Phase II study of the antiretroviral activity and safety of the glucocorticoid receptor antagonist mifepristone in HIV-1-infected patients

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Abstract. Preclinical studies have shown that the anti-glucocorticoid drug mifepristone effectively inhibits HIV replication both *in vitro* and *in vivo*. However, the drug did not demonstrate anti-HIV activity in a previous phase I/II study when administered at the daily dose of 75-225 mg. The aim of this study was to assess whether mifepristone may exert antiretroviral activity or influence immunological parameters when administered orally at daily doses of 150 or 300 mg in highly active antiretroviral therapy (HAART)-naïve HIV-infected patients. We performed an open label non-randomized phase II study that included 26 patients who underwent 28 days of once daily oral administration of 150 (12 subjects) or 300 mg (14 subjects) of mifepristone. A total of 3 patients dropped out of the study, respectively 1 in the 150 mg dose group and 2 in the 300 mg dose group. The main hemato-chemical alterations reported were hypokalemia and increase in the blood levels of cortisol, especially in those patients that received mifepristone at the dose of 300 mg/day. Although we observed a trend of reduced viral load along the study in both groups, statistical significance was not achieved for either the primary nor the secondary endpoints. In summary, mifepristone treatment was well-tolerated but it failed to significantly influence viro-immunological parameters in HAART-naïve HIV-infected patients.

Introduction

One of the HIV-1 accessory proteins, the 96-aa virion associated accessory protein, viral protein r (Vpr), has been recently implicated in a variety of functions. It has been demonstrated that Vpr is a vital constituent of the pre-integration complex (PIC), controls cellular functions, re-activates latent infection, and is involved in the infection of non-dividing target cells (1-5) (Muthumani K, *et al*, 12th CROI, 158, 2005).

Vpr enhances viral replication in monocyte- and lymphocyte-derived cell lines (6-8) and functions as a transcriptional activator of several viral promoters, including the HIV-1 long terminal repeat (LTR) promoter (9-12). Vpr is delivered to the infected cells with the viral particle at an early phase of HIV-1 infection, affecting infected lymphocytes, monocytes/macrophages, and dendritic cells (13). Thus, Vpr plays an important role in regulating the nuclear translocation of the HIV-1 pre-integration complex, and it possibly helps HIV-1 to efficiently also infect nondividing cells, such as macrophages and resting lymphocytes (14-17). Moreover, it was recently shown that Vpr contributes to the innate and cellular immunity deficits of HIV-1-positive individuals and AIDS patients (17), since the polypeptide is also expressed and secreted by infected cells of the host after successful integration of the HIV-1 provirus into the host genome (18).

It is thought that most Vpr functions are due to the interaction with the cellular glucocorticoid receptor (GR) (4) Vpr enhances GR2 activity functioning as a potent coactivator of the GR, through a classic LXXLL coactivator signature motif (10,17).

It was previously reported that the GR2 antagonist, mifepristone (or RU486), that is currently used as an abortive drug, may inhibit Vpr-induced transactivation of the HIV-1 LTR in a dose-dependent manner (4). Infectivity assays using X4 and R5 viruses demonstrated the dose-dependent antiviral effects of mifepristone regimen. Mifepristone abolished (>95%) viral replication in primary human peripheral blood mononuclear cells (PBMC)s at a concentration of 10 μM, whereas a 60-70% inhibitory effect was observed at a concentration of 1 μM (4). The effects of mifepristone were also examined in latently infected cells that could be activated with extracellular Vpr protein and the results indicated a specific inhibition of virus reactivation in the presence of this antagonist (4). Furthermore, in a simian immunodeficiency virus (SIV)-infected

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macaque model mifepristone injected intravenously clearly exhibited an antiviral activity (19).

Mifepristone is an orally available small molecule drug. Pre-existing toxicity data in animals have shown mifepristone to be safe at very high doses (5 mg/kg for 6 months in rats and macaques). In addition, chronic administration of up to 200 mg/day mifepristone for the experimental treatment of a variety of malignant and non-malignant conditions has been well tolerated in non-HIV-infected subjects for up to 1 year (20-24).

A previous phase I/II trial performed to assess the safety and anti-HIV activity of mifepristone concluded that a 75-225 mg mifepristone daily dose over a 28-day period was safe and well-tolerated, but failed to significantly influence plasma HIV-1 RNA levels and to alter $CD4^+$ lymphocyte count from baseline levels (25).

