

## Suboptimal CD4 gains in HIV-infected patients receiving didanosine plus tenofovir

Pablo Barreiro and Vincent Soriano\*

*Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain*

**The combination of nucleos(t)ide analogues (NAs) is essential for the design of effective antiretroviral regimens. Although there are currently many options for the selection of such drug backbones, not all combinations display optimal results. As the number of these compounds has increased, it has become clear that the concomitant administration of certain NAs should be avoided due to high rates of toxicity and/or greater risk of virological failure. As an example, the combination of didanosine and tenofovir has recently been associated with a paradoxical depletion of CD4+ T cells in the face of complete viral suppression. Interference between the pathways leading to the intracellular activation of didanosine and tenofovir, and their blocking of the purine nucleoside phosphorylase, seems to explain this phenomenon.**

Keywords: CD4+ T lymphocytes, purines, purine nucleoside phosphorylase

### Introduction

The association of tenofovir (tenofovir disoproxil fumarate) with didanosine seemed to be very attractive at first sight as a nucleos(t)ide analogue (NA) backbone, given that both drugs are administered once a day, they show a relatively high genetic barrier for resistance, they are relatively well tolerated and food restrictions can be avoided. The first unexpected problem using this combination came from the recognition of a pharmacokinetic interaction between the two drugs, which causes a significant elevation (40–60%) in plasma didanosine levels. Soon after this finding, it was recommended that the didanosine dose be reduced to 250 mg daily<sup>1</sup> in order to minimize the risk of developing complications such as pancreatitis and hyperlactataemia.

Two main mechanisms have been proposed to explain the pharmacokinetic interaction between didanosine and tenofovir. First, tenofovir seems to increase the gastrointestinal absorption of didanosine.<sup>2</sup> Although the underlying mechanism for this has not been fully elucidated, the lack of an effect of tenofovir on didanosine half-life and renal clearance, together with an increase in the  $C_{max}$ , AUC and cumulative urinary excretion of didanosine when given along with tenofovir, is a strong argument in favour of this hypothesis. Alternatively, recent data have been released proving that tenofovir may reduce the intracellular metabolism of didanosine. This second pathway, which is not incompatible with the first, has gained credibility over recent months and may provide some insights into other problems that have arisen when using the combination of didanosine and tenofovir.

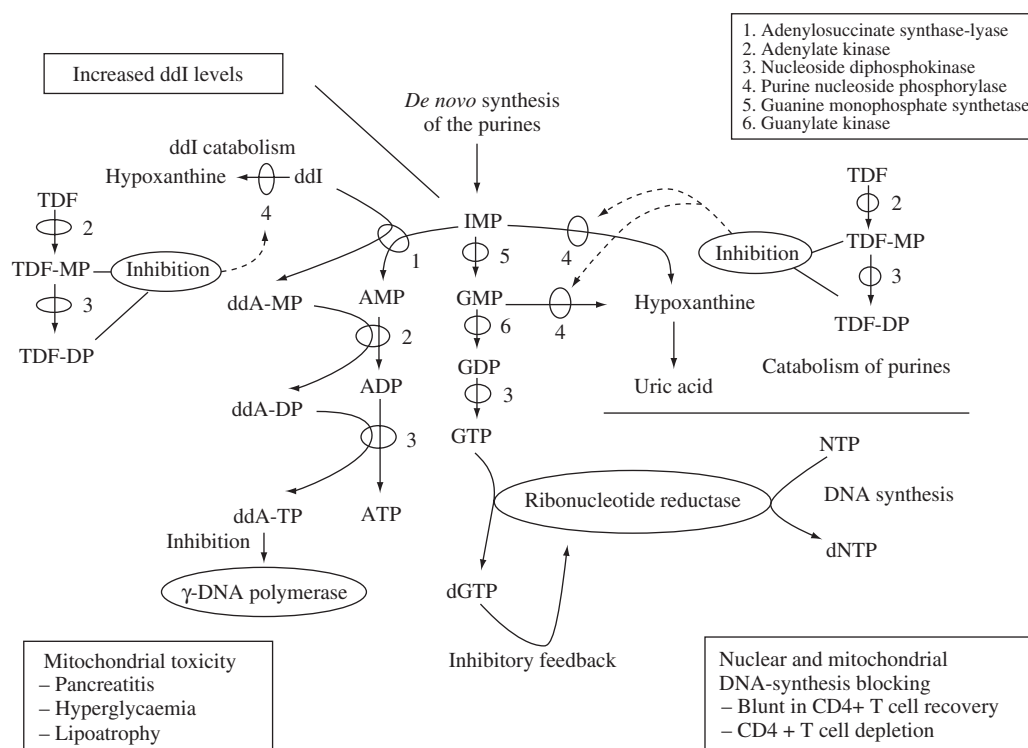
Purine nucleoside phosphorylase (PNP) is a cellular enzyme present in many tissues, but especially in lymphocytes. It is responsible for the metabolism of purines (inosine and guanine).

