspects of HIV infection such as the Kaposi sarcoma (93).

However, the addition of one TKI to ART during early HIV-1 infection might have the highest impact, reducing the final size of the reservoir, which has been associated to the time of virological control when ART is stopped (the lowest the reservoir, the longest the period without viral replication) (94, 95). Since the reservoir is formed very early during initial Fiebig stages (Figure 4) (96), only a short period (4 to 12 weeks) of TKI administration might be enough to obtain a clinical benefit, reducing the risk of increased toxicity by TKI. In addition, as previously described, doses required may be lower than those used in clinical practice for CML, decreasing even further the potential for side effects. Finally, if an immunomodulatory effect and increased long-acting immune control of HIV-1 replication effect induced by dasatinib is confirmed, this may be the basis for intermittent administration during brief periods, reducing further the potential for serious toxicity. Indeed, some CML patients exhibit what is called a treatment-free remission of the disease after treatment with TKI (89). In the particular case of HIV-1 infection, treatment with TKIs in acute infection could block the development of specific HIV-1 immune responses, mostly cytotoxic responses that are raised in the first weeks of infection. This delay in building up immune responses against HIV-1 could paradoxically be beneficial at mid-term by two different reasons: first, the immune responses will be generated in the absence of very high viremia levels

that result in abnormal immune activation and apoptosis (97). Actually, it has been demonstrated that very early treatment of HIV-1 infection blocks high peaks of viremia and results in better immune responses against viral replication (98). Second, it has been described that HIV-1 variants resistant to cytotoxic T lymphocyte (CTL)-specific responses are easily generated and stored in the reservoirs in the acute phase of infection due to the accelerated replication and the generation of escape variants (99). This viral escape that leads to failure of immune control compromises the future potential use of CTL-based therapeutic vaccines raised against immunodominant epitopes. The down-modulation of immune activation during the acute phase of infection could contribute to overcome the emergence of resistant variants by delaying specific HIV-1 responses within a context of lower viremia and normal cellular activation. Moreover, Shytaj et al (100) described that the restriction of CD4+ T cell activation in macaques infected with the simian immunodeficiency virus (SIV) was not only well tolerated, but even the SIV-specific cell-mediated immunity was increased. This suggests that likely TKIs would not negatively influence HIV-specific immune responses.

Available information regarding the antiretroviral activity of TKIs is very limited for all the drugs in this family (101). However, the anti-HIV-1 effects of dasatinib have been evaluated and it seems to be the most attractive drug of the class for this use (54, 102). There is not clinical experience with dasatinib in HIV-infected population. Therefore, the potential toxicity should be considered from the available information in CML patients and then extrapolated to the eventual clinical use in HIV-infected patients. Currently, dasatinib is indicated at usual doses ranging from 100 to 140 mg/day for the treatment of adult patients with Philadelphia chromosome positive (Ph+) CML in chronic, accelerated or blastic phase, and Ph+ acute lymphoblastic leukemia (ALL) (69). In patients who tolerate this medication and show haematological response, the

drug is administered indefinitely. Safety profile of dasatinib has been recorded from almost 3,000 patients included in clinical trials, most of them patients with CML who failed to or were intolerant to imatinib (69). The tolerability and rate of adverse reactions in patients with HIV-1 infection, using lower doses (ranging 20 to 70 mg) and for a shorter periods (up to 16 weeks) is unknown, but is expected to be lower.

However, it is essential to consider that in patients with both acute/recent or chronic HIV-1 infection, the addition of one TKI to ART regimen may represent a risk for increased toxicity and frequency of the side effects listed in Table 2. Potential raising issues include, first, bone marrow suppression, as HIV-1 infection itself has been associated with decreased numbers of red blood cells, leukocytes and platelets (103). Lymphopenia related to uncontrolled HIV-1 replication is especially prominent during acute infection (104) and, as previously explained, some TKIs such as imatinib induces lymphopenia. In chronic and advanced HIV-1 disease, there is a global dysfunction of bone marrow function and all three blood series may be reduced (103). The addition of a potential bone marrow suppressor such as the TKIs may increase this effect. Second, the high risk of infections should be considered as the most remarkable clinical expression of fully developed AIDS is the emergence of opportunistic diseases and tumors related to advanced immunodeficiency (105). Although infrequent, opportunistic infections may even arise as a complication of acute HIV-1 infection, when CD4 T cell count reaches the lowest level (nadir). In this context, the addition of one TKI to ART regimen may potentiate this risk. Hepatitis B reactivation has been described in patients receiving TKIs who were previously infected with hepatitis B virus (HBV), even those with no active replication (isolated anti-HBVc positive patients) (106). Since approximately 10% of HIV-1 infected patients are co-infected with HBV (107), particular attention should be paid to the potential reactivation of HBV in these patients,

although several drugs used in current antiretroviral regimens, such as lamivudine, emtricitabine or tenofovir have also anti-HBV activity.