To rule out the possibility that the lack of effect was due to the selection of an inappropriate dose regimen, we administered mifepristone to HIV patients at the two different doses of 150 and 300 mg day for 4 consecutive weeks. Our data show that similarly to the previous trial, this different dosing of mifepristone did not exhibit antiretroviral activity (decrease in plasma HIV-primary endpoint) and did not influence $CD4^+$ and $CD8^+$ T-cell counts (secondary endpoint) in HIV patients.

Materials and methods

Patients. To assess the antiretroviral efficacy of mifepristone in HIV-infected patients we performed an open label study including 26 patients that underwent 28 days of once daily oral administration of 150 (12 subjects) or 300 mg (14 subjects) of mifepristone.

Protocol inclusion criteria were: age ≥18 years; HIV-1 infection documented by ELISA test and confirmed by Western blot analysis at any time prior to study entry (HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA or a second antibody test by a method other than ELISA was acceptable as an alternative confirmatory test); $CD4^+$ cell count ≥200/mm^3 obtained within 90 days prior to study entry; plasma HIV-1 RNA of ≥2000 copies/ml as measured by any standard assay and performed within 90 days prior to study entry; stable hepatic (AST, ALT and alkaline phosphatase ≤2x ULN), renal (creatinine ≤2x ULN), and hematological (hemoglobin ≥10.0 g/dl) indices obtained within 30 days prior to study entry; negative spot urine pregnancy test result (girls who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months); use of two reliable methods of contraception simultaneously while on study drug and for 30 days after stopping the medication; and a Karnofsky performance score ≥80 within 30 days prior to study entry. Furthermore, any precautionary concomitant medications were to be on stable doses for >8 weeks prior to study entry with no plans to change medications or doses for the entire duration of the study.

Main exclusion criteria were the following: receipt of antiretroviral treatment within the 4 weeks prior to study entry or intent to initiate antiretroviral therapy within 60 days after entry; chronic adrenal failure; history of active hepatitis B or C; porphyrias; known moderate to severe cirrhosis; hemorrhagic disorders; concurrent anticoagulant therapy; any prior pituitary tumor, surgery, radiation treatment or pituitary failure; diabetes requiring treatment with oral hypoglycemics or insulin therapy; pregnancy within 90 days prior to study entry; breastfeeding and dysfunctional uterine bleeding within the 12 months prior to study entry. Subjects who were using other investigational agents, vaccines, chemotherapies, some antiarrhythmics, anticonvulsants, hormonal agents (systemic corticosteroids, estrogens and progesterones) and/or cytochrome P450 3A4 (CYP3A4) inhibitors or inducers were also excluded.

Trial design. Patients were screened -90 to -14 days prior to receiving drug and monitored on a weekly basis for safety and antiviral effects. The study was conducted at the Infectious Disease Unit, Cannizzaro Hospital, Catania, Italy and was approved by the local ethics committee. Patients were divided into two treatment doses groups: the 150 mg mifepristone once daily (75 mg, 2 tablets) and the 300 mg once daily (75 mg, 4 tablets). Mifepristone was supplied from Viral Genomix Pharmaceuticals, Inc. (Philadelphia, PA, USA). Patients were visited at the clinic at weekly intervals (Days 0, 7, 14, 21, 28 and, then Day 56) for evaluation of safety and to obtain a peripheral blood sample to assess viral loads and $CD4^+/CD8^+$ T-cell counts. A general clinical visit and blood sampling for determination of routine hematochemical analyses were also performed. The latter included routine hematology, glucose, creatinine, total proteins, bilirubin, Na^+, K^+, transaminases, LDH, amylases and cortisol determination. Tablet counts were performed at all study visits after the study medication was dispensed until subjects either completed or discontinued study treatment.

The following conditions/events were considered to result in treatment discontinuation: grade ≥3 drug-related toxicity, development of a malignancy requiring systemic chemotherapy, pregnancy, requirement for prohibited concomitant medications, failure by the subject to attend two consecutive clinic visits, ≥3 missed doses of study medication and clinical reasons believed to be life threatening by the physician, even if not addressed in the toxicity management of the protocol.

