CD4+ T cell evolution and predictors of its trend before and after tenofovir/didanosine backbone in the presence of sustained undetectable HIV plasma viral load

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Received 22 November 2006; returned 16 January 2007; revised 8 February 2007; accepted 13 March 2007

Background: Tenofovir with full-dose didanosine has been associated with paradoxical CD4+ T cell decrease despite virological suppression. We investigated whether tenofovir plus didanosine at a weight-adjusted dosage could be responsible for such an effect, and factors associated with CD4+ T cell count evolution under this combination.

Methods: This was a prospective observational multicohort study (Italian MASTER and Spanish Hospital Carlos III HIV cohorts). Patients with HIV plasma viral load suppression for ≥6 months who switched to an antiretroviral combination including tenofovir plus didanosine were studied, as long as virological success was maintained. CD4+ T cell count variations over time (slopes) were compared before and after switching to tenofovir plus didanosine using linear mixed models and segmented regression analysis.

Results: Annual time-weighted CD4+ T cell count slope did not change significantly after the prescription of tenofovir plus didanosine: it was 14 cells/mm³ (95% confidence interval (CI) −7 to 35) from month −24 to month −12, 12 cells/mm³ (95% CI −14 to 38) from month −12 to the time of switching, 30 cells/mm³ (95% CI 5−55) from switching to month +12 and 15 cells/mm³ (95% CI −8 to 39) from month +12 to month +24 after switching to tenofovir plus didanosine. No significant change in the slope of the segment after the switch to tenofovir plus didanosine-containing regimens when compared with the segment preceding the intervention was found (CD4+ T cell count slope change: 24 cells/mm³; 95% CI −10 to 58). Similar results were obtained using CD4+ T cell percentage over total lymphocytes. The significant independent predictors of lower CD4+ T cell count slope were older age (P = 0.006), lower nadir CD4+ T cell count (P < 0.001) and positive hepatitis C virus antibody (P = 0.03). Moreover, reduced estimated creatinine clearance was an additional independent predictor of lower CD4+ T cell count slope (P = 0.02), but only after excluding nadir CD4+ T cell count.

Conclusions: Tenofovir plus didanosine (weight-adjusted dosage) was not associated with paradoxical CD4+ T cell decrease in our patients maintaining undetectable HIV plasma viral load for a maximum of 24 months after switching. Several factors could explain variability in CD4+ T cell count evolution in these patients.

Keywords: immune recovery, CD4 cell count, immune toxicity, antiretroviral therapy

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Introduction

The use of tenofovir disoproxil fumarate in combination with didanosine has been considered an attractive option as a possible nucleoside reverse transcriptase inhibitor (NRTI) backbone of highly active antiretroviral therapy (HAART) because of its convenient posology (two pills once daily, regardless of food intake). Nonetheless, many studies have reported unexpected CD4+ T cell count decrease in patients treated with didanosine at unadjusted dosages in combination with tenofovir, and this was correlated with pharmacokinetic interactions, possibly resulting in a dose-related didanosine toxicity for lymphocytes. The same phenomenon could be responsible for the compromised immunological recovery reported in experienced patients treated with enfuvirtide in combination with tenofovir and full-dose didanosine. An analysis conducted by Karrer et al. demonstrated that immune depletion was less likely to occur if the didanosine dosage was adjusted to <4.1 mg/kg/day, which is close to the currently recommended dosage when didanosine is co-administered with tenofovir (i.e. 250 mg/day in patients weighing >60 kg). However, neither this analysis nor other available studies have fully accounted for the possible impact of HIV replication on CD4+ T lymphocytes, as they included patients either naive or experienced to antiretroviral drugs, with either detectable or undetectable HIV plasma viral load at baseline or during follow-up. Moreover, paradoxical CD4+ T cell count decrease was also evident in a minority of patients treated with weight-adjusted didanosine dosages, suggesting that variables other than per-kilo dosage may be responsible for this side effect.

Tenofovir plus didanosine is not considered a standard backbone combination because of high risk of virological failure in naive patients. Notwithstanding, this combination has been used in several patients when other options were precluded because of previous treatment failure or intolerance to alternative drugs. Therefore, the aim of our study was to investigate whether the introduction of tenofovir plus didanosine in a weight-adjusted dosage in fully virologically suppressed patients was associated with a paradoxical decrease in the CD4+ T cell count or with a reduction in their trend of increase. Moreover, we explored what factors may influence CD4+ T cell count evolution in these patients.

