Predictors of virological failure in HIV-1 infected patients switching to dolutegravir maintenance monotherapy.

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ABSTRACT

Introduction

The Dolutegravir Maintenance Monotherapy for HIV-1 (DOMONO; NCT02401828) study showed that maintenance monotherapy with dolutegravir (DTG) is associated with virological failure (VF) and leads to DTG resistance, and as a result should not be used. However, data on clinical and virological factors associated with VF during DTG monotherapy are lacking. We identified factors associated with VF during DTG monotherapy.

Methods

A **r**andomized trial was carried out in which patients on combination antiretroviral therapy (cART) with an HIV-1 RNA zenith <100.000 c/mL, a CD4 T-lymphocyte nadir ≥200 cells/mm^3, who had never experienced VF, switched to DTG monotherapy. Clinical and virological factors were compared between patients with and without VF, using univariable analyses.

Results

Eight of the 95 patients developed VF during DTG monotherapy. A total of 78 participants had reached week 48 when the study was discontinued. The median (IQR) CD4 T-lymphocyte nadir was lower in patients with VF than in patients without VF (260 (223-320) cells/mm^3 versus 380 (290-520) cells/mm^3, respectively, p=0.011). Patients with VF had a longer time between HIV-diagnosis and cART initiation than those without VF (49 (27-64) versus 15 (1-38) months, respectively, p=0.015). The median total peripheral blood mononuclear cells (PBMC) HIV-DNA copy number (min, max) PBMCs was higher in patients with VF than in those without VF (417 (85-4151) versus 147 (16-4132) copies/10^6 PBMCs, respectively, p=0.022).

Conclusions

A lower CD4 T-lymphocyte nadir, a longer time between HIV diagnosis and cART initiation, and a higher HIV-DNA copy number at the time of DTG monotherapy initiation were associated with VF. While there clearly is no future role for DTG monotherapy, ongoing and future studies on the efficacy of maintenance dual therapy (e.g. DTG with lamivudine) may have to take these variables into account in their study design and analysis.

INTRODUCTION

Dolutegravir (DTG) based combination antiretroviral therapy (cART) is one of the preferred treatment options in current guidelines for HIV-1 treatment. Given the high genetic barrier to resistance of DTG maintenance monotherapy based on previously determined *in vitro* data,[1–5] we studied its efficacy in the Dolutegravir Monotherapy for HIV (DOMONO, NCT02401828) study.[6] In the DOMONO study, 95 virologically suppressed patients on cART, selected on strict criteria regarding CD4 T-lymphocyte nadir and HIV-RNA zenith, started DTG monotherapy. The study was discontinued prematurely, because virological failure (VF) was observed in eight patients, of whom three had integrase inhibitor resistance associated mutations.[6–8] Previous studies on protease inhibitor (PI) maintenance monotherapy identified time on cART, drug adherence during monotherapy, the presence of very low-level viremia (plasma HIV-RNA less than 50 c/mL) at baseline, and CD4 T-lymphocyte nadir as predictors for failure.[9,10] Additionally, the peripheral blood mononuclear cell (PBMC) HIV-DNA copy number was associated with the risk of VF in the MONOI study and PROTEA study.[9,11] Predictors of VF during integrase inhibitor monotherapy have not been described. Here we determined which clinical and virological factors are associated with VF during DTG monotherapy.

METHODS

The DOMONO study was a randomized clinical non-inferiority trial. Participants provided written informed consent, and the study was approved by the ethics committee (METC Erasmus MC, MEC2015-043) and performed in accordance with the Helsinki Declaration. Details can be found elsewhere, but in brief, 95 patients, who were virologically supressed on cART, had never failed any antiretroviral regimen, had a CD4 T-lymphocyte nadir of at least 200 cells/mm^3 and an HIV-RNA zenith lower than 100.000 c/mL, consented to switch from cART to DTG monotherapy.[6] The primary outcome of this study was virological suppression at week 24 during DTG monotherapy, and we defined VF as a confirmed plasma HIV-RNA of more than 200 c/mL. Clinical and virological factors were compared between patients with and without VF using unpaired T-tests, Mann Whitney U Tests, and Fisher's exact tests, when applicable. As a consequence of the relatively low number of patients with VF at the time when the study was discontinued, a multivariable analysis could not be performed. Factors included were as follows: age, sex, the pre-cART HIV-RNA zenith and CD4 T-lymphocyte nadir, the CD4 T-lymphocyte count at the start of DTG monotherapy, and the time between HIV diagnosis and cART initiation. Other evaluated factors were the type of cART-regimen before switch to DTG monotherapy (non-nucleoside reverse transcriptase inhibitor versus PI- versus integrase strand transfer inhibitor-containing cART), the time on cART, whether