Finally, potential serious drug-drug-interactions (DDI) may occur as several antiretroviral drugs are metabolized by Cytochrome P450 3A4 (CYP3A4). In particular, non-nucleoside reverse transcriptase inhibitors (NNRTI) are inducers of this enzyme, whereas cobicistat and ritonavir are powerful inhibitors (108), which is the basis of pharmacokinetics enhancement of protease inhibitors (PI) and elvitegravir (an integrase-strand transfer inhibitor -InSTI-). Dasatinib, as well as other TKIs such as nilotinib, bosutinib and ponatinib, are also metabolized though CYP3A4 pathway (109-111). In consequence, standard doses of dasatinib or other TKIs might become toxic when co-administered with ritonavir, cobicistat or any other potent CYP3A4 inhibitor. In that context, ART regimens containing drugs such as dolutegravir or raltegravir should be considered if dasatinib is co-administered, since these molecules have no expected drug-drug interactions with TKI and are among the preferred regimens in the most recent guidelines (112, 113). On the other hand, non-specific, general, side effects, such as fatigue or gastrointestinal intolerance may also be relevant, since they could reduce the optimal adherence to ART, which is essential for treatment efficacy, particularly during ART initiation.

Conclusion:

At first, TKIs, and particularly dasatinib, may appear very attractive as adjunctive therapy for treating HIV-1 infection, especially in specific clinical settings such as acute/recent infection. However, the risk for increased toxicity and adverse events has to be carefully balanced. Initial evaluation should target patients who most likely could be helped by this intervention with the lowest risk for toxicity, such as patients with high CD4 T cell count, no active co-infections, and no previous conditions that could risk for

increased toxicity from TKIs (e.g., leucopenia). In addition, DDI should be carefully considered and drugs interfering with CYP3A4 inhibiting activity should be avoided; raltegravir or dolutegravir based-regimens can be used in combination with TKI. However, the potential virological benefit of TKIs and particularly dasatinib seems to be enough to consider performing controlled pilot trials, closely monitored and with carefully selected patients. Reduction of doses and/or shortening the duration of treatment, or even intermittent use to decrease the potential side effects rate, as well as avoiding DDI, might prove that using TKIs in HIV-1 infected individuals could be highly beneficial to reduce the reservoir size from the beginning, making possible longer structured treatment interruption of ART.

Expert opinion

A cure for HIV-1 infection is not available yet due to viral persistence in the reservoirs. To address this issue different strategies have been proposed (for a review see Coiras et al. (8) and Table 3). Shock and kill strategies with latency reversing agents (LRAs), aiming at the reactivation and destruction of the viral reservoirs, have been assayed in clinical trials with few success due to low drug potency and toxic effects. Bone-marrow transplantation approaches using HIV-resistant CCR5 deficient lymphocytes are only indicated in very special cases. Gene therapy tools are under development but in case an efficient system could be selected in the near future, this strategy is not affordable for all candidate patients due to economic and technical constrains. Finally, enhancement of immune responses aiming at the elimination of the HIV-1 reservoirs has shown interesting results in the SIV model but raises safety concerns for its use in humans (114). Therapeutic vaccination to increase specific cell responses against HIV-1 faces the challenge of hidden reservoirs carrying escape variants to immunodominant responses (99). Overall, there is not a clear strategy to tackle the HIV-1 reservoirs and achieve a functional cure of infected patients. Therefore, new approaches should be considered. In this context, the use of immunosuppressive/immunomodulatory drugs in a disease leading to immune suppression appears to be a paradox. However, although HIV-1 can infect both resting and activated CD4+ T cells, it only replicates in activated cells because they have adequate amounts of dNTPs, ATP, and active transcription factors to permit a complete replication cycle. Moreover, in activated CD4+ T cells the antiviral factor SAMHD1 is phosphorylated, which permits not only the progression of the cell cycle but also an effective viral reverse transcription. Therefore, HIV-1 replication is mostly successful due to the massive activation of CD4+ T cells that

occurs at very high levels during the first stages of the infection, allowing the early formation of viral reservoirs that renders the infection, from this moment, incurable.

