Dolutegravir as maintenance monotherapy for HIV (DOMONO): a phase 2, randomised non-inferiority trial.

I.E.A. Wijting, C. Rokx, C.A.B. Boucher, J.J.A. van Kampen, S.D. Pas, T.E.M.S. de Vries-Sluijs, C.A.M. Schurink, H.I. Bax, M. Derksen, E.R. Andrinopoulou, M.E. van der Ende, E.C.M. van Gorp, J.L. Nouwen, A. Verbon, W.F.W. Bierman, B.J.A. Rijnders

Lancet HIV. 2017;4:e547-554.

ABSTRACT

Introduction

The high genetic barrier to resistance of dolutegravir (DTG) might allow for its use as maintenance monotherapy in patients with HIV. We investigated whether DTG monotherapy was non-inferior to combination antiretroviral therapy (cART) for maintaining virological suppression in HIV-1 patients successfully treated with cART.

Methods

We did this open-label, phase 2, randomised non-inferiority trial at two medical centres in the Netherlands. Eligible patients (aged ≥ 18 years) were on cART, had been virologically suppressed (plasma HIV-RNA <50 c/mL) for at least 6 months, and had CD4-nadirs of 200 cells/mm^3 or higher, HIV-RNA zeniths of less than 100.000 c/mL, and no history of virological failure (VF). Patients were randomly assigned (1:1) via a web-based block randomization method (variable block sizes of 4 and 6) to switch to dolutegravir monotherapy (50 mg once a day) either immediately or after a delay of 24 weeks of continued cART. Randomisation was stratified by HIV-RNA zenith (<50.000 c/mL or 50.000-99.999 c/mL). Investigators and patients were not masked to group allocation. The primary endpoint was the proportion of patients with plasma HIV-RNA viral loads of less than 200 c/mL at week 24, with a non-inferiority margin of 12%. We did analyses in the on-treatment and intention-to-treat populations. This trial is registered with ClinicalTrials.gov, NCT02401828.

Results

Between March 10, 2015, and Feb 4, 2016, we randomly assigned 51 patients to the immediate switch group and 53 to the delayed switch group. One patient who received immediate monotherapy discontinued dolutegravir at week 12 because of disturbed sleep. At week 24, dolutegravir monotherapy was non-inferior to cART with plasma HIV-RNA loads of 200 c/mL or higher observed in 2% (1/50) during immediate dolutegravir monotherapy and in 0/53 patients in the delayed dolutegravir monotherapy group (difference 2%, exact 95% CI -5%,+12%). Of patients assigned to the delayed switch group, 47 of 53 patients (89%) switched to dolutegravir monotherapy at week 24, and two of them (4%) subsequently discontinued dolutegravir monotherapy because of headache (N=1) and disturbed sleep (N=1). Eight (8%) of the 95 patients who remained on dolutegravir monotherapy had VF; all had therapeutic DTG plasma-concentrations. In three of the eight patients, mutations associated with resistance were detected in the integrase gene. According to a predefined stopping rule, detection of these mutations led to premature study discontinuation.

Conclusions

Dolutegravir monotherapy was non-inferior to cART at 24 weeks. However VF continued to occur thereafter and led to dolutegravir resistance in three patients. Dolutegravir should not be used as maintenance monotherapy.

INTRODUCTION

Combination antiretroviral therapy (cART) regimens containing the second-generation integrase inhibitor dolutegravir (DTG) showed equal or superior virological suppression rates compared with raltegravir, efavirenz, or darunavir containing cART in treatment of HIV-1 infected adult patients.[1–3] This high virological efficacy, the favorable safety profile, and the high genetic resistance barrier of DTG has led to the recommendation of DTG-containing cART as first-line strategy in HIV treatment guidelines.[4,5] Although existing cART regimens are effective, maintenance therapy with one or two drugs might have advantages, including reduced side-effects, pill burden, and costs. Various factors make DTG a suitable candidate for maintenance monotherapy: the development of resistance is rare in integrase inhibitor-naive patients; the risk of drug-drug-interactions is low, the drug has a good tolerability, a once-daily dosing schedule, a small pill size, and a neutral effect on serum lipids.[1,6,7] Previous studies have not shown monotherapy with protease inhibitors to be virologically non-inferior to cART, although virological failure (VF) during protease inhibitor monotherapy has not been associated with an increased incidence of resistance to protease inhibitors.[8,9] However, in one study of virologically suppressed patients fulfilling strict criteria regarding HIV-RNA zenith (<100.000 c/mL) and CD4-nadir (>200 cells/mm^3) protease inhibitor maintenance monotherapy was non-inferior to cART.[10] We found DTG maintenance monotherapy to be promising in a retrospective observational study of five patients, although no control group was used.[11] Therefore, we conducted the randomized DOMONO trial to evaluate whether a switch to DTG monotherapy would be non-inferior to continuation of cART in maintaining virological suppression in HIV-1 infected patients.

