mTOR as a multifunctional therapeutic target in HIV infection

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Patients undergoing long-term highly active antiretroviral therapy treatment are probably at a higher risk of various HIV-related complications. Hyperactivation of The mammalian target of rapamycin (mTOR) has been found to contribute to dysregulated apoptosis and autophagy which determine CD4+-T-cell loss, impaired function of innate immunity and development of neurocognitive disorders. Dysregulated mTOR activation has also been shown to play a key part in the development of nephropathy and in the pathogenesis of HIV-associated malignancies. These studies strongly support a multifunctional key role for mTOR in the pathogenesis of HIV-related disorders and suggest that specific mTOR inhibitors could represent a novel approach for the prevention and treatment of these pathologies.

mTOR and its pharmacological inhibitors: multifunctional agonists and antagonists regulating immune functions, cancer, aging, viral infection and autism spectrum disorders

The mammalian target of rapamycin (mTOR), also known as mechanistic target of rapamycin or FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1), is a ~289 kDa protein originally discovered and cloned from Saccharomyces cerevisiae that shares sequence homologies with the phosphoinositide 3-kinase 3-kinase (PI3-kinase) family and represents a serine/threonine protein kinase that is present in all eukaryotic organisms [1].

mTOR plays a key part as an intracellular nutrient sensor that controls protein synthesis, cell growth and metabolism [2]; it is activated by different stimuli such as amino acids, growth factors and oxygen. mTOR represents the catalytic subunit of two distinct complexes, called mTORC1 and mTORC2 [2]. mTORC1 controls cell growth by maintaining a balance between anabolic processes, such as macromolecular synthesis and nutrient storage, and catabolic processes, such as autophagy and the utilization of energy stores [3,4]. mTORC2 regulates cell survival and cytoskeletal organization through the regulation of Akt and protein kinase C alpha (PKCα), respectively [5].

Rapamycin (Rapamune®; sirolimus) is a macrolide first isolated from the soil bacterium Streptomyces hygroscopicus [6]. It represents the first-in-class of specific mTOR inhibitors. This class also includes derivatives, termed rapalogs, which include temsirolimus, everolimus and ridaforolimus. Some of these drugs have been approved for the prevention of graft rejection and the treatment of cancers. However, in agreement with the pleiotropic role of mTOR in cell biology, several experimental studies indicate beneficial effects of mTOR inhibitors in other conditions that range from Parkinson’s disease to aging, viral infection and autism [7–9].

When rapamycin binds to its intracellular receptor FK-binding protein 12 (FKBP12) a complex is formed (rapamycin–FKBP12), and this inhibits the ability of mTOR to phosphorylate the p70 ribosomal protein S6 kinase 1 (p70S6K) [10]. In doing so, rapamycin interferes with the PI3K/Akt/mTOR axis which plays a key part in several cellular functions, including differentiation, viability and growth [2,11]. When the PI3K pathway is activated (e.g. by stress or growth factors), the consequent phosphorylation of Akt activates TSC2, a large protein that, together with TSC1
Can mTOR dysregulation link HIV-1 infection to CD4+-T-cell loss, impaired function of innate immunity, dementia, nephropathy and malignancies?

**mTOR and CD4+-T-cell loss**

mTOR activation seems to play a pathogenetic part in the dysregulated apoptosis of CD4+ T cells in HIV-1 patients through gp120-mediated activation of the CD4/CXCR4 (or CCR5)/mTOR/p53 axis [24]. In fact, mTOR can phosphorylate p53 on serine 15 involved in Env-induced syncytial apoptosis [25]. The complex of the Env glycoprotein expressed on the membrane of HIV-1-infected cells can also induce apoptosis of uninfected cells expressing CD4 and CXCR4 or CCR5 through interaction between Env-expressing infected cells and uninfected cells [24,25].

It has been proposed that autophagy also plays a pathogenetic part in the death of uninfected CD4+ T cells [26]. Because mTOR inhibits the cellular mechanism of autophagy, this makes the net contribution of mTOR to the mechanism of CD4+-T-cell depletion more complex to decipher. However, a study questioned the contribution of autophagy to CD4+-T-cell death [27] because the authors reported reduction of the autophagy proteins Beclin-1 and LC3-II following infection of human peripheral blood CD4+ T cells or U937 cells with HIV-1. Beclin-1 mRNA expression and autophagosomes were also reduced in HIV-1-infected cells [27,28]. The *in vivo* evidence indicating that the blockade of mTOR with rapamycin neither prevents CD4+-T-cell decline in HIV-1-infected SCID mice [13] nor fails to influence their numbers in HIV-infected individuals receiving liver transplantation [14] seems to suggest that promotion of autophagy via mTOR blockade does not influence HIV-1-induced CD4+-T-cell death *in vivo*.