Statistical analysis. The primary endpoint of the study was the evaluation of the antiretroviral activity of mifepristone, defined by the change in HIV-1 plasma viral load between baseline and Days 7, 14, 21, 28 and 56. The secondary endpoints were the effects of mifepristone on the immunological parameters measured as changes in the absolute numbers and percentages of $CD4^+$ and $CD8^+$ T lymphocytes relative to baseline levels. Data are expressed as mean ± SD. Statistical analysis was carried out by one-way ANOVA with Bonferroni's adjustement. P-values <0.05 were considered statistically significant.

Results

After the enrollment phase, the resulting 26 patients were randomly divided into 2 treatment arms (12 in the 150 mg dose group and 14 in the 300 mg dose one). A total of 23 patients completed the study follow-up. The main clinical and laboratory characteristics of these patients are summarized in Table I.

In the 150 mg dose group, there was only one drop-out patient who discontinued treatment on Day 7 because of elevated

Table I. Characteristics of patients and summary of results.

	150 mg	300 mg	All arms combined
N, Baseline (Day 28)	12 (11)	14 (12)	26 (23)
Male N, Baseline (Day 28)	10 (9)	11 (10)	21 (19)
Age, median (range), years	38±10 (27-65)	39±10 (23-65)	38±10 (23-65)
Viral load (copies/ml)			
Baseline	21964±19432	24383±20237	23173±19400
Day 28	17715±10803	21037±13085	19448±11897
Follow-up (Day 56)	38297±69411	17604±13356	26472±46210
Day 28 vs. baseline (p)	n.s.	n.s.	n.s.
$CD4^+$ (cells/mmc)			
Baseline	627±343	607±450	616±394
Day 28	581±304	535±315	558±303
Follow-up (Day 56)	568±250	478±304	517±280
Day 28 vs. baseline (p)	n.s.	n.s.	n.s.
$CD8^+$ (cells/mmc)			
Baseline	1304±505	1180±610	1239±553
Day 28	1063±484	984±430	1024±449
Follow-up (Day 56)	1077±484	883±511	966±537
Day 28 vs. baseline (p)	n.s.	n.s.	n.s.
Cortisol (m.U.I./ml)			
Baseline	465±92	380±132	404±126
Day 28	697±182	977±265	830±262
Follow-up (Day 56)	321±96	355±155	341±131
Day 28 vs. baseline (p)	0.02	<0.001	<0.001
Potassium (mEq/l)			
Baseline	4.3±0.4	4.0±0.24	4.1±0.3
Day 28	4.0±0.3	3.7±0.34	3.9±0.4
Follow-up (Day 56)	4.3±0.5	4.0±0.34	4.2±0.4
Day 28 vs. baseline (p)	n.s.	0.02	n.s.

n.s., not significant.

levels of transaminases that were not related to the treatment as they were present at baseline.

In the 300 mg dose group, two out of 14 patients dropped out of the study on Days 7 and 9 because of severe hypokalemia and gastrointestinal discomfort and due to the occurrence of multi-metameric herpes zoster, respectively. An additional patient was put in temporary suspension on Day 21 because of gastrointestinal discomfort. He commenced treatment the day after.

In spite of this overall good clinical tolerability of the drug, hematochemical abnormalities were observed throughout the study period and were primarily related to the development of hypokalemia that occurred during at least one visit in 1 of 12 patients (8.3%) in the 150 mg dose group and in 7 of 14 patients (50%) in the 300 mg dose group.

A substantial and significant increase in the blood levels of cortisol was observed in 5 of 12 (41.7%) patients of the 150 arm group. Its maximal mean value was 1.6-fold that of baseline and it declined to normal values in all the patients on Day 56 (Fig. 1, Table I).

In the group of patients receiving 300 mg/day mifepristone, increased blood levels of cortisol were found in 100% of the patients. Its maximal mean value was 2.7-fold that of baseline, it peaked on Day 21 and it appeared to decline to normal values in most (10/14) patients upon drug interruption at follow-up, Day 56 (Fig. 1, Table I).

Furthermore, relative to baseline values, there were also other significant abnormalities in the hematochemical analyses in the 300 mg dose group (hyperglycemia -14.3%, increase in AST/ALT blood levels, 42.3%) observed throughout the study period and most of them reverted to normal values at the follow-up visit 4 weeks after drug interruption.

