# Dose-dependent influence of didanosine on immune recovery in HIV-infected patients treated with tenofovir

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**Background:** Antiretroviral therapy (ART) containing tenofovir disoproxil fumarate (TDF) and didanosine (ddI) has been associated with poor immune recovery despite virologic success. This effect might be related to ddI toxicity since ddI exposure is substantially increased by TDF.

**Objective:** To analyze whether immune recovery during ART with TDF and ddI is ddI-dose dependent.

**Design and methods:** A retrospective longitudinal analysis of immune recovery measured by the CD4 T-cell slope in 614 patients treated with ART containing TDF with or without ddI. Patients were stratified according to the tertiles of their weight-adjusted ddI dose: low dose (< 3.3 mg/kg), intermediate dose (3.3–4.1 mg/kg) and high dose (> 4.1 mg/kg). Cofactors modifying the degree of immune recovery after starting TDF-containing ART were identified by univariable and multivariable linear regression analyses.

**Results:** CD4 T-cell slopes were comparable between patients treated with TDF and a weight-adjusted ddI-dose of < 4.1 mg/kg per day ($n = 143$) versus TDF-without-ddI ($n = 393$). In the multivariable model the slopes differed by −13 CD4 T cells/μl per year [95% confidence interval (CI), −42 to 17; $P = 0.40$]. In contrast, patients treated with TDF and a higher ddI dose (> 4.1 mg/kg per day, $n = 78$) experienced a significantly impaired immune recovery (−47 CD4 T cells/μl per year; 95% CI, −82 to −12; $P = 0.009$). The virologic response was comparable between the different treatment groups.

**Conclusions:** Immune recovery is impaired, when high doses of ddI (> 4.1 mg/kg) are given in combination with TDF. If the dose of ddI is adjusted to less than 4.1 mg/kg per day, immune recovery is similar to other TDF-containing ART regimen.



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**Keywords: immune recovery, antiretroviral therapy, CD4 cell count, drug interactions, reverse transcriptase inhibitor, didanosine, tenofovir**

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## Introduction

Once daily (OD) anti-retroviral therapy (ART) has attracted considerable interest in patients and physicians, since simple treatment regimens increase quality of life and improve adherence [1,2]. When used in combination, tenofovir disoproxil fumarate (TDF) and didanosine (ddI) can both be administered OD together with food. Therefore, OD-regimens containing ddI and TDF have become increasingly popular despite the lack of long-term data demonstrating their efficacy and safety. TDF increases ddI exposure, hence the recommendation to reduce the dose of ddI from 400 to 250 mg per day in patients treated with TDF and weighing $> 60$ kg [3,4].

Recent studies have reported declining CD4 T-cell counts despite fully suppressed HIV-RNA in patients treated with TDF and ddI [5–7]. However, at the time of these analyses most patients were treated with a higher ddI dose than is now recommended. Since this CD4 T-cell decline was associated with high ddI plasma levels [6], dose-related ddI toxicity for lymphocytes would be a plausible explanation. After dose reduction from 400 to 250 mg, subgroups of patients experienced partial recovery of CD4 T-cell (CD4-)counts [6,8].

Here, we analyze 614 patients from the Swiss HIV cohort study (SHCS) who were treated with TDF, with or without ddI. Our data suggest that ART-regimen containing TDF and ddI produce adequate immune recovery, provided that the daily ddI dose is less than 4.1 mg/kg body weight. This dose corresponds very closely to the current ddI dose recommendations of 250 mg for patients treated with TDF and weighing $> 60$ kg ($\leq 4.17$ mg/kg).

## Methods

### Data collection and patients

The SHCS is a prospective multi-center cohort study with continuous enrolment of HIV-infected patients aged 16 years and older. Information about demographics, HIV-associated diseases, medications, and laboratory parameters is collected according to standardized criteria at registration and at follow-up visits every 6 months. Informed consent is obtained from all participants. More than 12 600 patients have been enrolled and around 5400 are currently being followed ([9,10], www.shcs.ch).