the patient had a detectable viral load at the start of DTG monotherapy (defined as a plasma HIV viral load of more than 20 c/mL or a detectable HIV-RNA but lower than 20 c/mL), DTG plasma-concentration, and the total HIV-DNA copy number in PBMCs at the start of DTG monotherapy. Total HIV-DNA quantification was performed by droplet digital PCR (ddPCR), as described elsewhere, and could be done on 77 patients (eight patients with VF and 69 without VF) from whom PBMC had been successfully harvested.[12–14]

RESULTS

Seventy-eight of the 95 participants had reached the week 48 endpoint when the study was discontinued prematurely in accordance with one of the predefined stopping rules. At the time of study discontinuation, VF had been observed in eight patients. Median (IQR) follow up duration was 59 (48-71) weeks and for 17 patients, including five with VF, the follow-up was shorter than 48 weeks. The characteristics of the patients with and without VF are described in Table 1.

	no VF during DTG monotherapy (N=87)	VF during DTG monotherapy (N=8)	P-value (test)
Age, years, mean (SD)	47 (11.0)	47 (11.2)	0.891 (UTT)
Male sex, N (%)	80 (92)	8 (100)	1.00 (FET)
HIV RNA zenith, copies/mL	37000 (12950,65625)	27350 (17750,64325)	0.973 (MWU)
Viremia at start DTG*, N (%)	10 (11.5)	2 (25.0)	0.266 (FET)
HIV DNA, copies/10^6 PBMCs	147 (69,338)	417 (181,837)	0.022 (MWU)
Log10 HIV DNA/10^6 PBMCs, mean (SD)	2.16 (0.53)	2.57 (0.40)	0.037 (UTT)
CD4 T-lymphocyte nadir, cells/mm^3	380 (290,520)	260 (223,320)	0.011 (MWU)
CD4 T-lymphocyte count at start DTG, cells/mm^3	650 (540,825)	830 (573,1030)	0.153 (MWU)
CD4:CD8-ratio at start DTG	1.05 (0.74,1.50)	1.41 (0.74,2.00)	0.507 (MWU)
C-reactive protein at start DTG, mg/L	1.20 (0.40,2.70)	1.45 (0.73,3.08)	0.673 (MWU)
DTG plasma-concentration, mg/L	1.65 (1.23,3.75)	1.70 (1.05,2.40)	0.308 (MWU)
DTG plasma-concentration, % deviation from population average	12.9 (-43.2,55.2)	10.9 (-27.6,45.5)	0.879 (MWU)
cART before DTG, N(%)			
NNRTI	69 (79.3)	7 (87.5)	0.783 (CST)
PI	4 (4.6)	0 (0)	
INI	14 (16.1)	1 (12.5)	
Time between HIV-diagnosis and start cART, months	15 (1,38)	49 (27,64)	0.015 (MWU)
Time suppressed on cART, months	31 (20,54)	57 (28,94)	0.104 (MWU)

Table 1. Baseline characteristics of patients with and without VF during DTG maintenance monotherapy, including p-values resulting from univariate analysis. Data documented as median (Q1,Q3), unless stated otherwise, DTG=dolutegravir, VF=virological failure, SD=standard deviation, UTT=unpaired T-test, FET=Fisher's Exact test, MWU=Mann Whitney U test, * residual viremia is defined as HIV-RNA detectable but < 20 c/mL or > 20 c/mL, PBMC=peripheral blood mononuclear cells, cART=combination antiretroviral therapy, NNRTI=non-nucleoside reverse transcriptase inhibitor, PI=protease inhibitor, INI=integrase inhibitor, CST=Chi Square test.