With regard to virological and immunological data, there were no significant differences throughout the study period

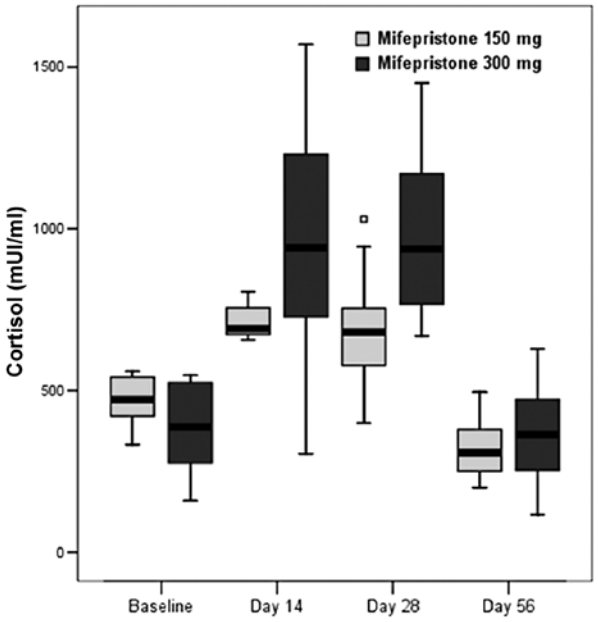

Figure 1. The effects of mifepristone treatment on blood cortisol levels in HIV-infected patients. HAART-naïve HIV-infected patients were screened -90 to -14 days prior to mifepristone administration and treated with once daily oral administration of 150 mg (12 subjects) or 300 mg (14 subjects) of mifepristone, starting on Day 0 for 28 consecutive days. A significant increase in the blood levels of cortisol was observed in 5 (41.7%) patients treated with 150 mg/day mifepristone and in all the patients treated with 300 mg/day. Cortisol blood levels declined to baseline values by Day 56.

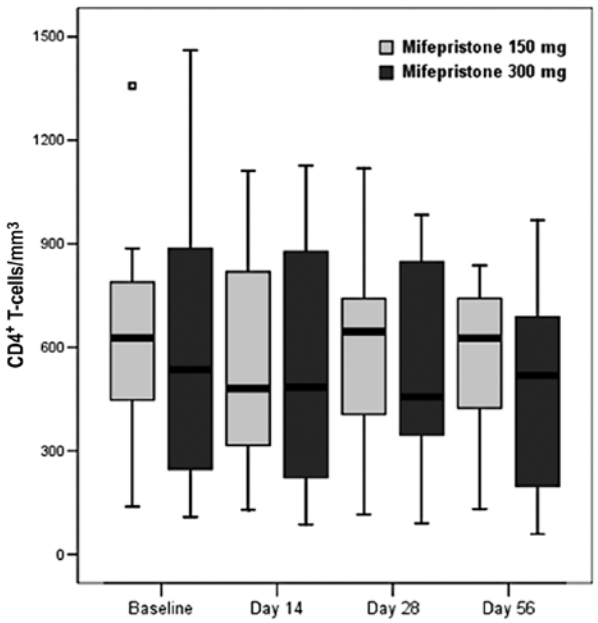

Figure 2. The effects of mifepristone treatment on $CD4^+$ T cell absolute number in HIV-infected patients. HAART-naïve HIV-infected patients were screened -90 to -14 days prior to mifepristone administration and treated with once daily oral administration of 150 mg (12 subjects) or 300 mg (14 subjects) of mifepristone, starting on Day 0 for 28 consecutive days. Treatment with both doses failed to influence blood $CD4^+$ T cell counts at all time points examined.

relative to baseline values in patients that received the 150 mg once daily dose of mifepristone, though there was an apparent trend for an ~20% reduction of the viral load on Day 28 along