**mTOR and impaired function of innate immunity during HIV infection**

Defective immunological function of cells of the macrophage lineage is known to contribute to the pathogenesis of HIV-1 infection. Thus, stopping phagocytosis of opportunistic pathogens such as *Mycobacterium avium* complex, *Pneumocystis carinii*, *Toxoplasma gondii* or *Candida albicans* by peripheral blood monocytes, tissue macrophages and monocyte-derived macrophages following *in vivo* and *in vitro* HIV-1 infection has been documented [29]. Late-stage development of opportunistic infections has therefore been attributed to defective monocyte/macrophage signalling and function in HIV-infected individuals [30].

mTOR activation inhibits the destruction of pathogenic microorganisms by macrophages by inhibiting autophagy; rapamycin restores this macrophage inhibition. Cells infected with HIV-1 blocked rapamycin-induced autophagy and CD40-induced autophagocytosis of *T. gondii* in bystander macrophage/monocytic cells. These studies show that a pathogenic microorganism can impair autophagy in non-infected cells by activating counter-regulatory pathways, and that activation of mTOR is involved in this process. In agreement with these findings it has also been demonstrated in dendritic cells (DCs) that rapamycin-mediated autophagy induction exerts a better anti-BCG vaccine response [31]. This raises the possibility of therapeutic manipulation of cell signalling to restore autophagy in HIV-1 infection [30].

A recent study also shows that HIV-1 can impair innate immune function by mTOR-mediated impaired autophagy of DCs [32]. The limited immune response seen in the early events of HIV-1 trans-
FIGURE 1
Overview of the PI3K/PTEN/mTOR pathway depicting the sites of action of various inhibitors that result in the regulation of protein translation. The PI3K/PTEN/Akt/mTOR pathway can affect protein translation by regulating the mTORC1 and mTORC2 complexes. Growth factor (GF) stimulation results in growth factor receptor (GFR) activation which can activate the Ras/PI3K/PTEN/Akt/mTOR pathway. Akt can phosphorylate and inhibit TSC2 and PRAS-40, resulting in mTORC1 activation. Rapamycin and rapalogs target mTORC1 and block its activity, also resulting in inhibition of downstream p70S6K. Also shown in this diagram are the sites of interaction with novel PI3K/mTOR dual inhibitors and mTOR inhibitors. These latter two classes of inhibitors directly inhibit the kinase activity of mTOR and/or PI3K, whereas rapamycin and rapalogs block mTOR activity. The mTOR pathway profoundly affects mRNA translation. Many mRNAs important in immunology, cancer and cell growth contain 5’ untranslated sequences that are difficult to translate and are referred to as ‘weak’ mRNAs. For efficient translation of these ‘weak’ mRNAs to occur, p70S6K and eIF4E must be active. Hence, rapamycin, rapalogs, PI3K/mTOR dual inhibitors and mTOR inhibitors target the translation of these crucial survival mRNAs.
mission could result from the shutdown of autophagy and immunoamphisomes in DCs that rely on mTOR-dependent pathways [32]. HIV-1-induced inhibition of autophagy in DCs increases cell-associated HIV-1 and transfer of HIV-1 infection to CD4+ T cells. HIV-1- and mTOR-mediated downregulation of autophagy in DCs impaired innate and adaptive immune responses. Immunoamphisomes in DCs engulf incoming opportunistic infections and amplify pathogen degradation as well as Toll-like receptor responses and antigen presentation [32]. The findings that HIV-1 downregulates autophagy and impedes immune functions of DCs in an mTOR-dependent manner could have therapeutic and prophylactic implications for the use of specific mTOR inhibitors.

**mTOR and HIV-associated neurocognitive disorders**

With the introduction of HAART, AIDS dementia complex – or HIV-associated dementia as it was termed later – largely disappeared from clinical practice. However, during the past few years long-term infected and treated patients, including those with well-controlled infection, started to complain about milder memory problems such as slowness and difficulties in concentration, planning and multitasking. Neuropsychological studies have confirmed that cognitive impairment occurs in 15–50% of patients, being one of the most feared complications of HIV-1-infection. Neurocognitive impairment can also affect adherence to treatment and, ultimately, result in increased morbidity [33,34].