As didanosine is a purine analogue, it is metabolized intracellularly, at least partially, through PNP. On the other hand, the phosphorylated forms of tenofovir (which is also a purine analogue) are potent PNP inhibitors, which may cause increased didanosine levels (see Figure 1). In a similar way, other antivirals that act as PNP inhibitors, such as ganciclovir, are known to elevate didanosine concentrations.<sup>3</sup> Interestingly, the reduction in the dose of didanosine from 400 to 250 mg, when combined with tenofovir, normalizes not only plasma levels of didanosine but also the concentration of intracellular active forms of didanosine, including dideoxyadenosine triphosphate (ddATP).<sup>4</sup> This observation indirectly supports the hypothesis that the main mechanism involved in the didanosine–tenofovir interaction occurs at the intracellular level.

### High rate of mitochondrial toxicity with didanosine plus tenofovir

The interference of tenofovir in the metabolism of didanosine could explain the observed higher rate in subjects taking these drugs in combination of didanosine-related toxicities, such as pancreatitis,<sup>5</sup> severe weight loss mimicking rapid progression of lipodystrophy<sup>6</sup> and hyperglycaemia.<sup>7</sup> *In vitro* studies have shown a direct relationship between didanosine levels and the intracellular concentration of ddATP,<sup>8</sup> the active form of didanosine. This metabolite is a potent inhibitor of the mitochondrial  $\gamma$ -DNA polymerase. Hypothetically, the reduction in the cellular catabolism of didanosine, through the inhibition of PNP by tenofovir, might enhance didanosine-related toxicities as a result of the inhibition of mitochondrial activity by ddATP (see

\*Corresponding author. Tel: +34-91-4532500; Fax: +34-91-7336614; E-mail: vsoriano@dragonet.es



**Figure 1.** Intracellular interaction between didanosine (ddI) and tenofovir (TDF).

Figure 1). In fact, pancreatic toxicity and lipoatrophy are side effects already known to occur in subjects exposed to other NAs due to their deleterious influence on mitochondrial metabolism.<sup>9</sup>

### Paradoxical CD4+ T cell declines under didanosine plus tenofovir

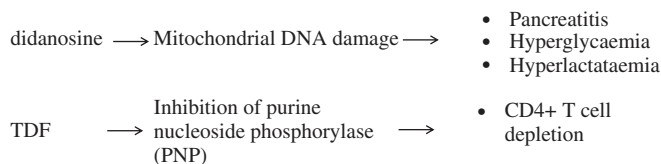
A further intriguing aspect related to the use of didanosine plus tenofovir in combination is the recent report of paradoxical CD4+ T cell declines despite optimal virological suppression. The first observation was made by Negredo *et al.*<sup>10</sup> in 2004. A total of 150 patients starting regimens with tenofovir plus didanosine 400 mg daily (or 250 mg daily in patients weighing <60 kg) who had undetectable viraemia under the previous antiretroviral regimen were followed for at least 48 weeks. While CD4 counts were stable or increasing with the prior regimen, a reduction in the mean CD4 count was noticed after switching to didanosine/tenofovir-based regimens. A control group of 152 patients with undetectable viraemia under didanosine and tenofovir separately was used for comparison. These individuals showed the expected CD4 gain during the follow-up. It should be noted that this study did not provide information on the immunological outcome of patients receiving reduced didanosine doses (250 mg daily, or 200 mg daily in patients weighing <60 kg). However, of the 10 patients changed from high to low didanosine doses, the CD4+ T cell count increased in six, but remained stable or continued to decrease in the other four patients.

