Potential use of rapamycin in HIV infection

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The strong need for the development of alternative anti-HIV agents is primarily due to the emergence of strain-resistant viruses, the need for sustained adherence to complex treatment regimens and the toxicity of currently used antiviral drugs. This review analyzes proof of concept studies indicating that the immunomodulatory drug rapamycin (RAPA) possesses anti-HIV properties both *in vitro* and *in vivo* that qualifies it as a potential new anti-HIV drug. It represents a literature review of published studies that evaluated the *in vitro* and *in vivo* activity of RAPA in HIV. RAPA represses HIV-1 replication *in vitro* through different mechanisms including, but not limited to, down regulation of CCR5. In addition RAPA synergistically enhances the anti-HIV activity of entry inhibitors such as vicriviroc, aplaviroc and enfuvirtide *in vitro*. RAPA also inhibits HIV-1 infection in human peripheral blood leucocytes-SCID reconstituted mice. In addition, a prospective nonrandomized trial of HIV patient series receiving RAPA monotherapy after liver transplantation indicated significantly better control of HIV and hepatitis C virus (HCV) replication among patients taking RAPA monotherapy. Taken together, the evidence presented in this review suggests that RAPA may be a useful drug that should be evaluated for the prevention and treatment of HIV-1 infection.

Introduction

Rapamycin, 'also' an immunosuppressant

Rapamycin (RAPA, Rapamune, sirolimus) is a natural product isolated from *Streptomyces hygroscopicus* [1]. It is a macrolide currently used to prevent transplant rejection and as an antitumour agent [2, 3]. RAPA is cytostatic to T cells as it disrupts molecular events resulting from signals initiated by interleukin (IL)-2 activation of the IL-2 receptor (IL-2R) [4]. Several studies have shown that the primary pharmacological mode of action of RAPA is to inhibit the mammalian target of rapamycin (mTOR) thus interfering with the phosphoinositide 3-kinase (PI3K)-Akt-mTOR axis that is a key to several cellular functions involving differentiation, viability and growth [5]. The pleiotropic effect of this pathway in cell biology and the key role played by RAPA in its modulation may explain its multiple pharmacological properties that vary from immunosuppression to immunostimulation, from anti-cancer and anti-viral effects to anti-ageing properties [6].

Molecular action

RAPA binds to its intracellular receptor FK-binding protein 12 (FKBP12), and the resulting complex (RAPA-FKBP12) binds to mTOR next to its kinase domain. This inhibits mTOR's ability to phosphorylate p70S6 kinase (p70S6K) [7].

mTOR forms two complexes, the mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 consists of mTOR, raptor, proline-rich protein 40 (PRAS40) and mLST8/Gβ2. This complex regulates cell growth by controlling the activity of p70S6K, 4E-binding protein1 (4E-BP1) and other proteins, and negatively regulates 'upstream' Akt [8]. mTORC2 is composed of LST8GβL, rapamycin-insensitive companion of mTOR (Rictor), mammalian stress-activated protein kinase interacting protein 1 (mSin1) and protein observed with Rictor (PROTOR)/proline-rich protein 5 (PPR5), and this complex phosphorylates Akt on S473 and, possibly, functions as a previously elusive protein known as PDK2 [9]. Additionally, mTORC2 is required for the development of prostate cancer in mice lacking phosphatase and tensin homolog (PTEN) [10].

Although RAPA directly inhibits mTORC1, prolonged treatment with this drug also results in mTORC2 down-regulation by unknown mechanisms [11–13]. This may occur by suppression of mTOR expression and the eventual depletion of mTOR in the mTORC2. Thus RAPA is a key inhibitor of both the IL2R signalling pathway and the PI3K/PTEN/Akt/mTOR pathway (Figure 1).

RAPA analogues (Rapalogs) such as temsirolimus, everolimus and ridaforolimus (AP-23573, Ariad-Merck) have been synthesized recently. These newer agents are

Figure 1

Interactions between the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/mTOR pathways result in the regulation of protein translation and sensitivity of HIV and HAART therapy to rapamycin treatment. The Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways can affect protein translation by complex interactions regulating the mTORC1 and mTORC2 complexes. GF stimulation results in GFR activation which can activate both the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways. Akt can phosphorylate and inhibit the effects of GSK-3 β , TSC2 and PRAS-40, which result in mTORC1 activation. ERK and PDK1 can phosphorylate p90^{Rsk1} which in turn can phosphorylate and inhibit TSC2. Rapamycin targets mTORC1 and inhibits its activity and also results in inhibition of downstream p70S6K. The effects of rapamycin are complex as long term administration of rapamycin may prevent mTOR from associating with mTORC2 and hence full activation of Akt is prevented. However, rapamycin treatment may result in activation of PI3K, by inhibiting the effects of p70S6K on IRS-1 phosphorylation which results in PI3K and Akt activation. Also rapamycin treatment may result in the activation of ERK in some cells, presumably by inhibition of the p70S6K mediated inhibition of IRS1. These later two effects of rapamycin could have positive effects on cell growth. Inhibition of PDK-1 activity can also result in activation of mTORC1, presumably by suppression of p70S6K and hence inhibition of IRS1 effects on PI3K activity. The PTEN, TSC1, TSC2 and LKB1 tumour suppressor genes all converge on the mTORC1 complex to regulate protein translation. Thus the Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR pathways can finely tune protein translation and cell growth by regulating mTORC1. Rapamycin can have diverse effects on these processes and affect the sensitivity to HIV and HAART therapy

derived from the structure of RAPA and have substitution of the C40 hydroxyl group of RAPA [7]. Although there are some pharmacokinetic differences, they appear to differ little from RAPA in terms of pharmacodynamic effects and overall tolerability [6]. Many of these compounds have progressed into clinical trials. Some are currently used to treat patients with renal cancer such as Torisel (temsirolimus, CCI-779 Wyeth-Pfizer) and Afinitor (everolimus, RAD001, Novartis) [14–18].

This review will evaluate the current literature suggesting that RAPA possesses both direct and indirect antiviral

effects that may provide ‘new’ therapeutic options for the treatment of HIV-1-infected patients.