Per study protocol, the median HIV-RNA zenith was low and the median CD4 T-lymphocyte nadir was relatively high with a minimum of 200 cells/mm^3. The median (IQR) CD4 T-lymphocyte nadir was significantly lower in the patients with VF at 260 (223-320) cells/mm^3 than in those without VF (380 (290-520) cells/mm^3, p=0.011). The median time between HIV-diagnosis and cART initiation was longer in patients with VF: 49 (27-64) months versus 15 (1-38) months for patients without VF on monotherapy (p=0.015). At the start of DTG monotherapy, no significant differences were observed between patients with and without VF regarding the number of patients with a detectable palsma HIV-RNA, the CD4 T-lymphocyte count, CD4:CD8-ratio or the C-reactive protein (CRP) concentration. In contrast, the median (min-max) total HIV-DNA copy number in PBMCs at the time of DTG monotherapy initiation differed significantly between both groups: 147 (16-4132) versus 417 (85-4151) copies

per 10^6 PBMCs respectively (p=0.022). DTG plasma levels were adequate (i.e. > 0.1 mg/L) in all patients with VF and no difference in median (min-max) DTG plasma-concentrations was observed between the patients with VF and 20 randomly selected patients without VF: 1.70 (0.70-2.90) mg/L versus 1.65 (0.70-4.50) mg/L. See Figure 1 for boxplots of CD4 T-lymphocyte nadir, time between HIV diagnosis and start cART, total HIV-DNA copy number in PBMCs, and DTG plasma-concentrations in patients without and with VF.

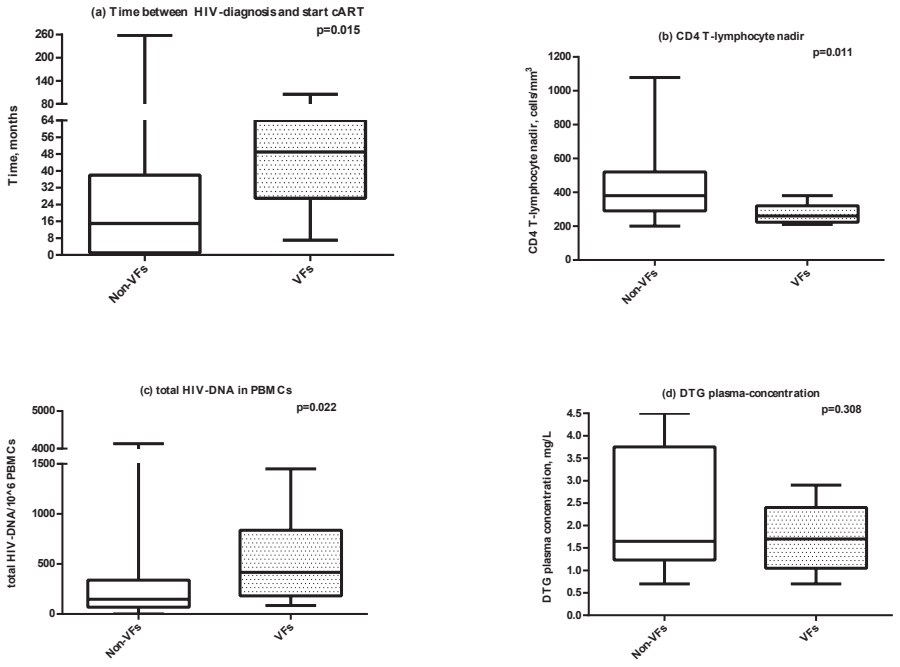

Figure 1. Distributions of time between HIV-diagnosis and start cART (a), CD4 T-lymphocyte nadir (b), total HIV-DNA in PBMCs (c), and DTG plasma-concentration (d) in patients without and with VF during DTG maintenance monotherapy. cART=combination antiRetroviral therapy, PBMC=peripheral blood mononuclear cell, DTG=dolutegravir.