We performed a retrospective analysis of all patients participating in the SHCS and treated with ART containing TDF. Baseline was defined as the date of starting TDF. Inclusion criteria were: (1) treatment with TDF as part of ART over a period of at least 180 days; and (2) two or more measurements of CD4 T cells and of HIV-RNA while TDF and/or ddI treatment remained qualitatively unchanged. ART changes other than TDF or ddI and dose adjustments of TDF and/or ddI were allowed. Exclusion criteria were concomitant use of immune response-modifying drugs such as interferon (IFN)-$\alpha$, ribavirin, hydroxyurea or interleukin (IL)-2. To examine the impact of the exact ddI dose on immune recovery measured by the CD4 T-cell (CD4-) slope from baseline, patients taking TDF and ddI (TDF+ddI) were stratified according to the tertiles of their weight-adjusted ddI dose, which was calculated according to the prescribed ddI dose and the body weight of the patient. The median ddI dose and the total dose ranges of the different treatment groups were the following: ddI low dose (ddI-low), 2.9 mg/kg (range, 1.9–3.3); ddI intermediate dose (ddI-intermediate), 3.7 mg/kg (range, 3.3–4.1); and ddI high dose (ddI-high), 5.0 mg/kg (range, 4.1–11.4; Table 1). These treatment groups were compared with patients treated with ART containing TDF without ddI (TDF-without-ddI). If the ddI dose was adjusted during the study, patients were assigned to their initial ddI dose group, as long as they were treated for $> 1$ month with the respective ddI dose.

### Statistical analyses

The CD4 T-cell slope after starting TDF was determined by linear regression for each individual patient. Cofactors modifying the CD4 T-cell slope were then analyzed by univariable and multivariable weighted linear regression. Individual weights were given according to the number of CD4 T-cell determinations for each patient.

As co-variables we considered tertiles of weight-adjusted ddI dose, gender, age, body weight, mode of HIV-transmission, CD4 cell count and CD4 percentage of total lymphocytes, treatment status at baseline (naive versus pre-treated), cumulative number of previous antiretroviral drugs, number of drugs in the new regimen which patients had never taken before, and co-infection with hepatitis C virus (HCV) [11,12]. In the univariable model we analyzed both baseline and follow-up HIV-RNA measured by the Amplicor HIV Monitor test, version 1.5 (Roche Diagnostics, Switzerland). The latter was defined as the lowest value measured between 3 and 9 months after starting TDF. Values were entered $\log_{10}$ transformed and divided in two groups: $< 50$ versus $> 50$ copies/ml. In the multivariable model the only viral parameter analyzed was baseline RNA $< 50$ versus $> 50$ copies/ml.

Chi-squared or Wilcoxon rank sum tests were used to determine significant differences between treatment groups. Analyses were performed using STATA 8.2 (StataCorp., College Station, Texas, USA).

## Results

### Baseline characteristics of patients

From the SHCS database we identified 614 patients meeting all inclusion and exclusion criteria: 221 patients