Recent advances on HIV-entry inhibition

Despite significant advances in the treatment of HIV infection by classical antiretroviral therapies, there remains a strong need for the development of alternative antiviral agents.

The emergence of strain-resistant viruses, the need for sustained adherence to complex treatment regimens and the toxicity of currently used antivirals warrant discovery of alternative therapies [19].

Much attention has recently been focused on the development of anti-HIV compounds targeting other life-cycle steps of viruses, for example viral entry into cells [20]. Infection by HIV is a complex multi-stage process involving attachment to host cells, CD4 binding, co-receptor binding and membrane fusion [21]. Drugs that block HIV entry are collectively known as entry inhibitors even though they may possess other actions depending on the level of entry inhibition and other processes or function that are inhibited.

Two entry inhibitors, enfuvirtide [22] and maraviroc [23], along with the integrase inhibitor raltegravir [24, 25] are being used as an option in the treatment of HIV patients who have developed resistance or exhibit poor response to classical active anti-retroviral therapy (ART).

Although enfuvirtide optimizes the response to new combinations of HIV-1 drugs in multiresistant patients, its use is hindered by high cost, the inconvenient subcutaneous route of administration and local reactions reported in more than 80% of patients [26, 27]. On the other hand, the recently approved orally available CCR5 receptor antagonist maraviroc has shown promise in patients infected with multidrug resistant CCR5-tropic HIV-1 [28]. Other orally available CCR5-antagonists such as vicriviroc and INCB009471, have been tested in phase III and IIb clinical trials, respectively [29, 30]. The anti-CCR5 antibodies PRO 140 and HGS004 are in the early stages of clinical development [31, 32]. The non-immunosuppressive monoclonal antibody that binds CD4, ibalizumab (TMB-355, previously known as TNX-355), the tetravalent CD4-immunoglobulin fusion protein (PRO-542), have also been tested or are currently being evaluated in phase II trials [33, 34].

In vitro and *in vivo* studies have indicated that the efficacy of CCR-5 inhibitors to treat HIV infection is related to the density levels (receptors/cells) of CCR5 on both CD4+ T cells and macrophages. In healthy individuals CCR5 density on CD4+ T cells ranges between 2×10^3 and 10×10^3 receptors/cell [35, 36], and the influence on disease progression and response to therapy in HIV-1 infection varies within this range [37, 38]. Moreover, cells from patients receiving vicriviroc in phase III studies showed an inverse correlation between CCR5 density and vicriviroc activity [39]. Hence, drugs capable of reducing CCR5 expression on CD4+ T cells and macrophages may also have positive effects in patients infected with HIV-1.

Inhibitory effects of RAPA on HIV-1 replication *in vitro*

Effects of RAPA monotherapy

Since CCR5 expression on T cells is strictly dependent on the binding of IL-2 to its receptor, drugs capable of

interfering with IL-2 signalling, for example RAPA, might also down-regulate the cellular expression of CCR5 [4]. Accordingly, Heredia *et al.* found that RAPA reduced CCR5 surface expression on T cells and macrophages at concentrations of 1 nM and 0.01 nM [40]. In addition, RAPA suppressed replication of CCR5 tropic (R5) strains of HIV-1 in both peripheral blood mononuclear cells (PBMCs) and macrophages cultures. At concentrations between 10 and 100 nM RAPA inhibited HIV-1 ADA infection from 88 to 99%, and at concentrations as low as 0.01 nM it inhibited macrophage infection with the R5 viruses HIV-1 ADA and HIV-1 SF162 by 64 and 45%, respectively [39]. Using the same experimental conditions, RAPA concentrations of 10 and 100 nM could also inhibit the CXCR4-tropic (X4) strain of HIV-1 IIIb by 13.5 and 32%, respectively [39]. This slight, though significant inhibition, of X4 strains indicates the relevance of targeting CCR5-dependent pathways but also suggests that additional CCR5-independent mechanisms of anti-HIV infectivity are regulated by RAPA. For example, RAPA is known to repress selectively the translation of a subset of mRNA bearing a 5'-polypyrimidine motif. This includes, among others, mRNA encoding ribosomal proteins and elongation factors. The presence of a suboptimal 5'-polypirimidine tract that is required for viral tat mRNA production, led Roy *et al.* to study the ability of RAPA to modulate replication of HIV-1 [41]. They demonstrated that treatment of established leukaemic T cell lines and human primary PBMCs with RAPA resulted in a significant decrease in production of both R5 and X4 strains of HIV-1. That this phenomenon was not due to interference with early steps of HIV-1 biology was suggested by reporter-gene assays and Northern blot analyses indicating that the HIV-1 long terminal repeat-mediated transcriptional activity was targeted by RAPA. This suggests that RAPA acts as an inhibitor of HIV-1 replication in human T cells by interfering with virus-mediated transcriptional events [41]. Oswald-Richter *et al.* subsequently demonstrated that RAPA blocked HIV-1 infection of activated T cells at stages after the reverse transcription phase [42].

Collectively, these data indicate that RAPA possesses multiple anti-HIV-1 properties primarily but not exclusively related to its ability to reduce CCR5 density levels on T cells and macrophages.