DISCUSSION

In the DOMONO study, we clearly showed that DTG maintenance monotherapy is associated with VF and the development of DTG resistance, and it should not be used as maintenance monotherapy. In the current study we evaluated potential predictors of VF during integrase inhibitor monotherapy. We showed that a higher level of cell-associated total HIV-DNA copy number at start of monotherapy, a lower CD4 T-lymphocyte nadir, and a longer time between HIV-diagnosis and start cART were significantly associated with VF. A lower CD4 T-lympho-

cyte nadir and a higher level of cell-associated HIV-DNA copy number have previously also been described as risk factors for virological failure during PI monotherapy.[9–11] In contrast, none of the following ten factors were associated with VF during DTG monotherapy: gender, age, CD4 T-lymphocyte count, CRP, CD4:CD8-ratio, the type of the cART-regimen, whether the patient had detectable plasma HIV-RNA (all at the time of DTG monotherapy initiation), DTG plasma-concentrations during monotherapy, the duration of viral suppression on cART, and the HIV-RNA zenith before DTG monotherapy initiation. Also, no differences were observed regarding plasma viral load detectability in the 12 months preceding DTG monotherapy initiation: three of 87 patients without VF versus zero of eight patients with VF had HIV-RNA more than 20 c/mL in the 12 months preceding the switch to DTG monotherapy.

Various PI monotherapy studies identified suboptimal adherence as a risk factor for VF.[9,15,16] We were unable to analyze adherence as a predictor of VF, because the inclusion and exclusion criteria of the study led to the selection of a very therapy-adherent study population: no history of VF on any previous cART regimen, a self-reported adherence during DTG monotherapy of more than 95%, and therapeutic DTG plasma-concentrations in all patients with and without VF. DTG plasma-concentrations were adequate in both groups and there was no significant difference in DTG plasma-concentrations between patients with and without VF, which consistent with previous studies which did not identify lower PI plasma-concentrations as a risk factor for VF in patients receiving PI monotherapy.[16,17] It must be noted that drug level measurement was only performed at single time-points, so the possibility of temporary non-adherence between study visits cannot be ruled out.

The limiting factor of this study is the relatively small number of patients who experienced VF in comparison to the previously mentioned PI studies. Even if we had considered an isolated and unconfirmed viral load of more than 50 c/mL as VF (as observed in 14 patients), the number of VF would have been too small to enable a multivariate analysis to be performed. Therefore, we were not able to assess whether CD4 T-lymphocyte nadir, time between HIV-diagnosis and start cART, and cell-associated HIV-DNA copy number are independent risk factors for VF during DTG maintenance monotherapy. Actually, CD4 T-lymphocyte nadir, time between HIV-diagnosis and start cART, and cell-associated HIV-DNA levels could very well be correlated. Indeed, Boulassel et al previously showed the inverse relationship between CD4 T-lymphocyte nadir and cell-associated HIV-DNA levels[18], and longer time between HIV-diagnosis and start cART is associated with higher cell-associated HIV-DNA levels.[19] This implies that the size of the viral reservoir is probably the most important determinant of VF, as the cell-associated total HIV-DNA level is a measure for the size of the viral reservoir in virologically suppressed patients. Reactivation of HIV from latently infected cells is a stochastic process, which occurs on average every five to eight days, and depends on the size of the replication competent viral reservoir.[20] Our observation that a higher HIV-DNA level

is associated with VF is in agreement with stochastic reactivation of pre-existing provirus harboring a single integrase inhibitor mutation. It would have been useful to provide data on the size of the reservoir at the time of VF. Unfortunately, we did not collect PBMCs at the time of VF and therefore, we are not able to provide these data.

In conclusion, a longer time between HIV diagnosis and cART initiation, a lower CD4 T-lymphocyte nadir, and a higher total HIV-DNA copy number increased the risk of VF during DTG monotherapy. While there clearly is no future role for DTG monotherapy, ongoing and future studies on the efficacy of maintenance dual therapy (e.g. DTG with lamivudine) should take these variables into account in their study design and analysis.

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