**Table 1. Baseline characteristics of included patients.**

| | | | TDF with ddI | | | |
|---|---|---|---|---|---|---|
| | All patients | TDF-without-ddI | ddI-low | ddI-intermediate | ddI-high | *P*-value |
| ddI-dose (mg/kg) | | | | | | |
| Median | | 0 | 2.9 | 3.7 | 5.0 | |
| Total range | | (0–0) | (1.9–3.3) | (3.3–4.1) | (4.1–11.4) | |
| No of patients | 614 | 393 (64%) | 73 (12%) | 70 (11%) | 78 (13%) | |
| Gender | | | | | | |
| Male | 448 (73%) | 286 (73%) | 64 (88%) | 58 (83%) | 40 (51%) | < 0.001 |
| Female | 166 (27%) | 107 (27%) | 9 (12%) | 12 (17%) | 38 (49%) | |
| Age (years) | 43 (38–49) | 43 (38–49) | 43 (38–49) | 42 (37–47) | 43 (38–49) | 0.61 |
| Transmission | | | | | | 0.003 |
| MSM | 266 (44%) | 169 (43%) | 38 (52%) | 30 (43%) | 29 (37%) | |
| IVDU | 126 (21%) | 86 (22%) | 4 (5.5%) | 22 (31%) | 14 (18%) | |
| Heterosexual | 221 (34%) | 133 (34%) | 27 (37%) | 17 (24%) | 34 (44%) | |
| Other | 11 (1.8%) | 5 (1.3%) | 4 (5.5%) | 1 (1.4%) | 1 (1.3%) | |
| Body weight (kg) | 68 (61–77) | 68 (61–77) | 84 (76–89) | 68 (64–71) | 59 (52–66) | < 0.001 |
| CD4 cell count (cells/μl) | 317 (204–484) | 330 (205–508) | 333 (234–477) | 304 (185–514) | 282 (184–419) | 0.27 |
| CD4 percentage (%) | 19 (13–26) | 19 (14–27) | 18 (13–25) | 21 (15–25) | 18 (13–25) | 0.46 |
| HIV RNA | | | | | | |
| $\log_{10}$ copies/ml | 2.2 (1.7–4.3) | 1.8 (1.7–4.2) | 2.8 (1.7–4.2) | 2.5 (1.7–4.2) | 2.7 (1.7–4.8) | 0.086 |
| < 50 copies/ml | 263 (43%) | 188 (48%) | 23 (32%) | 25 (36%) | 27 (35%) | 0.01 |
| HCV co-infection | 139 (23%) | 90 (23%) | 6 (8.2%) | 24 (34%) | 19 (24%) | 0.013 |
| ART naive | 24 (3.9%) | 19 (4.8%) | 0 (0.0%) | 2 (2.9%) | 3 (3.9%) | 0.24 |
| No of ARVs | 6.3 (0–14)[a] | 6.2 (0–14)[a] | 6.7 (3–14)[a] | 6.2 (0–11)[a] | 6.8 (0–13)[a] | 0.28 |

Numbers indicate the median value and the interquartile range or the absolute number of patients and their percentage in the different treatment groups, respectively.
[a]this data is given as mean and total range. ddI, didanosine; TDF, tenofovir disoproxil fumarate; ddI-low, ddI low dose; ddI-intermediate, ddI intermediate dose; ddI-high, ddI high dose, (the low, intermediate and high doses are specified in the text); MSM, men having sex with men; IVDU, intravenous drug users; HCV, hepatitis C virus; No of ARVs, number of previously used antiretroviral drugs;. *P*-values were calculated using chi-squared or Kruskal–Wallis tests and represent a global test for differences between the four treatment groups: TDF-without-ddI, ddI-low, ddI-intermediate and ddI-high.

were treated with ART containing TDF and ddI and 393 patients were treated with an ART regimen containing TDF but no ddI (Table 1). Demographic and disease-related parameters are depicted in Table 1. There were no significant differences at baseline between patient groups treated with TDF+ddI versus TDF-without-ddI concerning age, gender, mode of HIV-transmission, HCV co-infection and CD4 T-cell count or percentage. Median baseline HIV-RNA was significantly lower in the TDF-without-ddI group versus TDF+ddI ($\log_{10}$copies/ml, 1.82 versus 2.69; $P = 0.02$). Consequently, the proportion of patients with a baseline HIV-RNA < 50 copies/ml was higher in the group TDF-without-ddI versus TDF+ddI (48 versus 34%; $P = 0.001$). This difference most probably reflects why ART was changed. In the TDF-without-ddI group, TDF was more frequently used in a successful ART regimen to avoid side-effects of other nucleoside reverse transcriptase inhibitors (NRTIs) or to simplify treatment whereas the combination of TDF and ddI was more often used in a situation of virologic failure as part of a salvage regimen.

After subdividing the patients of the TDF+ddI group according to their weight adjusted ddI dose, significant baseline differences included body weight, gender, mode of HIV-transmission and HCV co-infection (Table 1). The median weight was 84 kg in ddI-low, 68 kg in ddI-intermediate and 59 kg in ddI-high compared with 68 kg in TDF-without-ddI. Due to their lower median body weight, women were over-represented in the group of ddI-high. As a consequence, modes of HIV-transmission were not evenly distributed over the treatment groups: men having sex with men (MSM) were over-represented in ddI-low and heterosexual transmission was more frequent in ddI-high. HCV co-infection was more prevalent in ddI-high and ddI-intermediate since intravenous drug users (IVDU) are more frequently malnourished. Baseline HIV-RNA and the proportion of patients with a baseline HIV-RNA < 50 copies/ml were comparable between the different ddI-dose groups but different from the TDF-without-ddI group of patients.