METHODS

Study design and participants

We conducted this open-label, phase 2, randomized, non-inferiority trial in two university medical centres in the Netherlands: the Erasmus MC and the University Medical Center Groningen (UMCG). Eligible patients were HIV-1 infected adults, on cART and virologically suppressed (HIV-RNA <50 c/mL) for at least 6 months at the time of screening, with an HIV-RNA zenith of less than 100.000 c/mL and a CD4-nadir of 200 cells/mm^3 or higher. A previous HIV-RNA zenith of 100.000 c/mL or more was allowed if measured during an untreated acute HIV-infection. We excluded patients with a chronic hepatitis B virus (HBV) infection or without anti-HBs antibodies and not willing to undergo HBV vaccination. We also excluded subjects with previous VF on any cART or with any documented HIV-1 resistance with at least low-level resistance according to the Stanford HIV drug resistance database.[12] Patients had to have a self-reported adherence of at least 95%. For a complete list of the inclusion

criteria and exclusion criteria, see Table 1 of the Supplementary Data. The study was approved by the Dutch competent authority and the Institutional Review Board of the Erasmus MC Rotterdam (NL51858.078.15). The study was done in accordance with Good Clinical Practice and the Helsinki Declaration. All participating subjects provided verbal and written informed consent in the language they could read (Dutch or English) before study procedures.

Randomisation and masking

We randomly assigned (1:1) eligible patients, via a web-based block randomization method (variable block sizes of 4 and 6) to switch to DTG monotherapy either immediately or after a delay of 24 weeks of continued cART (control). Randomization was stratified by HIV-RNA zenith (<50.000 c/mL or 50.000-99.999 c/mL). Because patients in the control group also switched to DTG monotherapy after 24 weeks, no randomized control group on cART was available after that timepoint. Therefore, we also collected data from a concurrent control group, which included eligible HIV-1 patients on cART who did not want to switch therapy. These patients remained in standard care for HIV-1, underwent no study procedures, and provided verbal consent for use of their clinical data for research purposes. Investigators and patients were not masked to group allocation.

Study procedures

We prescreened patients by reviewing their files for the inclusion and exclusion criteria. Eligible patients first received information about the study from their physician, and those who were interested in the study were referred to an investigator for a formal screening visit. We did clinical and laboratory assessments, including HIV-RNA, renal, urinary, and hepatic variables at weeks 0, 12, 24, 36, and 48. Additionally, HIV-RNA was also measured at weeks 4, 8, and 18 with the COBAS® ampliprep/COBAS®Taqman® HIV-1 v2 test (Roche diagnostics, Almere, The Netherlands). We defined VF as two consecutive HIV-RNA measurements of 200 c/mL or higher. We contacted all patients with an HIV-RNA of 200 c/mL or higher and retested them immediately to confirm the result. Patients with confirmed VF were taken off DTG monotherapy and restarted cART. We did Sanger sequence analysis of the integrase gene with in-house primers from EDTA-containing plasma that had been collected at the time of VF and before cART initiation, and we measured DTG plasma-concentrationsin these plasma samples. We contacted patients with a viral load above 20 but below 200 copies per mL and instructed them to take DTG with food to increase absorption, we then measured their plasma DTG levels concentrations in stored plasma to check for therapy compliance. After 48 weeks on DTG monotherapy, patients with viral loads less than 50 c/mL could choose to continue DTG monotherapy with plasma HIV-RNA measurements every 12 weeks, or to reinitiate cART. We informed patients that continuation of DTG monotherapy would be off label use and documented their consent again in the patient file. To protect the safety of the study participants, predefined stopping were the detection of resistance associated mutations

(RAMs) in the integrase gene in more than two patients during the study and failure of DTG monotherapy in more than 20 patients at any time during the study.

Outcomes

The primary endpoint of the study was the proportion of patients with plasma HIV-RNA of less than 200 c/mL at 24 weeks in the on-treatment (OT) population. The OT population consisted of all patients initiating DTG monotherapy except for those who discontinued DTG because of an adverse event while virologically suppressed at the time of DTG discontinuation. The intention to treat population (ITT) consisted of all patients who started DTG monotherapy. A temporary increase of the plasma HIV-RNA from less than 50 c/mL to 50-200 c/mL is not infrequent during cART. Furthermore, given the relatively small sample size of a phase 2 study, we expected only one to three patients with VF in each group. As such, the use of a cutoff of 50 c/mL could have led to inappropriate statistical conclusions about non-inferiority; therefore, we used 200 c/mL as cutoff for the primary analysis. Predefined secondary endpoints reported herein were the proportion of patients in the OT-population with plasma HIV-RNA of less than 50 c/mL at week 24, the proportion of patients in the entire population on DTG monotherapy after 48 weeks with plasma HIV-RNA less than 200 c/mL, the proportion of patients in the ITT population with plasma HIV-RNA of less than 200 c/mL at week 24, and the number and type of RAMs in the integrase gene of patients with confirmed HIV-RNA of 200 c/mL or higher at any time-point during DTG monotherapy. Other predefined secondary endpoints included bone, renal, and inflammatory markers and will be reported elsewhere. We registered adverse events according to the Common Terminology Criteria for Adverse Events version 4.0. Because of the study design, it would not have been fair to compare the groups for all adverse events that were not considered drug related (eg. Bronchitis, headache, diarrhea) because patients in the immediate switch group were seen or contacted seven times during the first 24 weeks, whereas patients in the delayed switch group were seen or contacted only twice. Therefore, adverse events would have been more frequent in the immediate switch group.