Synergistic effects of RAPA with CCR5 inhibitors

As expected, drug interaction studies revealed a synergistic antiviral activity of a combination of RAPA and the CCR5 antagonist vicriviroc in both multicycle and single-cycle infection of lymphocytes [39]. When used alone, RAPA and vicriviroc inhibited HIV-1 replication with dose at 50% inhibition values of 0.25 and 2.78 nM, respectively. However, when combined at a RAPA : vicriviroc ratio of 3:10, the EC_{50} value for RAPA was reduced to 0.03 nM and that of vicriviroc to 0.10 nM. Similar results were observed using a RAPA : vicriviroc ratio of 3:100. These reductions in EC_{50} values for each drug suggest a synergistic interaction and

Table 1EC₅₀ of RAPA and entry inhibitors in R5 HIV-1 replication assay

Drug	EC ₅₀ (nM)	Fold increase*
RAPA	0.15–0.20 [39, 46]	NA
Vicriviroc	2.78 [39]	NA
Aplaviroc	3.92 [44]	NA
Enfuvirtide	10–12.93 [46]	NA
RAPA + vicriviroc (ratio 3:10)	0.03/0.10 [39]	8.33/27.8 [39]
RAPA + aplaviroc (ratio 8.6:10)	0.1/1.16 [44]	2/3.37 [44]
RAPA + enfuvirtide (ratio 0.5:10)	0.12/2.48 [46]	1.66/4.62 [46]

*indicates fold increase in anti-HIV activity *in vitro* as measured as EC₅₀ in wild type strains of HIV.

the corresponding combination index values for the combination ranged from 0.174 to 0.048. Because combination index values <1 indicate synergy [43], and the values are proportional to the amount of synergy, the combination index values obtained for the RAPA/vicriviroc combination represent a high degree of synergy between the two drugs. The synergy translated into dose reduction index values of an 8 to 41-fold reduction for RAPA and 19 to 658-fold reduction for vicriviroc [39] (see Table 1). These effects demonstrated an enhanced antiviral activity of vicriviroc against both B and non-B clade isolates and allowed vicriviroc to acquire full antiviral activity against clade G viruses that were poorly responsive to both vicriviroc and the approved CCR5 antagonist maraviroc. Finally, RAPA-induced reduction of CCR5 density in lymphocytes sensitized vicriviroc-resistant strains to the drug inhibiting virus production by approximately 90% [39].

Similar synergistic interactions of RAPA were observed *in vitro* with aplaviroc [44], a CCR5 antagonist active against maraviroc-resistant strains, but whose clinical development has been terminated because of hepatotoxicity [45].

It was shown that reduction of CCR5 receptors/cell by RAPA enhanced the antiviral activity of aplaviroc, allowing lower, non-toxic effective doses. In the presence of RAPA, the concentration of aplaviroc required for 90% inhibition of R5 HIV-1 in primary CD4+ T-cells was reduced by as much as 25-fold [44]. The synergistic *in vitro* effects of RAPA and aplaviroc are shown in the Table 1.

It is interesting that RAPA also increased the activity of enfuvirtide against R5 strains of viruses in a cell-cell fusion assay and by quantification of early products of viral reserve transcription. Median effect analysis of drug interaction between RAPA and enfuvirtide in an infectivity assay using PBMCs demonstrated that the RAPA-enfuvirtide combination was synergistic against R5 strains of HIV-1 and that this synergy translated into enfuvirtide dose reduction of up to 33-fold (see Table 1). However, RAPA did not potentiate the activity of enfuvirtide against X4 strains [46].

It is worth noting that potentiation of antiviral activity by RAPA may not apply only to entry inhibitors as the

RAPA/efavirenz combination, at a ratio of 3:10, revealed an additive interaction between the two drugs with combination index values ranging from 0.9 to 1.2 [46].

Table 1 shows a summary of EC₅₀ values for these compounds against R5 HIV-1 replication either used alone or in combination with RAPA.

Anti-HIV-1 effects of RAPA *in vivo*

The effects of RAPA on CCR5 expression in vivo

On the basis of these *in vitro* data, a proof-of-concept study performed by Gilliam *et al.* evaluated the effects of 12 weeks administration of RAPA oral solution at 0.5 mg kg⁻¹ day⁻¹ to three healthy female cynomolgus macaques (*Macaca fascicularis*) on CCR5 expression in PBMCs and obtained from vaginal biopsies [47].

The regimen was well tolerated by the macaques with no changes in body weight, total white blood cell count and lymphocyte subset counts noted during the study, reaching a RAPA blood concentration between 2.5 and 3.8 ng ml⁻¹. RAPA treatment reduced CCR5 RNA concentrations in blood PBMCs in all three animals (fold reductions of 3, 8 and 33-fold at week 12 compared with baseline), and in two animals that underwent evaluation of vaginal biopsies (concentrations were reduced 0.6 and 7-fold at week 12). These data confirm the ability of RAPA to reduce CCR5 co-receptor expression in PBMCs and vaginal tissue biopsies *in vivo* thereby suggesting useful therapeutic activity against HIV infection [47].

RAPA in the Severe Combined Immunodeficiency (SCID) mouse model of HIV

The *in vitro* observations on the anti-HIV-1 effects of RAPA prompted us to evaluate its effects in a murine preclinical model of HIV infection [48]. RAPA (0.6 or 6 mg kg⁻¹ body weight) or its vehicle were administered daily by oral gavage to SCID mice reconstituted with human peripheral blood leukocytes (hu-PBL) starting 2 days before the intraperitoneal challenge with the R5 tropic SF162 strain of HIV-1 (1000 TCID₅₀ ml⁻¹). Relative to hu-PBL-SCID mice that had not received the viral challenge, HIV-infected Hu-PBL-SCID mice treated with the vehicle control for 3 weeks exhibited a 90% depletion of CD4+ T-cells, an increase in CD8+ cells, and an inversion of the CD4+ : CD8+ cell ratio. In contrast, treatment of HIV-infected mice with RAPA prevented the decrease in CD4+ T-cells and the increase of CD8+ T-cells, thereby preserving the original CD4+ : CD8+ T-cell ratio [48].

Viral infection was also witnessed from detection of HIV-RNA within peritoneal cells, spleen-, and lymph node cells of the vehicle-treated mice within 3 weeks of challenge. In contrast, treatment with RAPA decreased cellular provirus integration and reduced HIV-RNA concentrations in blood cells. Furthermore, in co-cultivation assays, spleen

cells from RAPA-treated mice exhibited a dose-dependent reduced capacity for infecting allogeneic T-cells [48]. These data demonstrated that RAPA possessed powerful antiviral activity against R5 strains of HIV *in vivo*.