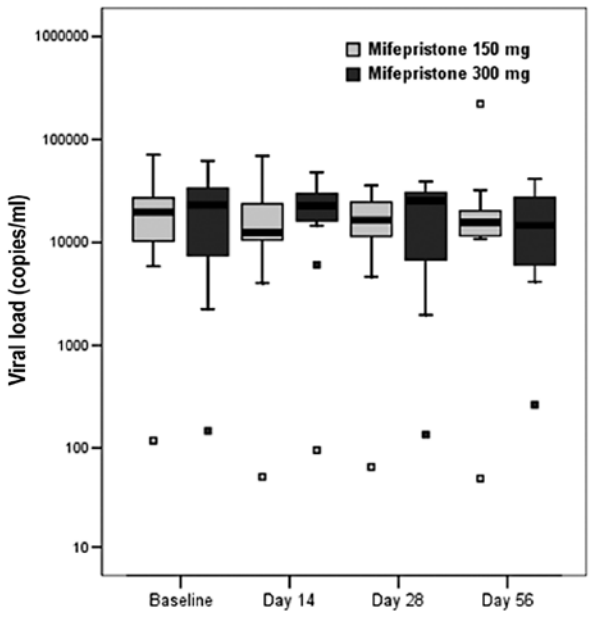

Figure 3. The effects of mifepristone treatment on HIV viral load. HAART-naïve HIV-infected patients were screened -90 to -14 days prior to mifepristone administration and treated with once daily oral administration of 150 mg (12 subjects) or 300 mg (14 subjects) of mifepristone, starting on Day 0 for 28 consecutive days. Treatment with both doses failed to influence blood HIV viral load at all time points examined.

with a slight reduction of $CD8^+$ T cells. None of the effects however were statistically significant (Figs. 2 and 3).

In spite of the most frequent hematochemical abnormalities observed with the highest dose of mifepristone (300 mg once daily) as compared to the 150 mg, this dose of the drug was also unable of significantly modifying either the primary or the secondary endpoints examined. There was a non-statistically significant trend towards decrease of the viral load (Table I, Fig. 3) together with a slight reduction of both $CD4^+$ and $CD8^+$ T cells, the maximal peak of each of them reached at the follow-up day 56 (Table I, Fig. 2).

Discussion

Mifepristone binds to the progesterone receptor five times more avidly than progesterone. It also binds to the GR receptor four times more strongly than dexamethasone. By contrast, mifepristone binds to the androgen receptor with only one quarter of the affinity of testosterone and has essentially no binding affinity for the mineralocorticoid or estradiol receptors (24).

Although mifepristone has been assessed in a number of medical applications other than in pharmacological termination of pregnancy including the treatment of uterine fibroids, endometriosis, psychotic major depression, glaucoma, tumors and Cushing's syndrome, it has not been approved by the FDA for any of these uses. However, even the long-term use of mifepristone as an antiprogestin in myoma, meningioma and other progesterone-dependent diseases was generally well-tolerated (24).

Our present data indicate that mifepristone when given as 150 mg once daily dose was well tolerated both from the clinical and the hematochemical point of view with the exception

of a substantial and homogeneous increase in the blood levels of cortisol that occurred in 5 of the 11 patients studied throughout the study period. The increase reverted at follow-up after discontinuation of the treatment and was not associated to clinical signs of hypercortisolism, such as hyperglycemia, hypertension or alteration of Na^+ and K^+ homeostasis.

On the other hand, the 300 mg dose of mifepristone given for 28 consecutive days was overall less well tolerated than the lower dose. This is consistent with the higher drop out rate as well as with the higher rate of hematochemical abnormalities observed in this study. This was particularly evident with regards to the occurrence of hypokalemia that developed in 7 of 14 patients as well as to the marked increase of cortisol blood levels that were observed in all of them.

Previous studies have already shown that mifepristone may affect either the central (through its negative feedback on CRH/ACTH secretion) or peripheral actions of cortisol. Therefore, mifepristone antagonizes the negative feedback of cortisol by blocking central GRs and leading to an increase of plasma ACTH and cortisol levels that is particularly evident in the early morning (26-29).

It has also been reported that, occasionally, long-term administration of mifepristone may be associated with low serum potassium levels, a slight increase in serum creatinine levels or with a moderate elevation of hepatic enzymes (30,31).

Since the GRs are blocked by mifepristone, while the mineralocorticoid receptors are not, the high cortisol levels associated with mifepristone-treatment may cause overstimulation of the latter. In light of our results, careful monitoring of potassium levels, as well as blood pressure values, during and eventually after mifepristone treatment are highly recommended. In the rare cases with clinically relevant anti-glucocorticoid side effects, concomitant glucocorticoid therapy may eventually be initiated (24).

Although neither primary nor secondary endpoints were achieved with either dose, it is worthy to mention that in the 150 mg/day group there was a non-significant trend towards reduction of the HIV-1 plasma viral load along with diminished $CD8^+$ T-cell number, that was more pronounced at the end of the drug treatment on Day 28. Moreover, a similar trend as regard to the $CD8^+$ T-cell count was observed in the 300 mg/day group, but it occurred with different kinetics.