Statistical analyses

The sample size calculation was based on a non-inferiority design comparing DTG monotherapy with cART. Assuming virological suppression (HIV-RNA < 200 c/mL) in 95% of patients in both groups (Pa=Pb=0.95), a non-inferiority margin of 12%, 80% power and a one-sided confidence interval of 97.5% (α=0.025), a sample size of 104 patients would be needed. For the primary endpoint, we calculated 95% exact confidence intervals for differences in proportions.[13] In a post-hoc analysis, we used Fisher's Exact test to compare virological suppression rates between the entire population on DTG monotherapy at study discontinuation and the concurrent control population. We did all analyses with the statistical software package R (version 3.3.1). This trial is registered with ClinicalTrials.gov (NCT02401828).

RESULTS

Between March 10, 2015 and February 4, 2016, we randomly assigned 104 patients to receive immediate (N=51) or delayed (N=53) DTG monotherapy (Figure 1). Baseline characteristics were similar between the groups (Table 1).

For our concurrent control group, we recruited 152 consecutive patients who had chosen not to participate in the DOMONO study. Patients in this group had to have been available for follow-up for at least one year and have a viral load measurement available between 44 and 56 weeks after they were initially considered eligible for the DOMONO study. The entire OT-population receiving DTG monotherapy consisted of 95 patients (N=50 in the immediate switch group and N=45 in the delayed switch group, Figure 1). The OT-population assessed for the week 24 primary endpoint consisted of 50 patients in the immediate switch group and 53 patients in the delayed switch group (Figure 1).

	Immediate DTG monotherapy (N=51)	**Delayed DTG monotherapy (N=53)**
Male sex, N(%)	47 (92)	48 (91)
Age, median (Q1,Q3)	46 (37,56)	45 (40,51)
Transmission route, MSM, N(%)	41 (80)	41 (77)
Ethnicity, Caucasian, N(%)	44 (86)	42 (79)
cART regimen before switch, N(%)		
NNRTI + 2 NRTI	41 (80)	43 (81)
PI + 2 NRTI	2 (4)	1 (2)
INI + 2 NRTI	7 (14)	9 (17)
Other	1 (2)	0 (0)
Receiving an STR, N(%)	32 (63)	41 (77)
On TDF before switch, N(%)	44 (86)	45 (85)
Median (Q1,Q3) time on cART, months	35 (24,61)	43 (25,68)
Median (Q1,Q3) time suppressed on cART, months	31 (20,54)	39 (21,60)
Median (Q1,Q3) HIV-RNA zenith, copies per mL	29.300 (14.800-76.900)	44.877 (16.100-63.100)
Median (Q1,Q3) CD4 T-lymphocyte nadir, cells/mm^3	320 (250-490)	380 (285-515)

Table 1. Baseline characteristics of the immediate DTG monotherapy and the delayed DTG monotherapy group. MSM=Men having Sex with Men, NNRTI=Non-Nucleoside Reverse Transcriptase Inhibitor, NRTI=Nucleoside Reverse Transcriptase Inhibitor, PI=Protease Inhibitor, INI=Integrase Inhibitor, STR=Single Tablet Regimen,TDF=Tenofovir Disoproxil Fumarate, cART= combination AntiRetroviral Therapy.