A few months later our group confirmed these observations and provided new insights into the immunological effect of tenofovir, with didanosine used at the recommended reduced doses.<sup>11</sup>

A total of 570 patients starting a didanosine plus tenofovir regimen were included. Among them, 98 were antiretroviral-naive and 472 switched to didanosine plus tenofovir as part of a simplification strategy, while having undetectable viraemia under other, more complex regimens. Overall, 378 received a non-nucleoside analogue (NNA) and 192 a third NA. It should be noted that 26 started therapy with the reduced doses of didanosine, whereas 269 received full didanosine doses initially, although they shifted to reduced didanosine doses during the follow-up. An overall reduction in the CD4 count was recorded in patients on didanosine plus tenofovir, even in those starting with the reduced didanosine doses. Of note, CD4+ T cell declines were more pronounced in certain circumstances:

- (i) In simplification regimens compared with first-line regimens. This may be because in first-line regimens a CD4 recovery is primed due to the reduction in viral load; in simplification regimens the viral load is already undetectable.
- (ii) In patients with high CD4 counts at baseline. There is no clear explanation for this, although it may be that the more CD4 cells present the easier it is to detect an absolute decrease.
- (iii) In subjects receiving high didanosine doses. Mitochondrial didanosine toxicity may be one of the causes of CD4 cell decay, and higher didanosine doses are more toxic.
- (iv) In patients with low weight (which may cause higher didanosine levels).
- (v) If the third drug was an NA rather than a NNA (as all NAs may produce mitochondrial toxicity).

Tenofovir interference with purine metabolic pathways may again be responsible for this unexpected finding. As shown in Figure 1, tenofovir-mediated inhibition of the PNP-mediated



**Figure 2.** Hypothetical mechanisms of toxicity using didanosine plus tenofovir.

catabolism of IMP and GMP would facilitate the final production of GTP. This molecule has high affinity for ribonucleotide reductase (RNR), the key enzyme for deoxyribonucleotide synthesis. These molecules are essential for the synthesis of cellular DNA during mitosis. Moreover, GTP plays a pivotal role in nucleotide versus deoxynucleotide intracellular equilibrium, because dGTP exerts a potent inhibitory feedback over RNR. As a result, increased dGTP levels, as the last consequence of PNP inhibition, will reduce RNR activity and, therefore, compromise the ability of T lymphocytes to proliferate. The final result will be CD4+ T lymphocytopenia, which mimics what is observed in experimental models using PNP knockout mice.<sup>12</sup> Further supporting this hypothesis is the recognition of a congenital immunodeficiency syndrome caused by the absence of PNP activity.<sup>13</sup> Among other signs and symptoms, children carrying this genetic defect usually die in their early infancy due to severe and selective CD4+ T cell depletion.

Alternatively, the accumulation of dGTP would preferentially cause the inhibition of mitochondrial RNR. The diminution in the capabilities for mitochondrial DNA repair caused by RNR malfunction would finally precipitate cellular apoptosis and CD4+ T cell depletion.

In summary, the association of didanosine plus tenofovir might produce unexpected toxicities by two different pathways: first, by enhancing didanosine-mediated mitochondrial toxicities (pancreatitis, hyperglycaemia, hyperlactataemia, etc.); second, through PNP inhibition mediated by tenofovir (i.e. CD4+ T cell depletion) (Figure 2).

### High rate of virological failure under didanosine plus tenofovir

Regimens containing didanosine plus tenofovir have shown other disadvantages also. Recent reports have recognized high rates of virological failure. The first observation came from a small open study in which 24 drug-naïve subjects started didanosine, tenofovir and lamivudine. The study was prematurely interrupted due to a 91% incidence of virological failure at week 12. Moreover, all patients had selected the M184V mutation (selected by lamivudine) and half of them K65R (selected by tenofovir).<sup>14</sup> Other studies have confirmed the weakness of the didanosine/tenofovir backbone when used along with other NAs or NNAs. As an example, one trial compared two once-daily NA backbones (didanosine/tenofovir versus didanosine/lamivudine), given along with efavirenz in drug-naïve subjects.<sup>15</sup> The study was interrupted at week 12 since the incidence of virological failure was significantly higher in the didanosine/tenofovir arm than in the didanosine/lamivudine arm (12% versus 0%). As good adherence to treatment was demonstrated, and all failing patients on didanosine/tenofovir harboured resistance mutations, interference between didanosine and tenofovir might explain this observation.