## Virologic response during follow-up in different treatment groups

Median HIV-RNA at follow-up was comparable and below 50 copies/ml in patients treated with TDF+ddI and TDF-without-ddI ($P = 0.38$). However, a higher proportion of patients reached a HIV-RNA < 50 copies/ml in the TDF-without-ddI than in the TDF+ddI group but this difference did not reach statistical significance (86 versus 81%; $P = 0.08$). The virologic response during follow-up was similar in all subgroups of TDF+ddI, irrespective of the ddI dose with 82, 80 and 79% of patients achieving < 50 copies/ml in ddI-low, ddI-intermediate and ddI-high, respectively.

**Table 2. Predictors of CD4 slopes in univariable and multivariable regression analyses.**

| | | Univariable model | | | Multivariable model | | |
|---|---|---|---|---|---|---|---|
| | Independent variable | Coeff.[a,b] | 95% CI | *P*-value | Coeff. | 95% CI | *P*-value |
| Overall CD4 slope | cells/μl per year | 60 | 48 to 72 | | [c] | | |
| ddI-dose (mg/kg) | TDF-without-ddI (0) | 67 | 52 to 83 | | | | |
| | ddI-low (1.9–3.3) | 6 | −33 to 45 | 0.77 | −16 | −57 to 25 | 0.44 |
| | ddI-intermediate (3.3–4.1) | −9 | −50 to 31 | 0.65 | −9 | −47 to 30 | 0.66 |
| | ddI-high (4.1–11.4) | −51 | −87 to −15 | 0.006 | −47 | −82 to −12 | 0.009 |
| Gender | Male | 60 | 46 to 75 | | | | |
| | Female | −1 | −28 to 27 | 0.97 | 18 | −17 to 53 | 0.32 |
| Age | per 10 year increase[e] | −20 | −34 to −7 | 0.004 | −24 | −37 to −10 | 0.001 |
| Body weight | per 10 kg increase[e] | 12 | 2 to 21 | 0.018 | 1.4 | 0.30 to 3 | 0.013 |
| HIV-transmission | All others | 57 | 41 to 74 | | | | |
| | MSM | 7 | −18 to 31 | 0.60 | 20 | −11 to 50 | 0.22 |
| | All others | 64 | 51 to 78 | | | | |
| | IVDU | −21 | −52 to 9 | 0.17 | −20 | −70 to 29 | 0.42 |
| HIV RNA– at baseline | per $\log_{10}$ reduction[e] | −28 | −21 to −34 | < 0.001 | | | |
| | > 50 copies/ml | 88 | 73 to 103 | | | | |
| | < 50 copies/ml | −72 | −96 to −47 | < 0.001 | −47 | −76 to −18 | 0.001 |
| HIV RNA– during follow-up[d] | per $\log_{10}$ reduction[e] | 15 | 3 to 26 | 0.013 | | | |
| | > 50 copies/ml | 20 | −10 to 50 | | | | |
| | < 50 copies/ml | 48 | 15 to 81 | 0.004 | | | |
| CD4 cells/μl (BL) | per 100 cells increase[e] | −17 | −22 to −13 | < 0.001 | −19 | −25 to −12 | < 0.001 |
| CD4/total Lc (BL) | per 1% increase[e] | −3 | −4 to −2 | < 0.001 | 1.0 | −1.0 to 2.7 | 0.28 |
| ART experience | experienced | 57 | 44 to 69 | | | | |
| | naïve | 97 | 33 to 161 | 0.003 | 45 | −23 to 112 | 0.20 |
| Number of previous ARVs | < 4 ARVs | 87 | 58 to 117 | | | | |
| | 4–5 ARVs | −7 | −46 to 32 | 0.74 | 32 | −9 to 73 | 0.12 |
| | 6–8 ARVs | −32 | −68 to 3 | 0.08 | 10 | −28 to 49 | 0.60 |
| | > 9 ARVs | −56 | −94 to −19 | 0.003 | −29 | −71 to 13 | 0.18 |
| Number of new ARVs | One new drug | 35 | 16 to 53 | | | | |
| | Two new drugs | 38 | 8 to 68 | 0.012 | 10 | −20 to 39 | 0.53 |
| | > two new drugs | 53 | 24 to 81 | < 0.001 | −0.2 | −34 to 34 | 0.99 |
| HCV co-infection | HCV negative | 62 | 48 to 76 | | | | |
| | HCV-Ab positive | 25 | −24 to 74 | 0.31 | 37 | −21 to 94 | 0.21 |
| | HCV-RNA positive | −22 | −56 to 11 | 0.19 | −8.4 | −56 to 39 | 0.73 |