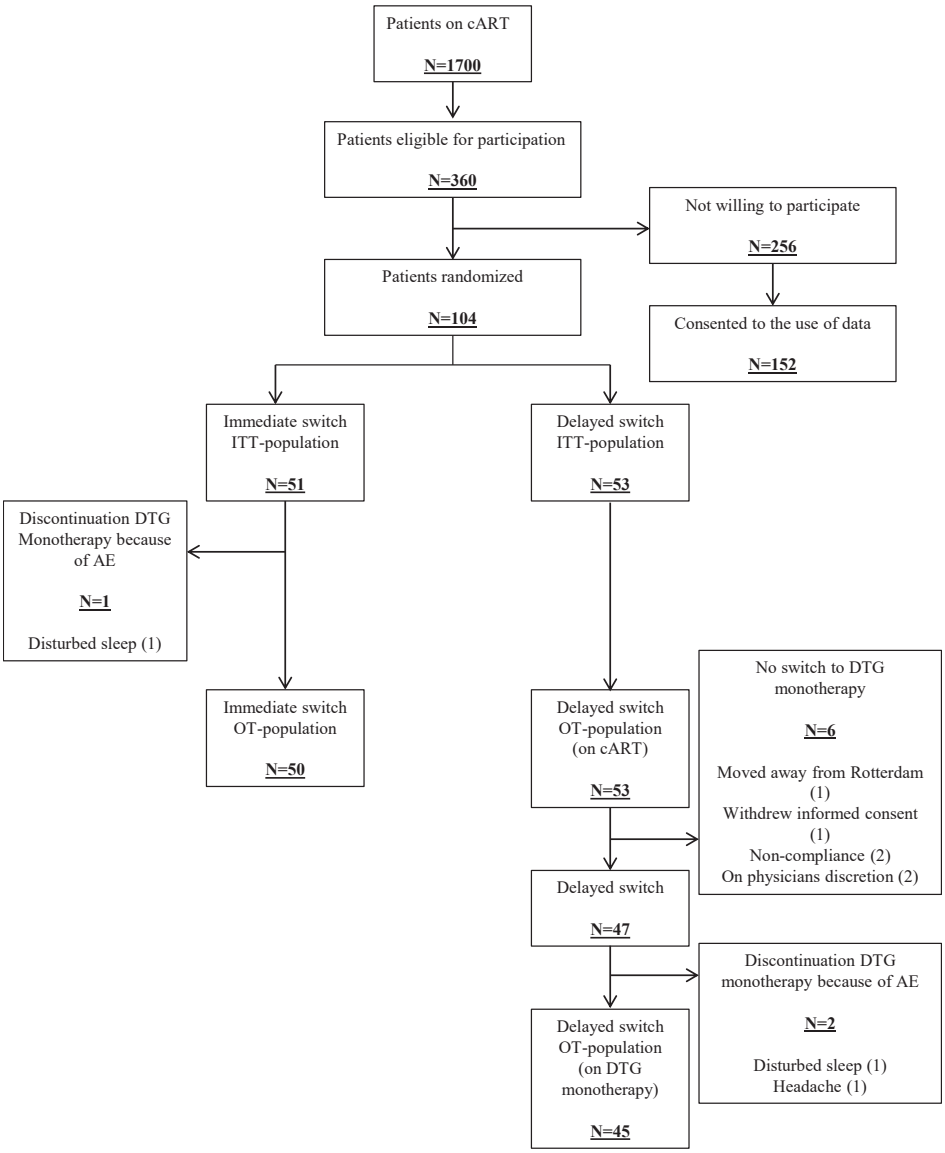


Figure 1. Patient disposition in the study. cART=combination AntiRetroviral Therapy, ITT=intention to treat, OT=on treatment, DTG=dolutegravir, AE=adverse event.

At 24 weeks, the proportions of patients with plasma HIV-RNA of 200 c/mL or higher were 2% (1/50) in the immediate DTG monotherapy group and 0% in the delayed DTG monotherapy group (difference 2%, exact 95% confidence interval -5% to +12%; Figure 2). The plasma HIV-RNA of the single patient in the immediate DTG monotherapy group who had VF was 71.600 c/mL, detected at week 4 of DTG monotherapy. The patient had a self-reported adher-

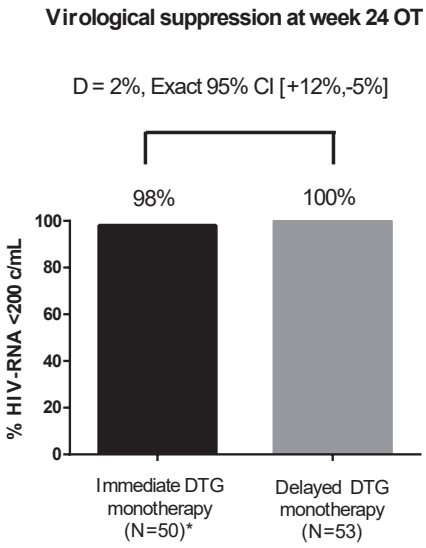


Figure 2. Percentages of virological suppression at week 24: on treatment analysis. * 1/51 patients discontinued DTG monotherapy at week 12 (HIV-RNA < 50 c/mL) because of disturbed sleep.

ence of 100%, an adequate DTG plasma-concentration (1.29 mg/mL, measured 14 hours after intake), and the integrase sequence showed no DTG-RAMs. However, we realize that even a 100% correct pill count and adequate plasma-concentrations at preplanned blood draws cannot exclude temporary incompliance with certainty. The patient reinitiated cART with single-tablet tenofovir disoproxil fumarate, emtricitabine, and rilpivirine, and had a plasma HIV-RNA of less than 50 c/mL within 12 weeks after cART reinitiation (Table 2). After 24 weeks of treatment, 8% (4/50) of the patients in the immediate DTG monotherapy group and 0% of the 53 patients in the delayed switch group had a plasma HIV-RNA of 50 c/mL or higher (difference 8%, exact 95% confidence interval [-1%, +20%]). In the ITT-analysis at week 24, 4% (2/51) of patients in the immediate switch group and no patients in the delayed switch group had a plasma HIV-RNA of 200 c/mL or higher (difference 4%, exact 95% confidence interval [-4%, 15%]).

Of the 95 patients on DTG monotherapy in the OT-population, 78 (82%) had reached the week 48 endpoint when we decided to discontinue the study early in agreement with the predefined stopping criteria. At that time, eight patients on DTG monotherapy had VF; N=6 in the immediate switch group and N=2 in the delayed switch group (Table 2). Two patients had VF before week 24 and the other six had VF after week 24 (Table 2). In all patients with VF, DTG plasma-concentrations were therapeutic and self-reported adherence was greater than 95% (Table 2). In patient 7, a period of suboptimal DTG plasma-concentrations might have occurred because of gastro-enteritis. Integrase sequencing was successful in six patients who had VF, three of whom had RAMs in the integrase gene, including a R263K in the patient