HIV-related neuropathology consists of microglial infiltration throughout white and grey matter, reactive astrogliosis in the cortex and in the central grey structures, and loss of neurons in the hippocampus and in the basal ganglia [35]. HIV enters the CNS through infected macrophages and resides in the microglia. The number of infected glial cells in HIV patients is highly variable but generally too low to explain the underlying process of encephalitis [33,34]. Thus, it is believed that immunoinflammatory and/or degenerative responses of the microglial cells to infection rather than virus-mediated cell death can explain AIDS-related neuropathogenesis [33,34].

Alizarei and co-workers have recently studied the role of autophagy in microglia-induced neurotoxicity in primary rodent neurons, as well as in primate and humans models [36,37]. They found that products of microglia infected with simian immunodeficiency virus (SIV) inhibited neuronal autophagy, resulting in decreased neuronal survival. This was associated with a decrease in autophagy-inducing proteins, a decrease in neuronal autophagy vesicles and an increase in sequestosome-1/p62. Signs of autophagy dysregulation associated with dementia and encephalitis were also found in brains from HIV-infected individuals and SIV-infected monkeys. Assessment of multiple biochemical markers of autophagic activity confirmed the inhibition of autophagy in neurons.

The contribution of dysregulated mTOR activity to this process was confirmed by the induction of autophagy in neurons after rapamycin treatment, and such treatment conferred significant protection to neurons [36,37]. This complements a previous study by Narducci and co-workers that showed that upregulation of mTOR and PUMA p53 inducible BH3-only protein, also occurs in neurons of patients suffering from HIV-associated encephalitis [38]. Taken together, these results prove that defects in autophagy are involved in neurodegenerative processes that arise from glial, as opposed to neuronal, sources, and that maintenance of autophagy could have a role in neuroprotection during HIV infection. The decreased neuronal autophagy might sensitise cells to proapoptotic and other damaging mechanisms, leading to neuronal dysfunction and death. Hence, new therapeutic approaches aimed at boosting neuronal autophagy including mTOR blockade with rapamycin are conceivable to prevent the development of HIV-associated neurocognitive disorders and to treat those suffering from these complications.

**mTOR and HIV-associated nephropathy**

HIV-associated nephropathy (HIVAN) is an important clinical manifestation of HIV infection that develops in ~33% of HIV-1 cases [39]. Clinically evident chronic kidney disease and subclinical renal pathology can occur in patients with long-lasting HIV infection. The pathogenesis of HIVAN involves the direct infection of the renal epithelium following HIV infection [40].

The introduction of combination HAART has been followed by a decline in the incidence of HIVAN [41] and with delayed progression of end-stage renal disease.

The fact that mTOR could be involved in the pathogenesis of HIVAN is suggested by a recent study carried out in Tg26 transgenic mice that harbour the proviral transgene pNL4-3:d1443, which encodes all of the HIV-1 genes with the exception of GAG and POL and serves as a preclinical model of HIVAN [42]. These animals develop proteinuria at ~24 days of age and progress into nephritic syndrome and renal failure. Parietal and visceral epithelial cells had increased levels of mTOR phosphorylation when compared with the FVB/N control mouse [42], and treatment with rapamycin reduced the renal lesions, proteinuria and uremia. Rapamycin also decreased tubular dilation, probably caused by inhibition of protein synthesis and cell cycle progression in tubular cells [42]. These findings concur with a role for mTOR in the proliferative phenotype and the development of HIVAN, and provide in vivo proof-of-concept for the beneficial effects of mTOR inhibitors in this setting.

**mTOR and HIV-associated malignancies**

The occurrence of HIV-associated malignancies represents a major complication for immunocompromised HIV-infected individuals [43]. Indeed, it has been shown that there is an association between HIV and cancer, particularly anal cancer, Kaposi sarcoma (KS), non-Hodgkin’s lymphoma (NHL), primary effusion lymphoma (PEL), primary brain lymphoma and Burkitt’s lymphoma (BL) [44]. Several factors have been identified as culprits of malignancies in the HIV patients, including impaired immunocompetence, dysregulated immune responses, genomic instability, chronic B-cell stimulation and opportunistic infections with onco-genic viruses [45]. The severity of immune suppression is predictive of mortality from AIDS- and non-AIDS-associated malignancies and it has been proposed that HAART is effective in preventing their occurrence only in the cases where CD4+ cell counts are restored to at least 500 cells per mm3 [45]. Indeed, circulating CD4+ cell count is the most predictive factor for all malignancies apart from anal cancer [46], which is better predicted by the duration of immunodeficiency.