[a]Constant term or fixed comparator from individual univariable models.
[b]Effect modifier by independent variable in relation to the constant term or comparator.
[c]Constant term for the multivariable model: 125 CD4 T cells/μl per year (10 to 238; $P = 0.032$). This is not comparable to the overall CD4 slope from the univariable model but rather represents an adjustment term to be applied after all variable-specific coefficients for an individual patient have been summed up.
[d]Not a baseline parameter, therefore not included in the multivariable analysis.
[e]All these parameters were analysed continuously. For the indicated change of the respective parameter from baseline or during follow-up (i.e. 10 years in age, 10 kg in body weight, $1 \times \log_{10}$ in HIV-RNA, 100 CD4 cells/μl or 1% CD4 cells/total lymphocytes) the subsequent CD4 slope difference is shown for the univariable and multivariable models. CI, confidence interval; Coeff., coefficient; ddI, didanosine; TDF, tenofovir disoproxil fumarate; ddI-low, ddI low dose; ddI-intermediate, ddI intermediate dose; ddI-high, ddI high dose; MSM, men having sex with men; IVDU, intravenous drug users; BL, baseline; Lc, lymphocytes; ARVs, antiretroviral drugs; HCV, Hepatitis C virus; Ab, antibody;

## Univariable regression analyses of baseline predictors of immune recovery during treatment

For the entire study population ($n = 614$) with a cumulative follow-up of 667.5 patient-years the average CD4 T-cell slope represented a gain of 60 CD4 T cells/μl per year [95% confidence interval (CI), 48–72]. In the univariable analyses gender, mode of HIV-transmission and HCV co-infection were not associated with the CD4 T-cell slope. In contrast, low HIV-RNA and high CD4 cell counts at baseline as well as increasing age, lower body weight and increased previous ART experience were significant predictors of a reduced CD4 slope (Table 2).

More importantly, poor immune recovery was clearly associated with a higher ddI dose (Fig. 1 and Table 2). Patients treated with TDF+ddI at a daily ddI dose > 4.1 mg/kg body weight gained 51 CD4 T cells/μl less per year (95% CI, −87 to −15; $P = 0.006$) compared with TDF-without-ddI. However, CD4 T-cell slopes were comparable with the TDF-without-ddI group if patients received a dose of ddI of < 4.1 mg/kg per day [CD4 T-cell slope difference, −1.5 CD4 T cells/μl and year (95% CI, −31 to 28; $P = 0.92$)].

The level of HIV-RNA reached after changing ART was the only parameter identified during follow-up which correlated significantly with the CD4 T-cell slope: per $\log_{10}$ reduction of HIV-RNA, 15 CD4 T cells were gained per year (95% CI, 3 to 16; $P = 0.013$). If a HIV-RNA of < 50 copies/ml was reached an additional CD4 gain of 48 CD4 T cells/year (95% CI, 15 to 81;

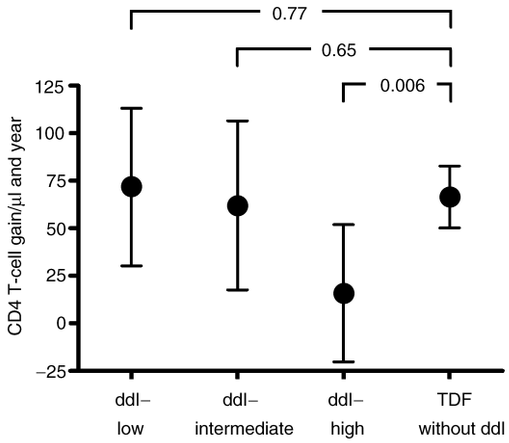


**Fig. 1. Univariable analysis of CD4 slopes of different ddI-treatment groups.** Symbols indicate the median gain in CD4 T cells/μl and year, error bars indicate the 95% confidence interval. The level of significance is indicated. ddI, didanosine; TDF, tenofovir disoproxil fumarate; ddI-low, ddI low dose; ddI-intermediate, ddI intermediate dose; ddI-high, ddI high dose, (the low, intermediate and high doses are specified in the text).