	Duration of DTG monotherapy at the time of failure (weeks)	HIV-RNA zenith (copies/ mL)	CD4-T-lymphocyte nadir (cells/ mm^3)	cART before DTG monotherapy	Time suppressed* on cART before DTG monotherapy (years months)	HIV-RNA at failure on DTG monotherapy (copies/mL)	DTG-plasma concentration at failure (mg/ mL)[$]	Adherence (self-reported)	IN sequence at failure
Failure 1	4	18.500	290	TDF/FTC/RPV	2Y5M	71.600	1·29 (+14h)	>95%	No RAM's
Failure 2	12	7.420	220	TDF/FTC/EFV	8Y7M	678	2·00 (+19h)	>95%	Not successful
Failure 3	30	17.500	280	TDF/FTC/RPV	3Y11M	3.510	2·59 (+16h)	>95%	No RAM's
Failure 4	30	99.270	330	TDF/FTC/RPV	1Y10M	1.570	2·96 (+22h)	>95%	**S230R**
Failure 5	36	56.300	210	TDF/FTC/DTG	4Y0M	1.440	1·00 (+24h)	>95%	Not successful
Failure 6	48	67.000	230	TDF/FTC/RPV	5Y4M	4.990	1·44 (+24h)	>95%	No RAM's
Failure 7	60	34.600	240	TDF/FTC/NVP	7Y0M	3.470	0·70 (+13h)	>95%**	**R263K**
Failure 8	72	20.100	380	TDF/FTC/NVP	1Y2M	4.180	2·15 (+9h)	>95%	**N155H**

Table 2. Overview of characteristics of the patients with virological failure. TDF=Tenofovir Disoproxil Fumarate, FTC=Emtricitabine, RPV=Rilpivirine, EFV=Efavirenz, DTG=Dolutegravir, NVP=Nevirapine, RAM=Resistance Associated Mutation, IN=integrase. *Suppressed is defined as HIV-RNA <50 copies/ml. [$]The hours mentioned after the DTG-plasma concentration was measured after the last DTG intake. **Probably suboptimal gastrointestinal uptake of DTG during 10 days due to gastroenteritis.

with VF at week 60 and the N155H in the patient with VF at week 72. In the patient with VF at week 30, the S230R mutation was detected (Table 2). Integrase sequencing of stored plasma collected before initiation of cART showed that these mutations were not present at that time. After the decision was made to stop the study prematurely, all participants were contacted by the study team and were instructed to reinitiate their previous cART regimen. Another informed option was addition of two nucleoside reverse transcriptase inhibitors to DTG, if the subject's plasma HIV-RNA was still undetectable.

We followed up 83 (the five patients who had VF before week 48 plus 78 other patients) of the 95 patients who received DTG monotherapy for at least 48 weeks. When the study was discontinued, 77 (93%) of these patients had an HIV-RNA of less than 200 c/mL, and the last HIV-RNA to be measured was less than 50 c/mL in 76 patients (92%). In only one of the eight patients who had VF, the VF was preceded by two consecutive HIV viral loads of greater than 50 c/mL. Therefore, in the seven other patients with a confirmed viral load of 200 c/mL or higher, VF occurred suddenly with no preceding low-level viral replication.

Of the 61 patients who completed the 48 week 48 follow-up and were virologically suppressed at that time, 59 chose to continue DTG monotherapy, whereas two preferred to switch back to cART. One patient had VF at week 48 and also reinitiated cART. Sixteen patients were in the 44-48 week follow-up window when the study was discontinued, and therefore, were not given the option to continue DTG monotherapy. We measured HIV-RNA measurements every 12 weeks thereafter, and the median follow-up in the 59 patients who continued DTG monotherapy was 64 weeks. In two of them, VF occurred after 48 weeks; the remaining 57 patients had HIV-RNA of less than 50 c/mL.

DTG monotherapy was inferior to cART in a post-hoc analysis comparing the overall virological suppression rate (<200 c/mL) in patients in the concurrent control group (149/152, 98%) with that in the 95 patients on DTG monotherapy in the OT-population at week 48 (87/95, 92%) (difference 6%, Exact 95% CI 0.5, 14.5, p=0·02, Figure 3 and Figure 3 of the Supplementary Data).

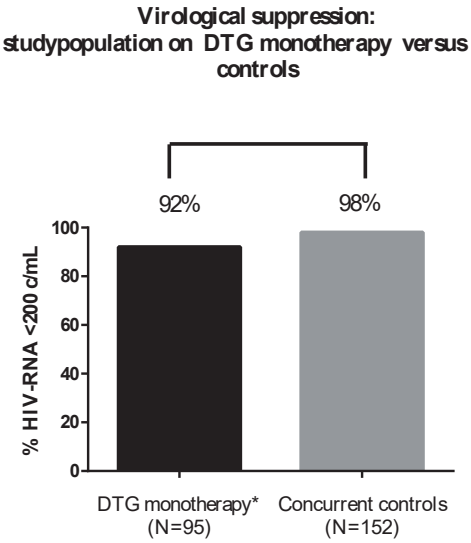


Figure 3. Percentages of virological suppression in entire study population: on treatment analysis. *8/53 patients in the delayed DTG monotherapy group did not switch to DTG monotherapy and were long enough to follow-up for inclusion in the OT-analysis, which brings the total number of patients on DTG to 95.