Finally, another study found higher than expected rates of viral rebound with didanosine plus tenofovir, even when used along with efavirenz (47%) or lopinavir/ritonavir (14%).<sup>16</sup> All together, these reports suggest that didanosine plus tenofovir should be considered as a combination with a low genetic barrier for resistance, as selection of just one mutation, K65R, compromises the antiviral activity of both the drugs.

In summary, the combination of didanosine plus tenofovir is associated with CD4+ T cell depletion in HIV-infected patients despite complete virus suppression. Although the reduction of didanosine doses seems to mitigate this paradoxical effect, the effect does not vanish. The recognition of this side effect, along with a greater risk of pancreatitis, hyperglycaemia and virological failure, should discourage the use of this NA combination.

### Transparency declarations

No declarations were made by the authors of this paper.

### References

1. Kearney B, Flaherty J, Shah J. Tenofovir disoproxil fumarate: clinical pharmacology and pharmacokinetics. *Clin Pharmacokinet* 2004; **43**: 595–612.
2. Pecora P, Kirian M. Effect of TDF on ddI absorption in patients with HIV. *Ann Pharmacother* 2003; **37**: 1325–8.
3. Ray A, Olson L, Fridland A. Role of purine nucleoside phosphorylase in interactions between 2',3'-dideoxyinosine and allopurinol, ganciclovir, or tenofovir. *Antimicrob Agents Chemother* 2004; **48**: 1089–95.
4. Pruvost A, Negro E, Benech H *et al.* Measurement of intracellular didanosine and tenofovir phosphorylated metabolites and possible interaction of the two drugs in HIV-infected patients. *Antimicrob Agents Chemother* 2005; **49**: 1907–14.
5. Martinez E, Milinkovic A, de Lazzari E *et al.* Pancreatic toxic effects associated with co-administration of didanosine and tenofovir in HIV-infected adults. *Lancet* 2004; **364**: 65–7.
6. Palacios R, Santos J, González M *et al.* Reduction in body weight and CD4 lymphocyte count with simplification regimens including TDF and ddI. A scheme to avoid? In: *Seventh International Congress on Drug Therapy in HIV Infection*. Glasgow, 2004. Abstract P19.
7. García-Benayas T, Barrios A, Sánchez-Conde M *et al.* Higher risk of hyperglycemia in patients under didanosine and tenofovir-containing regimens. In: *Twelfth Conference on Retroviruses and Opportunistic Infections*. Boston, 2005. Abstract 829.
8. Robbins B, Wilcox C, Fidland A *et al.* Metabolism of tenofovir and didanosine in quiescent or stimulated human peripheral blood mononuclear cells. *Pharmacotherapy* 2003; **23**: 695–701.
9. Brinkman K, ter Hofstede HJ, Burger DM *et al.* Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. *AIDS* 1998; **12**: 1735–44.
10. Negro E, Molto J, Burger D *et al.* Unexpected CD4 cell count decline in patients receiving didanosine and tenofovir-based regimens despite undetectable viral load. *AIDS* 2004; **18**: 459–63.
11. Barrios A, Rendón A, Negro E *et al.* Paradoxical CD4+ T cell decline in HIV-infected patients with complete virus suppression under tenofovir plus didanosine combinations. *AIDS* 2005; **19**: 569–75.
12. Arpaia E, Benveniste P, Di Cristofano A *et al.* Mitochondrial basis for immune deficiency. Evidence from purine nucleoside phosphorylase-deficient mice. *J Exp Med* 2000; **191**: 2197–207.
13. Markert ML. Purine nucleoside phosphorylase deficiency. *Immunodef Rev* 1991; **3**: 45–81.

## Leading article

14. Jemsek J, Hutcherson P, Harper E. Poor virological responses and early emergence of resistance in treatment naïve, HIV-infected patients receiving a once daily triple nucleoside regimen of didanosine, lamivudine and tenofovir DF. In: *Eleventh Conference on Retroviruses and Opportunistic Infections*. San Francisco, 2003. Abstract 51.

15. Maitland D, Moyle G, Hand J *et al*. Early virological failure in HIV-1 infected subjects on didanosine/tenofovir/efavirenz: 12-week results from a randomized trial. *AIDS* 2005; **19**: 1183–8.

16. Podzamczar D, Ferrer E, Gatell J *et al*. Early virological failure with a combination of tenofovir, didanosine and efavirenz. *Antivir Ther* 2005; **10**: 171–7.