HIV patients undergoing RAPA monotherapy after liver transplantation

Preliminary results of a prospective nonrandomized trial of HIV-infected patients receiving RAPA monotherapy after liver transplantation (LT) were recently reported by Di Benedetto *et al.* [49]. The 14 patients had received cadaveric donor LT due to end-stage liver disease (ESLD) associated or not associated with hepatocellular carcinoma, according to the Italian National Protocol for HIV recipients. ESLD occurred due to hepatitis C virus (HCV)-related hepatopathy for nine patients, hepatitis B virus-related hepatopathy for one patient, and hepatitis B virus-HCV hepatopathy for four patients. All patients except one were administered ART before transplantation, and this was discontinued on the day of transplantation. The same ART regimen was subsequently reinstituted between the first and the second week post-transplantation. Primary immunosuppression was based on calcineurin inhibitors (CIs), whereas switch to RAPA monotherapy occurred in cases of renal dysfunction or Kaposi's sarcoma for 6 of the 14 patients. Mean overall post-LT follow-up was 14.8 months (range 0.5–52.6). Mean preswitch period from CIs to RAPA was 67 days (range 10–225 days). Mean post-switch follow-up was 11.9 months (range 2–31 months). Significantly better control of HIV and HCV replication was observed among patients undergoing RAPA monotherapy ($P = 0.00001$ and 0.03 , respectively), but this treatment was not effective in maintaining a higher CD4 cell count than CI treatment [49]. However, Moreno *et al.* reported an increase in CD4 cell count after conversion to RAPA monotherapy in one LT recipient receiving co-administration with raltegravir [50].

This is the first clinical report showing beneficial signs of long-term suppression of HIV-1 amongst HIV-positive patients who underwent LT, and the data accord with the *in vitro* and *in vivo* results suggesting the anti-HIV-1 activity of RAPA. There is important information that cannot be deduced from the paper by Di Benedetto *et al.* [50], for example the ART regimen administered to the patients, the eventual use of ritonavir as booster and the measurement of the plasma concentrations of RAPA. These shortcomings impede drawing firm conclusions as to the benefits of simultaneous use of RAPA with ART. Nonetheless, this prospective nonrandomized trial hints towards a possible antiviral use of RAPA as part of ART.

Another interesting observation arising from the paper of Di Benedetto and colleagues [49] is that switch to RAPA monotherapy seems to exert a beneficial effect also on HCV infection, as indicated by the significantly lower viral load observed in the patients receiving RAPA monotherapy as compared with those who received CIs ($P = 0.03$

Figure 2

HCV viral load in patient series from the study by Di Benedetto *et al.* [47], before and after switch to RAPA monotherapy or treated with calcineurin inhibitors (CI). Fourteen patients had received cadaveric donor liver transplantation due to end-stage liver disease. Primary immunosuppression was based on calcineurin inhibitors, whereas switch to RAPA monotherapy occurred in cases of renal dysfunction or Kaposi's sarcoma for 6 of the 14 patients. Mean pre-switch period from calcineurin inhibitors to RAPA was 67 days (range 10–225 days). Mean post-switch follow-up was 11.9 months (range 2–31 months). * $P = 0.003$ by Dunnett's test vs. CI group last check. Last check indicates the last determination of HCV viral load in patients at the end of the observational period

by Dunnett's test) and the fact that two of them had undetectable HCV in their blood (see Figure 2). HCV genotype was 3a in seven cases, 4c/4d in three cases, 1a in two cases and 1b in one case. None of the patients was taking antiviral treatment for HCV before LT [49]. The HCV infection stage of these patients was not reported. Subsequent to LT 5 of the 13 patients received antiviral therapy based on peg-IFN and ribavirin (6 months for genotype 2 and 3, 12 months for 1 and 4) that was followed by reduction of viral load. For the other patients peg-IFN and ribavirin were contraindicated due to severe compromised clinical conditions [49]. It is interesting to observe that the two patients who developed HCV RNA clearance were free from peg-IFN and ribavirin treatment [49]. However, the possible beneficial effect of RAPA in controlling HCV replication in HIV-HCV co-infected patients should be carefully evaluated in light of studies suggesting that activation of the N-Ras-PI3K-Akt-mTOR pathway by HCV may control cell survival and viral replication. Hence, mTOR activation may play a defensive role during HCV infection [51]. In fact, the anti-HCV effect of interferon (IFN)-alpha occurs through an activation of mTOR that is independent of the PI3K-Akt pathway [52]. Both IFN-alpha-induced tyrosine phosphorylation of STAT-1 and expression of PKR and p48 were diminished when a normal human hepatocyte-derived cell line was treated with RAPA before treatment with IFN-alpha. RAPA inhibited the IFN-alpha-inducible IFN-stimulated regulatory element luciferase activity in a dose-dependent manner. However, the PI3K pathway

inhibitors wortmannin and LY294002 did not influence IFN- α -inducible luciferase activity. When the effect of PI3K-Akt-mTOR on the anti-HCV action of IFN- α was studied with the full-length HCV replication system, OR6 cells, pretreatment with RAPA attenuated its anti-HCV replicatory effect in comparison with IFN- α alone, whereas pretreatment with PI3K pathway inhibitors did not influence IFN-induced anti-HCV replication [52]. These results indicate that IFN-induced mTOR activity, independent of PI3K and Akt, is the critical factor for its anti-HCV activity. Caution should, however, be exercised to extrapolate these findings to the situation in the clinical setting as this work has been carried out on luc-engineered cell lines and luc is not a normal component of human cells