$P = 0.004$) was achieved in comparison with the patients not reaching this threshold.

### Multivariable regression analysis of baseline predictors of immune recovery during treatment

Factors which were significantly associated with the CD4 T-cell slope after start of TDF treatment in the multivariable regression analysis included age, body weight, CD4 cell count at baseline and an undetectable HIV-RNA at baseline (Table 2). As expected, increasing age was a negative predictor for immune recovery during ART. This has repetitively been demonstrated in large HIV-cohort studies [12–14]. Surprisingly however, lower body weight seemed to have an independent but small negative impact on the CD4 T-cell slope, which was not driven by ddI-high in patients with low body weight. Potentially, this effect was caused by a residual ddI dose-dependent effect within ddI dose groups, since patients received absolute ddI doses of 200, 250 or 400 mg.

A high absolute CD4 cell count and an undetectable HIV-RNA at baseline diminished the potential for subsequent immune recovery. Thus, these parameters were negatively associated with the CD4 T-cell slope. In comparison with the univariable model, the number of new drugs added to the new regimen and the previous treatment experience including treatment naivety were no longer significant predictors of the CD4 T-cell slope in the multivariable analysis. We did not identify any 'new' parameter of significance in the multivariable analysis compared with the univariable model.

Treatment with a high dose of ddI (> 4.1 mg/kg) was a strong predictor for a reduced CD4 T-cell slope. In the multivariable model, the association with poor immune recovery was highly significant with a CD4 T-cell slope difference of −47 CD4 T cells/μl per year (95% CI, −82 to −12; $P = 0.009$), confirming previous findings of Negredo and others [5–7]. Importantly, receiving a weight-adjusted dose of ddI of less than 4.1 mg/kg per day together with TDF resulted in an equivalent CD4 T-cell slope compared to TDF-without-ddI [ddI-intermediate, −9 CD4 T cells/μl per year (95% CI, 47 to 30; $P = 0.65$); ddI-low, −16 CD4 T cells/μl per year (95% CI, −57 to 25; $P = 0.44$); both combined: −13 CD4 T cells/μl per year (95% CI, −42 to 17; $P = 0.40$)].

To analyze whether the findings within the whole patient population were similar in more defined subgroups of patients, we performed additional multivariable regression analyses in patients maintaining HIV-RNA < 50 copies/ml throughout the whole observation period and in patients of the upper age tertile (> 46 years). These analyses confirmed the data of the whole population: patients treated with TDF and ddI-high had poorer immune recovery than patients treated with TDF-without-ddI. Treatment with ddI-intermediate and ddI-low resulted in a similar degree of immune recovery as treatment with TDF-without-ddI that was independent of HIV-RNA strata and across all age groups (data not shown). Since these subgroup analyses had a limited power, a more subtle influence of different treatments on the CD4 T-cell slope cannot be excluded.

## Discussion

We have examined the immunologic performance of ART regimens containing TDF with or without concomitant use of ddI based on the data of the SHCS. The main findings are that ddI used at a daily dose of > 4.1 mg/kg was significantly associated with poor immune recovery, whereas ddI used at a daily dose of < 4.1 mg/kg had no such negative impact when combined with TDF. Thus, at an adjusted dose of ddI, the combination of TDF and ddI appears to be immunologically safe. These data from a large cohort of very diverse patients confirm previous findings of poor immune recovery in patients concomitantly treated with TDF and ddI [5–8,15]. However, they suggest in addition that there is a ddI dose threshold for this negative effect.