Materials and methods

Patients

Patients were selected from the Italian MASTER cohort, which is a prospective longitudinal multicentre cohort composed of the general HIV patient population in referral centres throughout Italy. The distinguishing characteristic of this cohort is that data are collected by a common electronic database (Health & Notes version 3.5, Healthware S.p.A., Naples, Italy) in use in 11 Italian centres for clinical purposes since 1999. The electronic database is implemented to manage everyday activity of the outpatient HIV clinics in each centre. The resulting cohort is, therefore, an open cohort in which non-pre-selected patients are continuously enrolled. Demographics, medication and disease history are recorded at enrolment and updated on a 3 monthly basis. In order to increase the sample size, data from the MASTER cohort were merged with those from the Hospital Carlos III in Madrid (Spain), also recorded at similar intervals.

All HIV-1-positive patients who switched from an effective HAART regimen to a new regimen including tenofovir in combination with didanosine at a weight-adjusted dosage were eligible for the study, provided that HIV plasma viral loads were persistently undetectable (i.e. <50 copies/mL) during the previous ≥6 months. Patients were excluded from the analysis if they had previously experienced the tenofovir and didanosine combination, although they were still eligible if one or both drugs had been taken separately. Treatment intensification by the mere addition of either tenofovir or didanosine to the ongoing HAART was not allowed. Other exclusion criteria were the use of interferon-based treatment or bone marrow stimulating factors during the observation or lack of a weight determination within 180 days before or after switching. Right censoring of follow-up was applied when any change in the regimen prescribed at switching occurred, at the time of the first detectable HIV plasma viral load or when any clinical event (i.e. death or HIV-related diseases) occurred, whichever was the first to occur.

Statistical analysis

Two study outcomes were considered: absolute CD4+ T cell count and CD4+ T cell percentage value over total lymphocytes. As longitudinal data were available, linear mixed models were used to derive time-adjusted absolute CD4+ T cell count or percentage changes over time (i.e. slopes). MIXED procedure in SAS was used by fitting values of CD4+ T cell count or percentages from all study time points as dependent variables. Independent variables included the fixed effects of intervention, study visit time points and other co-variables assumed to have a potential confounding or residual effect on trends in CD4+ T cell counts over time. The following co-variables were considered: age, gender, nationality (European versus others), weight, risk factor for HIV acquisition [intravenous drug user (IVDU) versus others], clinical stage of HIV infection (CDC '93 class C versus others), serum reactivity for hepatitis C virus (HCV) antibodies (Ab), CD4+ T cell count at nadir (i.e. the lowest value ever recorded), CD4+ T cell count at the time of switching, creatinine clearance at the time of switching (calculated according to the Crockroft–Gault formula with actual body weight), per-kilo didanosine dosage (mg/kg), type of third drug associated with tenofovir plus didanosine [i.e. other NRTI, non-NRTI (NNRTI) or single or boosted protease inhibitor], previous exposure to tenofovir, previous exposure to didanosine, cumulative previous exposure to didanosine (months), number of drugs experienced (ritonavir and saquinavir used as booster were not considered), number of treatment lines experienced and duration of HIV plasma viral load undetectability prior to switching (months). Owing to co-correlation between explanatory variables, several multivariable models were generated and the model that was considered most clinically and statistically significant is presented.

A supplementary analysis (simple-segmented linear regression) of change in the CD4+ T cell count slope before and after tenofovir plus didanosine was conducted using Stata Software. The change in the annual slope of CD4+ T cell count was estimated using the
Model estimated a significant CD4+ T cell count levels at 1 year after tenofovir plus didanosine introduction and computing the difference between observed and predicted CD4+ T cell count values. Possible abrupt variation in the outcome, e.g. a jump or drop in CD4+ T cell count levels after tenofovir plus didanosine, was also investigated by comparing the value of the series at the beginning of the time interval (i.e. the y-intercept for the first segment) and the value immediately following at which the successive segment joins.

All statistical analyses were performed using SAS V8 and Stata 8.0 and all P values presented are two-tailed.

**Results**

**Patients**

One thousand, one hundred and seventy-five patients in the MASTER cohort and 463 patients in the Hospital Carlos III HIV cohort were prescribed tenofovir plus didanosine combinations. Among them, 1100 and 378 were experienced to antiretroviral drugs in the two cohorts, respectively; 230 and 157 were switched to this combination with undetectable HIV plasma viral load, of whom 99 and 18 had HIV plasma viral load persistently undetectable for at least 6 months before switching. Of these patients, six were excluded because they had undergone interferon-based treatment during the follow-up and three due to the absence of weight recorded within the stated time frame. The characteristics of the remaining 108 patients included in the study are summarized in Table 1. Median CD4+ T cell count at the time of switching was high (439 cells/mm^3) and the tenofovir plus didanosine backbone was prescribed mainly with an NNRTI. The median duration of follow-up prior to the switch was 14 [interquartile range (IQR) 9–24] months, whereas patients were followed for a median of 12 (IQR 6–19) months thereafter. Median number of CD4+ T cell count determinations per patient before and after the switch was 5 (IQR 3–8) and 4 (IQR 3–7), respectively. No significant differences were found comparing the MASTER patients with those from Hospital Carlos III.