Translational considerations

When and why to use RAPA during the course of HIV infection

Taken together, the above mentioned *in vitro* and *in vivo* studies and the preliminary results of a prospective non-randomized trial incorporating HIV-infected patients on RAPA monotherapy after liver transplantation indicate that RAPA possesses anti-HIV-1 properties that may qualify it as an additional drug in the armamentarium of antiretroviral compounds for treatment of HIV-1 infection. There are nonetheless important theoretical and pharmacokinetic issues to be evaluated when approaching the treatment of HIV patients with RAPA. Theoretically, it may not seem appropriate to use immunosuppressive drugs in HIV-1 infected individuals. However, RAPA exerted a potent antiviral activity *in vitro* at concentrations lower than those used to cause immunosuppression. Thus, renal transplant recipients receiving daily doses of 2 and 5 mg RAPA achieved trough concentrations of 4.4 nM and 18.9 nM, respectively [53]. When used alone at 1 nM, RAPA profoundly suppressed the replication of R5 strains of HIV-1 in PBMCs with mild antiproliferative effects on cells [40]. This indicates that RAPA doses lower than those used in transplantation may be effective in HIV-1 patients. In addition, RAPA-induced synergistic enhancement of vicriviroc, aplaviroc and enfuvirtide activity [39, 44, 46] may allow the use of lower and less toxic doses of these compounds. *In vitro* studies of the combined effect of RAPA and maraviroc are urgently needed to evaluate whether the synergistic effect observed with the other CCR5 inhibitors also applies to maraviroc. The additive effect of RAPA and efavirenz [46] also warrants studies aimed at evaluating a possible additive effect with other anti-HIV agents, including protease inhibitors (PIs), reverse transcriptase inhibitors (RTIs) and integrase inhibitors. In the study cited, RAPA reduced CCR5 density in lymphocytes sensitized by vicriviroc-resistant strains to vicriviroc. This should be clinically evaluated to determine if RAPA is effective (and safe) in patients with vicriviroc resistance. The demonstration that adding RAPA

to vicriviroc overcomes viral resistance to the latter [49] also warrants studies to evaluate the capacity of RAPA to revert viral resistance to other antiretrovirals.

The use of an immunosuppressant such as RAPA in HIV-1 infection is also supported by the clinical experience that hyperactivation of the immune system may favour HIV-1 infection and that HIV infection of human T cells is favoured by T cell activation [42]. The rapid shutdown of the early phases of primary HIV-1 infection achieved by combined therapy with ciclosporin A and ART may have long term beneficial effects on disease progression [54]. This has led to considering the use of immunosuppressive drugs as virostatics in the management of HIV infection [55].

The optimal timing of RAPA therapy needs to be determined during the course of HIV infection and whether it should be provided as monotherapy or in combination with other antiretroviral agents. The *in vitro* and *in vivo* studies mentioned above suggest that RAPA might be most effective in controlling the replication of R5 rather than X4 strains of viruses, suggesting a role for this drug as an early therapy for infections caused by the R5 strain of HIV-1. Hence, initial monotherapy with 'low' doses of RAPA in the early stages of infection might be particularly relevant as an alternative strategy to early initiation of ART. Indeed, starting ART therapy in any given patient requires careful evaluation of the potential risks and the potential benefits of such treatment, and optimal timing for ART initiation in asymptomatic adults with CD4 counts ranging between 350 and 500 cells μL^{-1} is still debated [56].

As suggested by Heredia *et al.* [40], the antiviral properties of RAPA could be especially important in geographical areas where subtype C HIV-1 is present as these viruses use CCR5 as a major co-receptor [57]. Subtype C HIV-1 infections have increased in prevalence over the last decade and they currently constitute the predominant subtype worldwide [58].

The antiviral properties of RAPA might also open novel therapeutic opportunities for suppression of allograft rejection in HIV-1-infected subjects undergoing solid organ transplantation. This is strengthened by conflicting results obtained with CIs such as ciclosporin A as regards drug toxicity and virus control [54, 59]. Thus, the antiviral properties of RAPA, coupled with its recently recognized antiangiogenic properties [60, 61], may offer a better choice in transplanted HIV patients, as recently shown by Di Benedetto *et al.* [49] and Moreno *et al.* [50].

Drug-drug interaction and pharmacokinetic considerations for the possible use of RAPA in combination with ART regimes

To avoid emergence of drug-resistant strains of HIV, the current treatment of the infection requires the adoption of therapeutic regimes consisting of multidrug combinations. It can therefore be expected that RAPA will also even-

tually be used in HIV infected patients together with other anti-HIV agents such as the ART drugs included in the current guidelines and/or entry inhibitors for those patients who require treatment with these drugs. This raises the issue of possible drug–drug interaction and pharmacokinetic considerations.

In fact, the potential combined use of RAPA within ART regimens including PI or ritonavir boosted PIs should be carefully evaluated for pharmacokinetic and drug–drug interaction effects as these therapies consist of a combination of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or PIs, the latter of which are substrates the cytochrome P450 3A (CYP3A) enzyme system also responsible for RAPA metabolism [62]. The same is true for new immunosuppressive regimens in HIV-infected organ transplant recipients. Frassetto *et al.* [63] evaluated the pharmacokinetics and dosing modifications in 35 patients (20 kidney, 13 liver, and two kidney-liver HIV-infected subjects with end-stage kidney or liver disease), on both immunosuppressants and NNRTIs, PIs, and combined NNRTIs + PIs in studies done at weeks 2–4 and/or 12 weeks after transplantation or after a change in immunosuppressants or ART ($n = 97$ studies). HIV-infected transplant recipients using PIs with immunosuppressants had marked increases in RAPA blood trough concentrations, and also of ciclosporin A and tacrolimus concentrations, compared with those on NNRTIs alone or to patients not on ART.

The elevated RAPA plasma concentrations could lead to side-effects such as mucositis, pneumonitis, altered thrombocyte function and hyperlipidaemia [64]. Micro-dose formulation of RAPA might overcome or reduce this problem. The feasibility of this dosing regime is supported from a retrospective study from Bickel *et al.* who showed that decreasing the dose of tacrolimus to 0.03–0.08 mg daily in HIV patients with concomitant boosted PIs therapy resulted in stable tacrolimus blood concentrations without alteration of PI drug concentrations [65].