Because the first reports between the association of HIV infection and neoplasia were published, there has been a dramatic change in the incidence and epidemiology of AIDS-related malignancies. KS,
NHL and cervical cancer are classified by the Centers for Disease Control and Prevention as AIDS-defining malignancies. However, as the development and use of HAART continues, especially protease inhibitors, there has been a steady increase in non-AIDS-defining malignancies, such as Hodgkin’s lymphoma (HL), lung cancer, hepatocellular cancer and anal cancer – and a decline in AIDS-defining neoplasias. These emerging malignancies present a new challenge in the care of patients with HIV infection, and require optimal treatment protocols that take into consideration the interaction between HAART and systemic chemotherapy [47].

**Anal cancer**

The incidence of anal cancer is progressively increasing among HIV-infected patients undergoing HAART [48]. The treatment of anal cancer relies upon traditional chemo- and radio-therapeutic approaches, which are associated with high morbidity and limited effectiveness in patients with high-grade disease. The role of mTOR in this setting has recently been investigated by Stelzer and colleagues [49] in preclinical models of murine and human anal cancer. The first model was based on the use of the HPV16 transgenic mouse, in which expression of the E6 and E7 oncogenes in the epithelium of the anus, along with the topical application of the carcinogen DMBA, causes the formation of a progressive neoplastic disease that leads to anal carcinoma. The second model comprises HPV16-positive human anal cancer xenografts passed subcutaneously in immunodeficient SCID and nude mice. In both models, anal cancers were associated with activation of the mTOR pathway, and rapamycin significantly reduced their growth rates. In the transgenic mouse model the prophylactic administration of rapamycin also significantly reduced the incidence of the overall onset of tumours, which include benign and malignant lesions.

**Kaposi’s sarcoma**

In the HAART era, KS remains the second most frequent tumour in HIV-infected patients worldwide, and the most common cancer in Sub-Saharan Africa. Patients with KS in Sub-Saharan Africa have high tumour burden and rapid disease progression with a life expectancy <6 months. KS has a variable clinical course ranging from extremely indolent forms, requiring no or minimal therapy, to rapidly progressive disease. Several different therapeutic options are available but the optimal therapy is still unclear. HAART, including protease inhibitors, could represent the first treatment step for slowing progressive disease; chemotherapy plus HAART is indicated for visceral and/or rapidly progressive disease, whereas maintenance HAART after systemic chemotherapy could be an effective anti-KS measure. The angiogenic nature of KS makes it particularly suitable for therapies based on targeted agents such as metallocproteinase inhibitors, angiogenesis inhibitors and tyrosine kinase inhibitors [50].

The identification of the KS-associated herpes virus (KSHV or HHV8) as the viral etiologic agent of KS has prompted renewed interest in the molecular pathogenesis of this disease. Evidence now points to a single KSHV gene, vGPCR, as being essential for KS development, and recent work has identified the Akt/TSC/mTOR signalling cascade as a crucial pathway in vGPCR sarcomagenesis. Indeed, pharmacological inhibition of mTOR with rapamycin has shown promising results in preventing vGPCR-induced tumourigenesis in an animal model (see Ref. [51] for a review). These observations are further validated by coincidental reports demonstrating the efficacy of rapamycin as an immunosuppressive and antitumoural solution for post-transplant KS patients [52].

Although confirmation of these observations in controlled clinical trials is needed, these data seem to point to an important role for mTOR in the pathogenesis of KS and suggest that mTOR inhibitors could represent a novel therapeutic option for the treatment of this condition in HIV-infected individuals.

**Lymphomas**

Lymphomas comprise a large proportion of the malignancies that affect the HIV-infected population in developed countries. In particular, NHL represents the most common tumour in HIV-infected patients and represents an AIDS-defining illness since the establishment of HAART therapy [53].