**CD4+ T cell evolution**

As shown in Figure 1(a), the introduction of tenofovir plus didanosine was not associated with any paradoxical decline in the CD4+ T cell count using the linear mixed model. In fact, the model estimated a significant CD4+ gain after 12 (30 cells/mm^3; P = 0.02) and 24 months (45 cells/mm^3; P = 0.003) from switching. Similar results were obtained when the CD4+ percentage value over total lymphocytes was considered as the outcome measure (Figure 1b). When compared with pre-switch levels, the CD4+ percentage was significantly higher at 12 and 24 months after switching (0.9 percentage-point; P = 0.02 and 1.6 percentage-point; P = 0.001, respectively).

**Slope of CD4+ T cells**

CD4+ T cell count slope did not change significantly after prescription of tenofovir and didanosine when the linear mixed model was used. The annual time-weighted CD4+ T cell count slope before switching was +14 cells/mm^3 (95% CI -7 to 35) from month -24 to month -12 and +12 cells/mm^3 (95% CI -14 to 38) from month -12 to the time of switching. After the introduction of tenofovir plus didanosine, the CD4+ T cell count slope was +30 (95% CI +5 to +55) from switching to month +12 and +15 (95% CI -8 to +39) from month +12 to month +24.

Likewise, the slope of percentage CD4+ T cell count among total lymphocytes did not vary significantly from the introduction of tenofovir plus didanosine. Before switching,
Figure 1. Rate of change of absolute (a) and percentage (b) CD4+ T cell counts before and after tenofovir plus didanosine (estimated by unadjusted and adjusted linear mixed models). Mixed model estimated CD4+ T cell count slopes were based on 108 patients (month 2 to baseline), 108 patients (baseline to month 12), 59 patients (month 2 to month 12) and 53 patients (month 12 to month 24). Adjustments for the following factors were made: gender, age, CDC classification, HCV antibody serostatus, nadir CD4+ T cell count, per-kilo didanosine dosage and creatinine clearance at switch (model A) and nadir CD4+ T cell count (model B). TDF, tenofovir disoproxil fumarate; ddI, didanosine.
the time-weighted percentage CD4\(^+\) T cell slope was +1.3 percentage-points (95% CI 0.7–1.9) from month −24 to month −12 and +0.4 percentage-points (95% CI −0.4 to 1.1) from month −12 to time 0. Following the switch, the slope was +0.9 percentage-points (95% CI +0.1 to +1.6) and +0.7 percentage-points (95% CI 0 to +1.4) from introduction of tenofovir plus didanosine to month +12 and from month +12 to month +24, respectively.

We further investigated these trends using the segmented regression analysis method. This also demonstrated no significant change in the slope of the segment after the switch to the tenofovir plus didanosine-containing regimens when compared with the segment prior to the intervention (slope change: +24 cells/mm\(^3\); 95% CI −10 to +58). Furthermore, no significant level change (i.e. abrupt variation in CD4\(^+\) T cell count immediately after intervention) was detected (level change: +11 cells/mm\(^3\); 95% CI −7 to +28).

**Predictors of CD4\(^+\) T cell evolution**

Table 2 shows a multivariable MIXED model showing factors adjusted for each other. Older age (years) \((P = 0.02)\), lower nadir CD4\(^+\) T cell count \((P < 0.001)\) and positive HCV-Ab \((P = 0.03)\) were significantly associated with decreasing CD4\(^+\) T cell count slope across the follow-up. Moreover, lower creatinine clearance was significantly correlated with lower CD4\(^+\) T cell count slope using univariate analysis, but could not be input in the multivariable analysis because of a significant co-correlation with nadir CD4\(^+\) T cell count. Nonetheless, its independent predictive value was demonstrated in a model without nadir CD4\(^+\) T cell count (2.2 cells/mm\(^3\) per mL/min increase; 95% CI 0.4–4; \(P = 0.02\)).