Concluding remarks

Taken together, the *in vitro* and *in vivo* data discussed herein provide valuable proof of concepts that warrant clinical trials to evaluate the safety, efficacy and pharmacokinetic interactions of RAPA in HIV-infected patients. The experimental evidence demonstrating synergistic actions of RAPA with CCR5 inhibitors and enfuvirtide anticipate a role for this drug in the context of these therapeutic regimes. However, the observed additive effects of RAPA with a NNRTI such as efavirenz also warrant studies aimed at evaluating the possible efficacy of RAPA together with ART regimens in patients who develop resistance to the treatment. We are also presently preparing a proof of concept study in HIV naive patients with viral load $>50\,000$ copies ml^{-1} and CD4+T cells ranging between 350 and

500 ml^{-1} to evaluate the impact of a 30 day course of RAPA monotherapy. Although combined treatment of HIV with multiple drugs is envisaged to avoid emergence of resistant strains, it may be interesting to evaluate the impact of RAPA monotherapy in early HIV in naive individuals. Since RAPA seems to exert its antiviral effects at concentrations lower than those required to exert immunosuppressive effects in transplanted patients, it would be helpful to evaluate if RAPA monotherapy would be effective in reducing viral load and preserving CD4 T cell loss in this selected group of patients. This may represent an important novel therapeutic option that may delay the natural course of the disease before initiating ART.

Finally, the demonstration that systemic treatment with RAPA decreased CCR5 mRNA expression in cervicovaginal tissue of cynomolgus macaques [47] also opens the possibility of using this drug in the prevention of sexual transmission of HIV and eventually also as a topical formulation. Promising data showing a moderate protective effect against HIV sexual transmission have been reported from clinical trials on the use of topical microbicides [66, 67].

In conclusion, this review indicates that RAPA may be a useful therapeutic option in the treatment of HIV infection. Examples include:

- Combined with ART (RTIs and PIs) to enhance efficacy
- Combined with entry inhibitors to improve response and reduce toxicity
- Combined with selected ART in patients who may be difficult to treat, who are treatment naïve, who have progressed to AIDS or who have predominantly CCR5 HIV
- Used either alone or with other agents to prevent sexual transmission of HIV

Competing interests

There are no competing interests to declare.

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REFERENCES

- 1 Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* 1975; 28: 721–6.
- 2 Patel SJ, Elliott EN, Knight RJ, Gaber LW, Gaber AO. Considerations in sirolimus use in the early and late post-transplant periods. *Expert Opin Drug Saf* 2009; 8: 421–34.

- 3 Baldo P, Cecco S, Giacomini E, Lazzarini R, Ros B, Marastoni S. mTOR pathway and mTOR inhibitors as agents for cancer therapy. *Curr Cancer Drug Targets* 2008; 8: 647–65.
- 4 Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem* 1998; 3: 335–40.
- 5 Bai X, Jiang Y. Key factors in mTOR regulation. *Cell Mol Life Sci* 2010; 67: 239–53.
- 6 Hartford CM, Ratain MJ. Rapamycin: something old, something new, sometimes borrowed and now renewed. *Clin Pharmacol Ther* 2007; 82: 381–8.
- 7 Easton JB, Houghton PJ. mTOR and cancer therapy. *Oncogene* 2006; 25: 6436–46.
- 8 Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst D, Sabatini DM. Rictor a novel binding partner of mTOR, defines the rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol* 2004; 14: 1296–302.
- 9 Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM. Prolong rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 2006; 22: 159–68.
- 10 Guertin DA, Stevens DM, Saitoh M, Kinkel S, Crosby K, Sheen JH, Mulholland DJ, Magnusson MA, Wu H, Sabatini DM. mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. *Cancer Cell* 2009; 15: 148–59.
- 11 Phung TL, Ziv K, Dabydeen D, Eyiah-Mensah G, Riveros M, Perruzzi C, Sun J, Monahan-Earley RA, Shiojima I, Nagy JA. Pathological angiogenesis is induced by sustained Akt signaling and inhibited by rapamycin. *Cancer Cell* 2006; 10: 159–70.
- 12 Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; 307: 1098–101.
- 13 Zeng Z, Sarbassov DD, Samudio IJ, Yee KW, Munsell MF, Jackson CE, Giles FJ, Sabatini DM, Andreeff M, Konopleva M. Rapamycin derivatives reduce mTORC2 signaling and inhibit Akt activation in AML. *Blood* 2006; 109: 3509–12.
- 14 Saylor PJ, Michaelson MD. New treatments for renal cell carcinoma: targeted therapies. *J Natl Compr Canc Netw* 2009; 7: 645–56.
- 15 Fouladi M, Lunningham F, Wu J, O'Shaughnessy MO, Molina K, Broniscer A, Spunt SL, Luckett I, Stewart CF, Houghton PJ, Gilbertson RJ, Furman WL. Phase I study of everolimus in pediatric patients with refractory solid tumors. *J Clin Oncol* 2007; 25: 4806–12.
- 16 Ihle NT, Powis G. Take your PI3K: phosphatidylinositol 3-kinase inhibitors race through the clinic towards cancer therapy. *Mol Cancer Ther* 2009; 8: 1–9.
- 17 Brachmann S, Fritsch C, Maria SMM, Garcia-Echeverria C. PI3K and mTOR inhibitors – a new generation of targeted anticancer agents. *Curr Opin Cell Biol* 2009; 21: 194–8.
- 18 Marone R, Cmiljanovic V, Giese B, Wymann MP. Targeting phosphoinositide 3-kinase – moving towards therapy. *Biochim Biophys Acta* 2008; 1784: 159–85.
- 19 Taiwo B, Hicks C, Eron J. Unmet therapeutic needs in the new era of combination antiretroviral therapy for HIV-1. *J Antimicrob Chemother* 2010; 65: 1100–7.
- 20 McKinnell JA, Saag MS. Novel drug classes: entry inhibitors [enfuvirtide, chemokine (C-C motif) receptor 5 antagonists]. *Curr Opin HIV AIDS* 2009; 4: 513–7.
- 21 Copeland KF. Inhibition of HIV-1 entry into cells. *Recent Pat Antiinfect Drug Discov* 2006; 1: 107–12.
- 22 Makinson A, Reynes J. The fusion inhibitor enfuvirtide in recent antiretroviral strategies. *Curr Opin HIV AIDS* 2009; 4: 150–8.
- 23 Lieberman-Blum SS, Fung HB, Bandres JC. Maraviroc: a CCR5-receptor antagonist for the treatment of HIV-1 infection. *Clin Ther* 2008; 30: 1228–50.
- 24 Hicks C, Gulick RM. Raltegravir: the first HIV type 1 integrase inhibitor. *Clin Infect Dis* 2009; 48: 931–9.
- 25 Schafer JJ, Squires KE. Integrase inhibitors: a novel class of antiretroviral agents. *Ann Pharmacother* 2010; 44: 145–56.
- 26 Marr P, Walmsley S. Reassessment of enfuvirtide's role in the management of HIV-1 infection. *Expert Opin Pharmacother* 2008; 9: 2349–62.
- 27 Myers SA, Selim AA, McDaniel MA, Hall R, Zhang Y, Bartlett JA, True AL. A prospective clinical and pathological examination of injection site reactions with the HIV-1 fusion inhibitor enfuvirtide. *Antivir Ther* 2006; 11: 935–9.
- 28 Latinovic O, Kuruppu J, Davis C, Le N, Heredia A. Pharmacotherapy of HIV-1 infection: focus on the CCR5 antagonist maraviroc. *Clin Med Ther* 2009; 1: 1497–510.
- 29 Kümmerle T, Lehmann C, Hartmann P, Wyen C, Fätkenheuer G. Vicriviroc: a CCR5 antagonist for treatment-experienced patients with HIV-1 infection. *Expert Opin Investig Drugs* 2009; 18: 1773–85.
- 30 Cohen C, DeJesus E, Mills A, Pierone G Jr, Kumar P, Ruane P, Elion R, Fusco G, Levy R, Solomon K, Erickson-Viitanen S. Potent antiretroviral activity of the once-daily CCR5 antagonist INCB009471 over 14 days monotherapy. 4th IAS Conference on AIDS Pathogenesis, Treatment and Prevention, 22–25 July 2007, Sydney, Australia (Abstract TUAB106).
- 31 PRO 140 by IV administration in adults with HIV-1 infection. Sponsor: Progenics Pharmaceuticals, Inc. ClinicalTrials.gov Identifier: NCT00613379. Available at <http://www.clinicaltrials.gov/ct2/show/NCT00613379?term=pro-140&rank=1> (last accessed 29 March 2010).
- 32 Lalezari J, Yadavalli GK, Para M, Richmond G, DeJesus E, Brown SJ, Cai W, Chen C, Zhong J, Novello LA, Lederman MM, Subramanian GM. Safety, pharmacokinetics, and antiviral activity of HGS004, a novel fully human IgG4 monoclonal antibody against CCR5, in HIV-1-infected patients. *J Infect Dis* 2008; 197: 721–7.
- 33 Dose-Response Study of Ibalizumab (Monoclonal Antibody) Plus Optimized Background Regimen in Patients With HIV-1