According to the current ddI dose recommendation for patients also treated with TDF [3,4], the ddI dose was too high for all patients of the initial Negredo study [5], whereas in the present study, 64% of patients were treated with the recommended dose, 7% received a lower and 29% a higher than recommended ddI dose. Therefore, our cohort is more likely to represent the currently used ddI dose spectrum. In addition, we decided to use

weight-adjusted ddI doses for our analysis, since we hypothesized, that the influence of ddI exposure on CD4 T cells is likely to be continuous. However, when we tried to correlate continuous ddI-doses with subsequent CD4 T-cell slopes in patients treated with TDF and ddI, we did not find a significant linear correlation. Only after subdividing the patients into weight-adjusted ddI-dose tertiles, we clearly demonstrated poor immune recovery in ddI-high but not in ddI-low or ddI-intermediate. To further support the evidence of a ddI dose threshold we performed additional univariable and multivariable regression analyses using five different ddI dose groups (0–2, 2–3, 3–4, 4–5 and 5–11.4 mg/kg ddI per day). Again, patients in the highest ddI dose group (5–11.4 mg/kg) experienced a significantly poorer immune recovery of −62 CD4 T cells/μl per year (95% CI, −108 to −16; $P = 0.009$) than patients treated without ddI. For patients treated with 4–5 mg/kg ddI we identified a trend towards poorer immune recovery [−37 CD4 T cells/μl per year (95% CI, −81 to 7; $P = 0.099$)], whereas patients treated with less than 4 mg/kg ddI per day exhibited similar immune recovery compared to patients treated without ddI. For a follow-up period of 56 weeks the finding of a ddI dose threshold between 4 and 5 mg/kg for having a negative impact on CD4 T-cell recovery seems to be robust. Further support for a dose threshold is provided by a recent retrospective study of 95 patients, where the use of TDF and ddI at a dose $> 5.5$ mg/kg was associated with a significant loss of CD4 T cells [7].

Clearly, the breakpoint of 4.1 mg ddI per kg body weight identified in our study is arbitrary and mainly dependent on the body weight distribution of our patients and the absolute ddI doses which were used in our cohort. Therefore, individual patients may still experience poor immune recovery with ART containing TDF and ddI despite ddI dose adjustment to $< 4.1$ mg/kg. Particularly elderly patients and those with mild renal impairment might be at increased risk for such an effect. Moreover, prolonged concomitant use of TDF and ddI over more than 2 years might represent an additional risk factor for poor immune recovery as suggested by Lacombe *et al.* [7]. Nevertheless, the breakpoint of 4.1 mg/kg estimated from our data comes close to the current dose recommendations for ddI if used together with TDF; that is, to use a ddI dose of 250 mg in patients $> 60$ kg, which is equivalent to 4.17 mg/kg or less. In patients weighing less than 60 kg the ddI dose should be further reduced.

Importantly, the virologic response was equivalent across all ddI-dose ranges suggesting comparable efficacy. Within the limits of our study there seemed to be no virologic advantage to use a higher than currently recommended dose of ddI. Therefore, the dose of ddI should be adjusted to $< 4.1$ mg/kg body weight if used together with TDF. This is also supported by a recent analysis of Tung *et al.* who found equivalent virologic efficacy but fewer adverse events in patients treated with dose-adjusted ddI [15].

Our study has some inherent limitations due to the retrospective design and due to the great variability of included patients. On the other hand, we argue that these results are likely to be more representative of daily clinical practice than a prospective clinical trial with stringent inclusion and exclusion criteria. We would like to emphasize that the difference in baseline HIV-RNA between treatment groups may lead to a bias in some of our results. At baseline, HIV-RNA was significantly lower in patients treated with TDF-without-ddI compared to the different TDF+ddI dose groups, but at follow-up there was no significant difference anymore. Consequently, a higher proportion of patients in the TDF+ddI groups experienced a substantial decrease in HIV-RNA, which is associated with a higher potential to increase their CD4 cell counts during ART. Therefore, we may underestimate the negative effect of treatment with TDF and ddI on the CD4 T-cell slope in ddI-low and ddI-intermediate.

In the multivariable model we have addressed this potential bias by including a variety of parameters that might independently influence immune recovery such as age, body weight, mode of HIV-transmission, HIV-RNA and CD4 cell count at baseline, previous ART experience, number of new drugs added to the regimen and possibly HCV co-infection [11,12,16]. Nevertheless, after maximal correction of the multivariable model according to these co-variables, only ddI-high was associated with poor immune recovery ($P = 0.009$), whereas ddI-intermediate and ddI-low were not. In addition, the CD4 T-cell slope coefficients for the different ddI dose groups were similar in the univariable and in the multivariable model suggesting reasonable robustness for our model (Table 2). Finally, the subgroup analysis of patients without detectable HIV-RNA throughout the study did not reveal a negative impact of ddI-intermediate or ddI-low in comparison with TDF-without-ddI (not shown). However, the limited number of patients and duration of follow-up in this subgroup analysis might not detect minor negative effects on immune recovery.