The change in point estimates produced by these factors in the adjusted model was very slight, however, thus indicating little or no confounding effect (Figure 1a). Similarly, no significant changes occurred in the CD4\(^+\) T cell percentage model (Figure 1b) when it was adjusted for nadir CD4\(^+\) T cell count, the only significant independent predictor of percentage CD4\(^+\) T cell slope (0.04; 95% CI 0.03–0.05; \(P < 0.001\)). The adjusted segmented regression models also showed similar findings. This indicated that CD4\(^+\) T cell count slope or CD4\(^+\) T cell count level did not change significantly after switching to tenofovir plus didanosine, even adjusting for possible explanatory variables.

**Discussion**

In our cohort, the use of tenofovir in combination with didanosine was not associated with a significant change in the trend of CD4\(^+\) T cell count which was ongoing before switching to this combination. In other terms, we did not observe a significant drop in CD4\(^+\) T cell count as a result of tenofovir plus didanosine introduction, although a great interindividual variability was present. Different hypotheses may be formulated to explain why our findings partially differed from those of the studies that demonstrated the immune-toxicity of this combination.\(^1\)\(^-\)\(^6\),\(^7\)

First, in our cohort, didanosine was never used at 400 mg/day, a dosage that is no longer recommended when didanosine is administered in combination with tenofovir. In other studies, a proportion varying from 30% to 100% of patients received a didanosine dose higher than that now recommended. Therefore, our cohort is more likely to represent current clinical practice and our results suggest that immune recovery is not impaired when didanosine is prescribed at weight-adjusted dosages. This is consistent with previous observations of partial CD4\(^+\) recovery after didanosine dosage reduction\(^1\)\(^3\) and with the identification of a threshold for didanosine dosage, below which immune-toxicity is unlikely to occur.\(^7\)

Secondly, only patients who switched to tenofovir and didanosine with full virological suppression were studied. In a previous study, a greater decline in HIV plasma viral load was associated with a steeper CD4\(^+\) T cell increase and this could have influenced the results.\(^7\) Moreover, in some studies, HIV replication was more frequently detected among subjects taking tenofovir plus didanosine,\(^9\) thus hampering CD4\(^+\) T cell gain in this group of patients. In our study, a possible effect resulting from different levels of HIV replication was removed by considering patients provided that their HIV plasma viral load remained <50 copies/mL.

Thirdly, even though switching to tenofovir plus didanosine-containing treatment was not associated with a paradoxical CD4\(^+\) T cell loss, other factors, which were not invariably taken into account in previous studies, were associated with a reduced immune recovery, despite full virological suppression induced by HAART. In particular, patients with advanced HIV disease, as proved by a low nadir CD4\(^+\) T cell count, had smaller CD4\(^+\) T cell gains. The reduced immune recovery observed in some patients was therefore more likely to be due to the degree of the previous immune damage, rather than to the introduction of a particular drug combination, as already suggested by others\(^1\)\(^2\). The association between higher per-kilo didanosine dosage and reduced CD4\(^+\) T cell slope was present at univariate analysis, but disappeared after adjustment for nadir CD4\(^+\) T cell count. This was probably due to the fact that weight of patients with low nadir CD4\(^+\) T cell count was lower than that of the others (mean weight 71.4 and 65.1 kg among patients with nadir \(\geq\) or <200 cells/mm\(^3\), respectively; \(t\)-test \(P = 0.015\)).

Older age, HCV co-infection and renal function impairment were also independently associated with blunted CD4\(^+\) T cell recovery. Some hypotheses can be raised to explain these findings. Ageing is known to compromise immunological gain after HAART,\(^1\)\(^3\) and this phenomenon is probably related to a decreased proliferation of lymphocytes,\(^1\)\(^4\) as well as to their increased susceptibility to apoptosis.\(^1\)\(^5\),\(^1\)\(^6\) Similarly, higher rates of apoptosis of naive CD4\(^+\) T cells have been demonstrated in HCV-co-infected than in HIV-monoinfected individuals.\(^1\)\(^7\) The association between impaired immune reconstitution and reduced creatinine clearance may be interpreted in different ways. Renal dysfunction could be an epiphenomenon of severe immune damage, thus explaining why it was co-linear with disease stage and associated with lower CD4\(^+\) T cell gain. Renal dysfunction could be directly responsible for reduced CD4\(^+\) T cell gain because of cytokine unbalance, as suggested by a previous study.\(^1\)\(^8\) In contrast, renal dysfunction could act through an indirect mechanism, increasing didanosine plasma levels and its dose-related toxicity on lymphocytes.