- (TMB-202). Sponsor: TaiMed Biologics Inc. ClinicalTrials.gov Identifier: NCT00784147. Available at <http://clinicaltrials.gov/ct2/show/NCT00784147?term=HIV+Houston&recr=Open&rank=6> (last accessed on 16 July 2010).
- 34 Jacobson JM, Israel RJ, Lowy I, Ostrow NA, Vassilatos LS, Barish M, Tran DN, Sullivan BM, Ketas TJ, O'Neill TJ, Nagashima KA, Huang W, Petropoulos CJ, Moore JP, Maddon PJ, Olson WC. Treatment of advanced human immunodeficiency virus type 1 disease with the viral entry inhibitor PRO 542. *Antimicrob Agents Chemother* 2004; 48: 423–9.
 - 35 Hladik F, Lentz G, Delpit E, McElroy A, McElrath MJ. Coexpression of CCR5 and IL-2 in human genital but not blood T cells: implications for the ontogeny of the CCR5+ Th1 phenotype. *J Immunol* 1999; 163: 2306–13.
 - 36 Lee B, Sharron M, Montaner LJ, Weissman D, Doms RW. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. *Proc Natl Acad Sci USA* 1999; 96: 5215–20.
 - 37 Reynes J, Portales P, Segondy M, Baillat V, André P, Réant B, Avinens O, Couderc G, Benkirane M, Clot J, Eliaou JF, Corbeau P. CD4+ T cell surface CCR5 density as a determining factor of virus load in persons infected with human immunodeficiency virus type 1. *J Infect Dis* 2000; 181: 927–32.
 - 38 Reynes J, Baillat V, Portales P, Clot J, Corbeau P. Relationship between CCR5 density and viral load after discontinuation of antiretroviral therapy. *JAMA* 2004; 291: 46.
 - 39 Heredia A, Latinovic O, Gallo RC, Melikyan G, Reitz M, Le N, Redfield RR. Reduction of CCR5 with low-dose rapamycin enhances the antiviral activity of vicriviroc against both sensitive and drug-resistant HIV-1. *Proc Natl Acad Sci USA* 2008; 105: 20476–81.
 - 40 Heredia A, Amoroso A, Davis C, Le N, Reardon E, Dominique JK, Klingebiel E, Gallo RC, Redfield RR. Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV beta-chemokines: an approach to suppress R5 strains of HIV-1. *Proc Natl Acad Sci USA* 2003; 100: 10411–6.
 - 41 Roy J, Paquette JS, Fortin JF, Tremblay MJ. The immunosuppressant rapamycin represses human immunodeficiency virus type 1 replication. *Antimicrob Agents Chemother* 2002; 46: 3447–55.
 - 42 Oswald-Richter K, Grill SM, Leelawong M, Unutmaz D. HIV infection of primary human T cells is determined by tunable thresholds of T cell activation. *Eur J Immunol* 2004; 34: 1705–14.
 - 43 Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 1984; 22: 27–55.
 - 44 Latinovic O, Heredia A, Gallo RC, Reitz M, Le N, Redfield RR. Rapamycin enhances aplaviroc anti-HIV activity: implications for the clinical development of novel CCR5 antagonists. *Antiviral Res* 2009; 83: 86–9.
 - 45 Nichols WG, Steel HM, Bonny T, Adkison K, Curtis L, Millard J, Kabeya K, Clumeck N. Hepatotoxicity observed in clinical trials of aplaviroc (GW873140). *Antimicrob Agents Chemother* 2008; 52: 858–65.
 - 46 Heredia A, Gilliam B, Latinovic O, Le N, Bamba D, Devico A, Melikyan GB, Gallo RC, Redfield RR. Rapamycin reduces CCR5 density levels on CD4 T cells, and this effect results in potentiation of enfuvirtide (T-20) against R5 strains of human immunodeficiency virus type 1 in vitro. *Antimicrob Agents Chemother* 2007; 51: 2489–96.
 - 47 Gilliam BL, Heredia A, Devico A, Le N, Bamba D, Bryant JL, Pauza CD, Redfield RR. Rapamycin reduces CCR5 mRNA levels in macaques: potential applications in HIV-1 prevention and treatment. *AIDS* 2007; 21: 2108–10.
 - 48 Nicoletti F, Lapenta C, Donati S, Spada M, Ranazzi A, Cacopardo B, Mangano K, Belardelli F, Perno C, Aquaro S. Inhibition of human immunodeficiency virus (HIV-1) infection in human peripheral blood leucocytes-SCID reconstituted mice by rapamycin. *Clin Exp Immunol* 2009; 155: 28–34.
 - 49 Di Benedetto F, Di Sandro S, De Ruvo N, Montalti R, Ballarin R, Guerrini GP, Spaggiari M, Guaraldi G, Gerunda G. First report on a series of HIV patients undergoing rapamycin monotherapy after liver transplantation. *Transplantation* 2010; 89: 733–8.
 - 50 Moreno A, Bárcena R, Quereda C, Casado JL, Pérez-Elías MJ, Fortún J, Nuño J, Arranz I, Moreno S. Safe use of raltegravir and sirolimus in an HIV-infected patient with renal impairment after orthotopic liver transplantation. *AIDS* 2008; 22: 547–8.
 - 51 Mannová P, Beretta L. Activation of the N-Ras-PI3K-Akt-mTOR pathway by hepatitis C virus: control of cell survival and viral replication. *J Virol* 2005; 79: 8742–9.
 - 52 Matsumoto A, Ichikawa T, Nakao K, Miyaaki H, Hirano K, Fujimoto M, Akiyama M, Miuma S, Ozawa E, Shibata H, Takeshita S, Yamasaki H, Ikeda M, Kato N, Eguchi K. Interferon-alpha-induced mTOR activation is an anti-hepatitis C virus signal via the phosphatidylinositol 3-kinase-Akt-independent pathway. *J Gastroenterol* 2009; 44: 856–63.
 - 53 Rapamune package insert 2002, Wyeth.
 - 54 Rizzardì GP, Harari A, Capiluppi B, Tambussi G, Ellefsen K, Ciuffreda D, Champagne P, Bart PA, Chave JP, Lazzarin A, Pantaleo G. Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. *J Clin Invest* 2002; 109: 681–8.
 - 55 Romanelli F, Hoven AD. Use of virostatics as a means of targeting human immunodeficiency virus infection. *Curr Pharm Des* 2006; 12: 1121–7.
 - 56 Zolopa AR. The evolution of HIV treatment guidelines: current state-of-the-art of ART. *Antiviral Res* 2010; 85: 241–4.
 - 57 Björndal A, Sönnernborg A, Tscherning C, Albert J, Fenyo EM. Phenotypic characteristics of human immunodeficiency virus type 1 subtype C isolates of Ethiopian AIDS patients. *AIDS Res Hum Retroviruses* 1999; 15: 647–53.
 - 58 Buonaguro L, Tornesello ML, Buonaguro FM. Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. *J Virol* 2007; 81: 10209–19.