Our data add to the published literature suggesting that poor immune recovery associated with concomitant use of TDF and ddI is mainly caused by a dose-dependent ddI toxicity [5,7,8,15]. Increased plasma ddI levels correlated best with poor immune recovery in a recent study by Barrios *et al.* [6]. This is further supported by reports of an increased risk of pancreatitis in patients treated with TDF and ddI [17,18], since this risk is known to be ddI-dose dependent [19]. Intracellular inhibition of ddI-phosphorolysis by TDF anabolytes has been proposed as a molecular mechanism for this pharmacologic interaction [20].

Remarkably, the initial ddI dose-finding studies and subsequent randomized clinical trials did not report a negative impact of high ddI doses on T-lymphocyte counts, although patients were treated with up to 9.6 mg ddI/kg for more than 1 year [21–23]. To corroborate these findings for patients treated within the SHCS during the same observation period, we identified 69 patients, who started an ART regimen containing a new NRTI (not TDF) and ddI at a median dose of 5.6 mg/kg. This is significantly higher ($P < 0.001$) than the median ddI dose of patients treated with TDF and ddI within the highest ddI dose tertile (ddI-high, 5.0 mg/kg). The average CD4 T-cell increase of these 69 patients was 82 cells/μl per year, which is comparable with the patients treated with TDF-without-ddI. Moreover, there was no indication of a ddI dose dependence of CD4 T-cell recovery without concomitant use of TDF. Therefore, as proposed by Kakuda *et al.*, the toxic effects on lymphocytes caused by TDF and ddI-high seem to be cumulative and may be explained by an accumulation of endogenous purines impairing T-cell maturation and differentiation [24]. This postulated mechanism is reminiscent of the purinogenic immunodeficencies which lead to T-cell depletion [25]. Clearly, further research is needed to clarify the mechanism of this unexpected immunologic side-effect of treatment with TDF and ddI.

Other unresolved problems with ART containing TDF and ddI concern virologic efficacy: two recent clinical trials in treatment-naive patients have been halted prematurely due to poor efficacy of ART combining TDF, ddI and efavirenz [26,27]. Similarly, Leon *et al.* reported early virologic failure in 50% of patients started on similar regimens in a retrospective cohort analysis [28]. A low barrier to resistance development of these ART regimens has been proposed as a potential mechanism for the high rate of virologic failure. Thus far, no reports have been published suggesting a poor virologic efficacy of regimen combining TDF, ddI and a boosted protease inhibitor.

In summary, if used in combination with TDF the dose of ddI should be adjusted to less than 4.1 mg/kg per day. Such a combination appears to be immunologically safe leading to a treatment response comparable with regimens containing TDF and other NRTIs. Nevertheless, we would caution against the use of novel combinations of individual antiretroviral drugs in daily clinical practice prior to the availability of results from large clinical trials.

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## Appendix

The members of the Swiss HIV Cohort Study are M. Battegay, E. Bernasconi, J. Böni, H. Bucher, Ph. Bürgisser, S. Cattacin, M. Cavassini, R. Dubs, M. Egger, L. Elzi, P. Erb, M. Fischer, M. Flepp, A. Fontana, P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, CH-1011- Lausanne), H. Furrer (Chairman of the Clinical and Laboratory Committee), M. Gorgievski, H. Günthard, B. Hirschel, I. Hösli, Ch. Kahlert, L. Kaiser, U. Karrer, O. Keiser, C. Kind, Th. Klimkait, B. Ledergerber, B. Martinez, N. Müller, D. Nadal, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, J.-C. Piffaretti, M. Rickenbach (Head of Data Center), C. Rudin (Chairman of the Mother & Child Substudy), P. Schmid, J. Schüpbach, R. Speck, P. Taffé, P. Tarr, A. Telenti, A. Trkola, P. Vernazza (Chairman of the Scientific Board), R. Weber, S. Yerly.