In spite of a higher rate of hematochemical alterations and patient drop-out, the highest dose of mifepristone failed to significantly modify viro-immunological parameters, such as the viral load or the $CD4^+$ and $CD8^+$ T-cell counts in these patients. The trend toward a reduced viral load and $CD8^+$ T cells was comparable to that observed in the 150 mg/day dose group, but it occurred with different kinetics; it peaked in the follow-up period on Day 56, e.g. 4 weeks after interruption of the drug. A slight reduction of $CD4^+$ T cells was also observed and it reached its maximal value at the follow-up period on Day 56.

These data prove that when given at the doses examined in the present study, mifepristone is not able to significantly influence viro-immunological parameters in HIV-infected patients.

The discordance of the results of the *in vitro* system and macaque model and those in our present trial may depend on pharmacokinetic differences between the two experimental settings that have been discussed elsewhere (25) as well as on possible differences in the pathogenesis of SIV infection in macaques and HIV infection of humans. In a more general context, these data reinforce the need of exerting caution when preclinical data generated with SIV in macaque are translated to the clinical setting.

The lack of antiviral activity of mifepristone in our study is consistent with a previous phase I/II trial that showed that administration of mifepristone at 75-225 mg daily doses was safe and well-tolerated, but it did not significantly influence plasma HIV-1 RNA levels and $CD4^+$ lymphocyte count compared to the baseline (25).

The trend to reduced HIV viral load observed with the 150 mg dose of the drug along with the strong preclinical data and coupled to the good clinical tolerability would justify additional studies of the additive or synergistic effects of mifepristone with other anti-HIV drugs if there were not so many alternative options for the treatment of HIV-infected patients that also successfully cover, and with increasingly limited side-effects, those HIV patients that develop resistance to one therapeutic strategy. Nonetheless, the slight (if any) anti-retroviral activity of mifepristone coupled with its pharmacological profile may allow the postulation of an alternative use for mifepristone in the HIV setting that relates to the prevention and treatment of HIV-associated Kaposi sarcoma (KS). In fact, it has recently been shown that cultured KS cells from AIDS patients express an unusually high level of GR protein, that is further up-regulated by glucocorticoid treatment. In addition, *in vitro* treatment with a potent agonist of the GRs, such as dexamethasone significantly stimulated the proliferation and survival of sarcoma cells and these stimulatory effects were completely abolished by treatment with mifepristone (32).

The development of novel therapeutic options in the treatment of KS remains an important medical need even during the HAART era. In fact, KS remains the second most frequent tumor in HIV-infected patients worldwide, and it has become the most common cancer in Sub-Saharan Africa. In western countries the risk for KS in homosexual men is 5-10 times higher compared to other groups of individuals practicing other HIV-risk behaviors. Patients with KS in Sub-Saharan Africa have very high tumor burdens and rapid disease progression with a diminished life expectancy of less than 6 months. Several different therapeutic options are available, but the optimal therapy is still unclear. HAART including protease inhibitors may represent the first treatment step for slowly progressive disease; chemotherapy plus HAART is indicated for visceral and/or rapidly progressive disease, whereas maintenance HAART after systemic chemotherapy may be an effective anti-KS measure after debulking chemotherapy (33).

Taken together and in light of our present findings we believe that preclinical studies may be warranted to assess the hypothesis that treatment with mifepristone may represent a novel therapeutic strategy for the treatment of some cases of HIV-related KS.

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Note added in proof

At the stage of proofreading a paper has been published describing an AlphaScreen®-based assay for high-throughput screening for specific inhibitors of nuclear import of HIV through the integrase molecule of HIV-1 (1). By screening for specific inhibitors of the interaction between integrase and its nuclear transport receptor importin α/β, the authors have identified mifepristone as one of the inhibitors of the integrase/importin α/β interaction. Mifepristone was effective in preventing active nuclear transport of integrase in transfected cells. This study sheds new lights on the *in vitro* anti-HIV-1 activity of mifepristone. Additional studies with this drug in HIV-1 infected patients are required in order to understand and possibly overcome the factors limiting its activity *in vivo* as well as to evaluate its efficacy in the context of HAART regimes.

1. Wagstaff KM, Rawlinson SM, Hearps AC and Jans DA: An AlphaScreen®-based assay for high-throughput screening for specific inhibitors of nuclear import. J Biomol Screen 16: 192-200, 2011.