The incidence of AIDS-related lymphoma (ARL) has been found to be 200-fold higher than the incidence of lymphoma in uninfected individuals. ARLs are often associated to herpes virus-γ, EBV or HHV-8 [43]. It is believed that B-cell proliferation stimulated by chronic antienaemia can result in polyclonal and eventually monoclonal lymphoproliferation [43]. Standard treatment for HIV lymphomas includes immune reconstitution using HAART, anti-CD20 monoclonal antibodies, radioimmunotherapy, stem cell transplantation, cytokine therapy and vaccination. Because the prognosis for HIV-associated NHL nears that of the general population, these approaches are of increasing importance. However, treatment of HIV-associated lymphomas could be hindered by the difficulty of delivering full-dose chemotherapy to patients with compromised bone marrow or those suffering from infectious diseases. This might explain the lower responses and survival rates in HIV-associated lymphomas as compared with those of HIV-seronegative patients [54]. Novel therapeutic options for HIV-associated lymphomas are therefore warranted.

El-Salem and colleagues [55] showed that the mTOR signalling pathway is activated in all patients with ARLs, regardless of their histological classification. In particular, they found that mTOR is activated in germinal centre cells and in the interfollicular areas, but not in the resting lymphocytes of the benign HIV-associated lymphadenopathy. Along with the reported occurrence of mTOR activation in non-HIV-associated lymphomas [56], these data suggest that the use of mTOR inhibitors could have potential therapeutic effects in HIV-associated and non-HIV-associated lymphomas. Although large studies to support the potential use of mTOR inhibitors against HIV-associated lymphomas are not available, it is interesting to observe that post-transplant lymphoproliferative disorder (PTLD)-type cell lines are highly sensitive to mTOR inhibition [57] and that substitution of immunosuppressive therapies based on inhibitors of calcineurin with mTOR-inhibitor-based therapies in organ-transplanted patients reduced the occurrence of PTLD lesions [58].

PEL constitutes a subset of NHL and PEL incidence is highly increased in HIV-infected patients. KS-associated herpes virus is the causative agent of PEL. PEL has a poor prognosis with reported median survival times of <6 months. PEL displays activated PI3K, Akt and mTOR. Although single modulation of the mTOR pathway inhibited PEL proliferation, it has been reported that dual inhibition of PI3K and mTOR was significantly more efficacious in culture and in a PEL xenograft tumour model [59]. These data
provide important preclinical proof-of-concept on the suitability of targeting the PI3K/mTOR axis with specific inhibitors for the treatment of PEL in HIV-infected patients [59].

Mantle-cell lymphoma (MCL), a well-defined subtype of B-cell non-Hodgkin's lymphoma (B-NHL), accounts for 6% of all lymphoid neoplasms, and has a median survival of 3–4 years. Recent studies show that most cases of MCL exhibit constitutive activation of PI3K/Akt/mTOR pathways, which promotes tumour proliferation and survival. In agreement with this, relapsed or refractory MCL patients in a Phase III clinical study have shown a better response to treatment with the specific mTOR inhibitor temsirolimus than the investigator's choice therapy [60]. These data warrant the use of mTOR inhibitors in HIV-associated MCL.

**Concluding remarks**

The data discussed indicate that hyperactivation of mTOR occurs in different pathologies associated with HIV infection such as nephropathy and AIDS-associated and non-AIDS-associated malignancies. Hyperactivation of mTOR could also be responsible for defective autophagy that can contribute to the pathogenesis of neurocognitive disorders during HIV infection (Fig. 2). Preclinical evidence indicates that selective inhibitors of mTOR such as rapamycin could represent a novel therapeutic approach for the treatment of these pathologies. HIV-infected patients could additionally benefit from the use of rapamycin because of its non-mTOR-mediated capacity to inhibit HIV replication [12]. Although, the use of an immunosuppressive drug such as rapamycin might raise the concern of increasing the immunodeficiency of HIV-infected patients, the fact that mTOR inhibitors have already proven effective in preventing some lymphoproliferative disorders in transplant patients, who are also immunocompromised [52,58], suggests that the direct effects of mTOR inhibitors could outweigh their immunosuppressive benefits in the clinical setting.

These data indicate that the potential use of mTOR inhibitors, either alone or in association with HAART, warrants that they are studied in Phase II proof-of-concept trials designed to determine whether the inhibition of mTOR ameliorates the natural course of the infection and reduces the risk of developing HIV-associated disorders.

**References**