DISCUSSION

DTG monotherapy was non-inferior to cART in maintaining virological suppression for 24 weeks, with VF recorded in only one patient in the group of patients who switched immediately to DTG monotherapy and in no patient in the delayed switch group. No RAMs in the integrase gene were detected in the virus of the one patient with VF at week 24, and viral replication in this patient was re-suppressed soon after reinitiation of cART. Despite these promising results, VF was observed in seven additional patients after week 24, which led to virological suppression rate in 92% of patients at the time of study discontinuation. This result was statistically inferior to the 98% suppression rate observed in the concurrent control group. Because all eight patients with VF achieved re-suppression of the plasma viral load soon after reinitiation of cART, this observation alone would not contraindicate DTG monotherapy. However, the results of integrase sequencing at the time of VF clearly showed that DTG monotherapy cannot replace cART, even in patients with a CD4-nadir above 200 cells/mm^3 and an HIV-RNA zenith of less than 100.000 c/mL. In two of the six patients in whom integrase sequencing was successful, well defined resistance associated mutations were detected at position 263 and 155 of the integrase gene. Additionally, in one patient, a change at position 230 was observed, which is an accessory mutation that has been previously described in combination with other RAMs in the integrase gene of patients with VF on raltegravir or elvitegravir.[14] The three mutations could not be detected in viral RNA from

stored plasma collected before initiation of cART. The presence of acquired RAMs in the integrase gene in three of the 95 patients was inconsistent with the results from three phase 3 studies on DTG-containing cART.[2,3,15] No mutations associated with decreased susceptibility to integrase inhibitors was observed in any of the 1067 treatment-naive patients who started DTG-containing cART in the phase 3 FLAMINGO, SINGLE, and SPRING-2 studies.[2,3,15] Given the development of RAMs in the integrase gene of more than two patients on DTG monotherapy in our study, with the potential for cross-resistance to other available and future integrase inhibitors, one of the stopping rules was met and the study was terminated. Development of RAMs in the integrase gene has been extremely rare in patients on DTG previously untreated with integrase inhibitors and with a history of VF on other antiretroviral drugs; in the SAILING study, such mutations were observed in only two of 354 patients during the first 48 weeks of follow-up.[1] More recently, five small observational studies found that VF led to the development of a new RAM in the integrase gene in five of 118 patients treated with DTG monotherapy.[11,16–19] However, these studies were done in patients undergoing routine clinical care with non-standardized monitoring and without formal approval from any ethics committee.

We documented good self-reported adherence and therapeutic DTG plasma-concentrations in all patients who had VF on DTG monotherapy, and no other patient-related causes (such as intercurrent diseases or use of concomitant medications) could be identified as a possible causes of VF. However, we realize that self-reported adherence is not always reliable. The development of VF, with or without RAMs, is possibly the result of ongoing (low-level) viral replication. Therefore, DTG monotherapy seems to be insufficiently potent, and its genetic barrier to resistance insufficient, to decrease viral replication in all tissues to levels low enough to avoid development of resistance. Notably, in two of the three patients who developed resistance, we observed VF after plasma HIV-RNA levels had been below 20 c/mL at eight consecutive visits, and was therefore not preceded by documented viral replication. We can only speculate about the possible mechanisms of virological escape in the three patients in whom known RAMs in the integrase gene were not detected and therapeutic DTG plasma-concentrations were measured. Possibly, resistance to integrase inhibitors can also develop outside the integrase gene as recently described in *in vitro* experiments.[20] A similar outcome has been described in the context of resistance against protease inhibitors, wherein mutations at the protease cleavage side were involved in protease inhibitor resistance.[21]

Several hypotheses might explain the occurrence of VF in our cohort. First, the decision to start cART in patients was more often based on a decreasing CD4 T-lymphocyte count than on HIV-RNA or other factors. Therefore, HIV-RNA measurements from a time close to cART initiation were not available in four of the eight patients who had VF, meaning that the HIV-RNA zeniths in these patients just before cART intiation might have been greater than

100.000 c/mL. The multivariate analysis in the PROTEA-trial showed the relevance of this criterion, darunavir monotherapy was inferior to darunavir-containing cART in patients with an HIV-RNA of greater than 100.000 c/mL before initiation of cART.[22] However, in all three patients with RAMs in the DOMONO study, the HIV RNA plasma viral load on the day of cART initiation had been measured and was less than 100.000 c/mL in all patients, and was as low as 20.000 c/mL in one patient.

Besides the HIV-RNAzenith, an estimation of the size of the viral reservoir might be a more reliable predictor of VF during maintenance therapy with fewer drugs.[23] In the MONOI-trial, a higher baseline total HIV-DNA copy number was associated with virological rebound on darunavir monotherapy.[24] If this finding can be confirmed in future studies of maintenance therapy with fewer drugs, quantification of HIV-DNA as marker of the viral reservoir has the clear advantage as marker of the reservoir that it can be measured before simplification of cART.