- 59** Calabrese LH, Lederman MM, Spritzler J, Coombs RW, Fox L, Schock B, Yen-Lieberman B, Johnson R, Mildvan D, Parekh N; AIDS Clinical Trials Group 334 Investigators. Placebo-controlled trial of cyclosporin-A in HIV-1 disease: implications for solid organ transplantation. *J Acquir Immune Defic Syndr* 2002; 29: 356–62.
- 60** Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 2002; 8: 128–35.
- 61** Xue Q, Nagy JA, Manseau EJ, Phung TL, Dvorak HF, Benjamin LE. Rapamycin inhibition of the Akt/mTOR pathway blocks select stages of VEGF-A164-driven angiogenesis, in part by blocking S6Kinase. *Arterioscler Thromb Vasc Biol* 2009; 29: 1172–8.
- 62** Marfo K, Greenstein S. Antiretroviral and immunosuppressive drug-drug interactions in human immunodeficiency virus-infected liver and kidney transplant recipients. *Transplant Proc* 2009; 41: 3796–9.
- 63** Frassetto LA, Browne M, Cheng A, Wolfe AR, Roland ME, Stock PG, Carlson L, Benet LZ. Immunosuppressant pharmacokinetics and dosing modifications in HIV-1 infected liver and kidney transplant recipients. *Am J Transplant* 2007; 7: 2816–20.
- 64** Meier-Kriesche HU, Kaplan B. Toxicity and efficacy of sirolimus: relationship to whole-blood concentrations. *Clin Ther* 2000; 22 (Suppl. B): B93–100.
- 65** Bickel M, Anadol E, Vogel M, Hofmann WP, von Hentig N, Kuetscher J, Kurowski M, Moench C, Lennemann T, Lutz T, Bechstein WO, Brodt HR, Rockstroh J. Daily dosing of tacrolimus in patients treated with HIV-1 therapy containing a ritonavir-boosted protease inhibitor or raltegravir. *J Antimicrob Chemother* 2010; 65: 999–1004.
- 66** Minces LR, McGowan I. Advances in the development of microbicides for the prevention of HIV infection. *Curr Infect Dis Rep* 2010; 12: 56–62.
- 67** Abdool Karim SS. Results of effectiveness trials of PRO 2000 gel: lessons for future microbicide trials. *Future Microbiol* 2010; 5: 527–9.