Based on these observations, some attempts have been made to try to control immune activation during HIV-1 infection. However, although the rationale for using immunosuppressants as adjuvants of conventional ART was solid, the results were controversial as the experimental design and the stages of infection of the patients that entered in the different clinical trials were very diverse and hardly comparable. Moreover, there was reluctance to use immunosuppressants in a disease that already causes immunosuppression, even in the presence of the antiretroviral treatment. However, one important conclusion was obtained from these clinical trials: the control of viral replication during STI was improved in those patients previously treated with a combination of immunosuppressants and ART, in whom a delay in viral rebound was observed in comparison with patients only treated with ART. But this improvement was only achieved when lymphocyte proliferation was inhibited by the immunomodulatory/cytostatic agent, suggesting that administering immunosuppressants during acute infection could be potentially useful to avoid massive CD4+ T cell activation and consequently, to restrain the formation of viral reservoirs. In fact, the smaller the size of the reservoir, the later the viral rebound during STI, and the better the prognosis of the disease. Besides, decreasing immune activation during acute infection can result in lower lymphocyte destruction by HIV-1 through different mechanisms and contributes to preserve specific HIV-1 immune responses and avoid viral escape mutants. Although the rationale for using immunosuppressants with ART in patients with early acute infection can be considered, their use in chronically infected patients is more controversial as they would not directly beneficiate from this regimen by having reduced the size of the viral reservoir. However, it could be recommendable to

administer the cytostatic drugs in these patients in order to avoid low-level viral replication and the chronic activation of the immune system that eventually, leads to the failure of the immune response. TKIs could also be used to limit the viral reservoir replenishment during STI following the administration of LRAs.

The family of tyrosine kinase inhibitors (TKIs) that are currently used in clinic for the treatment of several types of cancer such as chronic myeloid leukemia, emerges as a potential adjuvant of ART in acutely HIV-infected patients to reduce or impede the formation of the viral reservoir, as well as in chronically infected patients to avoid viral replication and to help the immune system to preserve its function. Some of these TKIs are directed against SRC kinases that are specifically expressed in the main viral targets: the CD4+ T cells. Therefore, their use would not cause a general immunosuppression like the immunosuppressants that were used in previous clinical trials. In fact, they would achieve two specific goals, depending on the targeted tyrosine kinase: 1) to avoid the phosphorylation – and subsequent inactivation – of SAMHD1 through the interference with LCK activity; and 2) to restrict CD4+ T cell activation due to their cytostatic effect, impeding HIV-1 replication.

From a clinical point of view, several aspects should be considered. The most adequate TKI should display a broad range of inhibition of tyrosine kinases to make possible the interference with HIV-1 replication in vitro by preserving the antiviral effect of SAMHD1 and by inducing a cytostatic effect on CD4+ T cells. This would ensure a resistance to infection in the most important targets of HIV-1, as well as to avoid the formation of the viral reservoir. Dasatinib, a TKI shares these characteristics but although is generally well tolerated, several serious adverse effects have been described for this drug and the possible interaction with the antiretroviral treatment should be carefully considered. Moreover, dasatinib is a potent cytostatic of T cells, and although

it does not appear to make CML patients on chronic treatment more susceptible to infection, is unknown whether they could enhance opportunistic infections in immunodeficient patients. A pilot clinical trial with a small number of patients carefully selected would be advisable to determine the feasibility of this approach, evaluating both immunological and virological benefits for the patients with this chronic infection.

Figure legends

Figure 1. Factors that induce high restriction to HIV-1 infection and replication in resting/non-activated CD4+ T cells.

Figure 2. (A) PBMCs isolated from healthy donors were activated with PHA/IL-2 in the presence of dasatinib at different concentrations before infecting them in vitro with NL4-3_Renilla strain (1ng p24 per million of cells) for 5 days. The presence of Renilla in this strain allowed the monitorization of the infection with luminescence (relative light units, RLUs). IC50 and CC50 were calculated by using GraphPad software. The selectivity index (SI = CC50/IC50) was calculated to determine the therapeutic index, giving maximum antiviral activity with minimal cytotoxicity. R^2 is a measure of goodness-of-fit of linear regression. (B) PBMCs isolated from healthy donors were activated with IL-7 for 5 days in the presence of dasatinib at different concentrations and then SAMHD1 phosphorylation was analyzed by immunoblotting using specific antibodies. β-actin was used as loading control.

Figure 3. Mechanisms of inhibition of the HIV-1 cycle by TKIs. TKIs specifically block SAMHD1 phosphorylation and inhibits viral reverse transcription (1). Through inhibition of T-cell activation, TKIs inhibit full viral replication (2) and the reservoir expansion by interfering with the homeostatic proliferation of latently infected T cells (3). Finally, enhancement of the antiviral activity of Natural Killer (NK) cells can contribute to the destruction of HIV-infected CD4+ T lymphocytes (4).

Figure 4. Fiebig stages of HIV-1 infection (modified from Fiebig et al. (96)).

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