Whether other mechanisms, such as differences in drug-concentrations between plasma and sanctuary sites (eg lymphoid tissue), are involved in failure of DTG maintenance monotherapy remains unknown.[25,26] We examined other more obvious factors that could have been associated with VF during DTG monotherapy, such as time on type of cART, CD4-nadir, and height of the peak viral load before initiation of cART. Given the strict inclusion and exclusion criteria of the study, and the few events, it is not surprising that the eight patients with VF were similar to the 87 other patients with regard to these factors ($p>0.05$ for all). Another explanation for the development of mutations associated with resistance to DTG could be that patients had archived replication-competent viruses with pre-existing RAMs, and that this virus was reactivated during monotherapy. Finally, although plasma-concentrations of DTG were therapeutic in all patients, intermittent non-adherence could not be entirely excluded. However, even if patients had been intermittently non-adherent, a switch to DTG monotherapy in those who had been virologically suppressed on cART for years led to an unacceptable number of patients with VF and RAMs in the integrase.

Our study has several limitations. First, the study was small and was only intended to be a proof-of-concept study before designing a larger study with a smaller non-inferiority margin and a primary endpoint of 50 c/mL. Second, the 24 week delayed-switch design has been used in most randomized studies of treatment switches and has the advantage that study results are available 48 weeks after the last patient is randomized. However, several patients in this study had VF between week 24 and 48. Therefore, future switch studies should use week 48, rather than week 24, as the primary endpoint. Finally, given the very strict inclusion and exclusion criteria, the external validity is limited to a small proportion of HIV patients in care.

In conclusion, although DTG monotherapy was non-inferior to cART after 24 weeks, it led to VF in a relatively high number of patients during longer-term follow-up. Moreover, three patients with VF developed resistance to integrase inhibitors. DTG should therefore not be used as maintenance monotherapy.

REFERENCES

1. Cahn P, Pozniak AL, Mingrone H et al. Dolutegravir versus raltegravir in antiretroviral-experienced, integrase-inhibitor-naive adults with HIV: week 48 results from the randomised, double-blind, non-inferiority SAILING study. *Lancet* 2013; **382:**700–708.
2. Walmsley S, Baumgarten A, Berenguer J et al. Brief Report: Dolutegravir Plus Abacavir/Lamivudine for the Treatment of HIV-1 infection in Antiretrovial Therapy-Naive Patients: Week 96 and Week 144 Results From the SINGLE Randomized Clinical Trial. *J Acquir Immune Defic Syndr* 2015; **70:**515–519.
3. Molina JM, Clotet B, van Lunzen J et al. Once-daily dolutegravir is superior to once-daily darunavir/ritonavir in treatment-naïve HIV-1-positive individuals: 96 week results from FLAMINGO. *J Int AIDS Soc* 2014; **17:**19490.
4. Brenner BG, Wainberg MA. Clinical benefit of dolutegravir in HIV-1 management related to high genetic barrier to drug resistance. *Viruses* 2016; published online Jul 12. DOI: 10.1016/j.virusres.2016.07.006.
5. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. URL http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentsGL.pdf.
6. Marcelin AG, Grude M, Charpentier C et al. French national survey of resistance to integrase inhibitors shows high differences of resistance selection rate in case of virological failure in a context of routine hospital care (ANRS-AC11 virology network). Oral presentation of abstract O332 at 'HIV Glasgow 2016.' Abstract available at URL http://jiasociety.org/index.php/jias/issue/view/1485.
7. Mesplède T, Wainberg MA. Resistance against Integrase Strand Transfer Inhibitors and Relevance to HIV Persistence. *Viruses* 2015; **7:**3703–3718.
8. Paton NI, Stöhr W, Arenas-Pinto A et al. Protease inhibitor monotherapy for long-term management of HIV infection: a randomized, controlled, open-label, non-inferiority trial. Lancet HIV 2015; **2**: e417–426
9. Arribas JR, Girard PM, Paton N et al. Efficacy of protease inhibitor monotherapy vs. triple therapy: meta-analysis of data from 2303 patients in 13 randomized trials. *HIV Med* 2016; **17**: 358–367
10. Girard PM, Antinori A, Arribas JR et al. Week 96 efficacy and safety of darunavir/ritonavir monotherapy vs. darunavir/ritonavir with two nucleoside reverse transcriptase inhibitors in the PROTEA trial. *HIV Med* 2017; **18:**5–12.
11. Rokx C, Schurink CA, Boucher CA, Rijnders BJ. Dolutegravir as maintenance monotherapy: first experiences in HIV-1 patients. *J Antimicrob Chemother* 2016; **6**:1632–1636.
12. Stanford University. HIV Drug Resistance Database. https://hivdb.stanford.edu/hivseq/by-mutations/.
13. Wang W. On construction of the smallest one-sided confidence interval for the difference of two proportions. *The Annals of Statistics* 2010; **38:**1227–1243W.
14. Goethals O, Clayton R, Van Ginderen M et al. Resistance mutations in human immunodeficiency virus type 1 integrase selected with elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. *J Virol* 2008;**82**:10366–10374.
15. Raffi F, Rachlis A, Stellbrink HJ et al. Once-daily dolutegravir versus raltegravir in antiretroviral-naive adults with HIV-1 infection: 48 week results from the randomized, double-blind, non-inferiority SPRING-2 study. *Lancet* 2013; **381:**735–743.
16. Gubavu C, Prazuck T, Niang M et al. Dolutegravir-based monotherapy or dual therapy maintains a high proportion of viral suppression even in highly experienced HIV-1-infected patients. *J Antimicrob Chemother* 2016; **71**:1046–1050.