Our study has several limitations that should be acknowledged. First, the analysis was conducted with an as-treated approach, with observation of patients restricted to those with complete virological suppression and the censoring of the observation if the patient changed treatment. Therefore, our study was
not designed to investigate virological effectiveness of this combination. This was done deliberately, because patients were heterogeneous in terms of clinical and treatment history and a carefully matched control group would have been difficult to obtain. Moreover, only a careful monitoring of patient adherence to treatment would give reliable information in this respect. However, it should be kept in mind that a higher risk of virological failure has been associated with tenofovir plus didanosine use, especially among naive patients and in combination with NNRTI.8–11,19 In the light of these observations, antiretroviral treatment guidelines contraindicated this backbone as part of first-line regimens.20 Risk of virological ineffectiveness should also be taken into account when this combination is used in experienced patients.

Secondly, the small sample size and the relative shortness of the follow-up do not have the potential to detect minor or delayed variations in the CD4+ T cell slope, although patients had been followed up to a maximum of 24 months after switching.

Thirdly, long-term tolerability of tenofovir plus didanosine was not assessed in this study. Although tolerability and toxicity profiles could be improved by using weight-adjusted doses of didanosine, a possible increased risk of toxicity is still present.21–23 Therefore, careful surveillance is needed when tenofovir plus didanosine is used.

In conclusion, paradoxical CD4+ T cell loss was not related to the introduction of tenofovir plus didanosine (low dose) after excluding the possible influence of positive HIV plasma viral load in our series of patients. Factors explaining interindividual variability could explain blunted CD4+ T cell recoveries and/or paradoxical declines in former studies using higher doses of didanosine and/or longer follow-ups. In the meantime, our results are reassuring about the safety of this combination, as far as immune-toxicity is concerned. Therefore, we suggest that the use of tenofovir plus didanosine as backbone in experienced patients with lack of alternative options should not be avoided just because of immune-toxicity risk.

Acknowledgements
This work has been presented in part to the ‘Eighth International Congress on Drug Therapy in HIV Infection’, Glasgow, UK, 12–16 November 2006 (Abstract P48). This was an independent study not funded by private or public institution.

The Italian MASTER Cohort is a large national project involving the major centres providing care to HIV/AIDS patients and includes the following doctors (towns where the clinical centres are placed, all in Italy, are in brackets): Prof. L. Minoli, Dr S. Novati and Dr R. Maserati, (Pavia); Prof. G. Pastore and Dr N. Ladisa (Bari); Prof. G. Carosi, Dr G. Cristini, Dr S. Casari, Dr F. Castelnuovo, Prof. M. Puoti, Dr G. Paraini, Dr E. Quiros-Roldan and Dr C. Torti (Brescia); Dr T. Quirino and Dr G. Migliorino (Busto Arsizio); Dr F. Mazzotta, Dr S. Lo Caputo and Dr N. Marino (Florence); Dr F. Ghinelli and Dr L. Sighinolfi (Ferrara); Prof. R. Cauda and Dr A. De Luca from Università Cattolica del Sacro Cuore (Rome); Dr A. Antinori and Dr F. Antonucci from Istituto Nazionale di Malattie Infettive (Rome) and Prof. A. D’Arminio Monforte and Dr P. Cicconi (Milan).

Transparency declarations
None to declare.

References


Table 2. Predictors of absolute CD4+ T cell count slope

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted Est. (95% CI)</th>
<th>P value</th>
<th>Adjusted Est. (95% CI)</th>
<th>P value</th>
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<td>Age (years)</td>
<td>-9 (-14 to -3)</td>
<td>0.003</td>
<td>-6 (-10 to -2)</td>
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<td>CD4 nadir (cells/mm³)</td>
<td>1.2 (0.9–1.4)</td>
<td>&lt;0.001</td>
<td>1.1 (0.9–1.3)</td>
<td>&lt;0.001</td>
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<td>CDC class C (yes versus no)</td>
<td>-95 (-200 to 9)</td>
<td>0.07</td>
<td>-39 (-112 to 33)</td>
<td>0.29</td>
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<td>HCV-Ab (positive versus negative)</td>
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<td>0.16</td>
<td>-69 (-132 to 5)</td>
<td>0.03</td>
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<tr>
<td>Per-kilo ddI dosage (mg/kg)</td>
<td>-86 (-150 to -22)</td>
<td>0.008</td>
<td>-38 (-83 to 7)</td>
<td>0.10</td>
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<tr>
<td>Creatinine clearance (mL/min)</td>
<td>2.7 (1.4–4)</td>
<td>&lt;0.001</td>
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<td></td>
</tr>
</tbody>
</table>

Est., estimate; HCV, hepatitis C virus; Ab, antibodies; ddI, didanosine.

*aCreatinine clearance was not input in the model because of co-correlation with nadir CD4+ T cell count. Its independent predicting value was demonstrated in a separate multivariable model not including nadir CD4+ T cell count (Est. 2.2; 95% CI 0.4–4; P = 0.02).