17. Rojas J, Blanco JL, Marcos MA et al. Dolutegravir monotherapy in HIV-infected patients with sustained viral suppression. *J Antimicrob Chemother* 2016;**71**: 1975–1981
18. Katlama C, Soulié C, Caby F et al. Dolutegravir as monotherapy in HIV-1-infected individuals with suppressed HIV viremia. *J Antimicrob Chemother* 2016; **71**: 2646–2650.
19. Oldenbuettel C, Wolf E, Ritter A et al. Dolutegravir monotherapy as treatment de-escalation in HIV-infected adults with virological control: DoluMono cohort results. *Antivir Ther* 2016; DOI: 10.3851/IMP3082.
20. Malet I, Subra F, Charpentier et al. A high-level resistance to integrase inhibitors selected outside integrase hiv-1 gene. Poster presentation of poster 499 at CROI 2017, Seattle. Poster available at URL http://www.croiconference.org/sessions/high-level-resistance-integrase-inhibitors-selected-outside-integrase-hiv-1-gene.
21. Maguire MF, Guinea R, Griffin P et al. Changes in human immunodeficiency virus type 1 Gag at positions L499 and P453 are linked to I50V protease mutants in vivo and cause reduction of sensitivity to amprenavir and improved viral fitness in vitro. *J Virol* 2000; **76:**7398–7406.
22. Antinori A, Arribas JR, Fehr J et al. The PROTEA trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV-1 RNA below 50 copies/mL. *J Int AIDS Soc* 2014; **17:**19525.
23. Avettand-Fènoël V, Hocqueloux L, Ghosn J et al. Total HIV-1 DNA, a Marker of Viral Reservoir Dynamics with Clinical Implications. *Clin Microbiol Rev* 2016; **29:**859–880.
24. Lambert-Niclot S, Flandre P, Valantin MA et al. Factors associated with virological failure in HIV-1-infected patients receiving darunavir/ritonavir monotherapy. *J Infect Dis* 2011; **204:**1211–1216.
25. Fletcher CV, Staskus K, Wietgrefe SW et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proc Natl Acad Sci U S A.* 2014; **111:**2307–2312.
26. Dellamonica P, Di Perri G, Garraffo R. NNRTIs: pharmacological data. *Med Mal Infect* 2012; **42:**287–295.

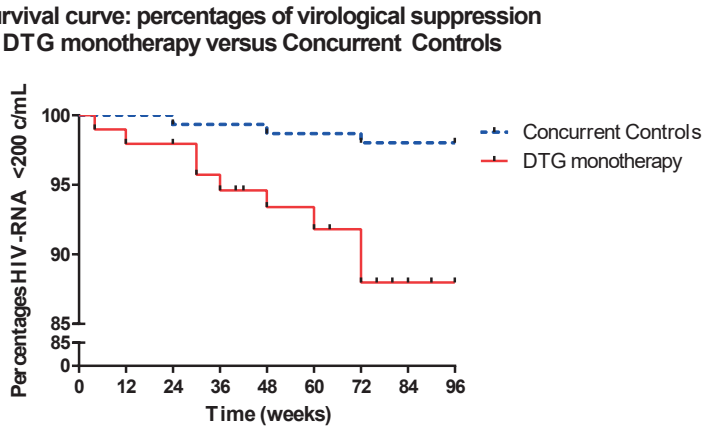
SUPPLEMENTARY DATA CHAPTER 2

Inclusion criteria	Exclusion criteria
Documented HIV-1 positive by ELISA or Western Blot or Plasma HIV-RNA >1000 copies/mL	Previous virological failure on any cART
18 years or older	Patients without anti-HBs antibodies who are not willing to undergo hepatitis B vaccination
HIV-RNA <50 copies/mL for >24 weeks	Subjects positive for hepatitis B at screening (HBsAg+)
Historical baseline HIV-RNA plasma load <100.000 copies/mL. A HIV-RNA plasma load >100.000 copies/mL is allowed, if measured during an acute HIV infection. Acute means within 6 months after a negative HIV-1 test or during the documentation of an incomplete HIV-1 Western Blot antibody-test.	No record of the historical baseline plasma viral load available
CD4-T-lyphocyte nadir ≥200 cells per µL	Subjects with concomitant CDC-C opportunistic infections within 90 days of screening
Not on strong UGT1A1 or CYP3A4 inducing agents as stated in DTG SPC	Subjects with history of allergy to INI
General medication is not interfering with trial procedures (on investigators' discretion)	Subjects with creatinine clearance <50 ml/min according to CKD-EPI
Females should have no plans of becoming pregnant during the next 18 months after baseline visit	Subjects with hepatic impairment of at least Child-Pugh B
	Exposure to experimental drug or experimental HIV-1 vaccine within 90 days of start DTG
	Screening ALT >5x ULN or ALT >3x ULN and bilirubin >2x ULN
	Patient planning or hoping to conceive a child or become pregnant during the study
	Patients who cannot take DTG 2 hours before or 6 hours after antacids, calciumcarbonate, or iron supplements

Table S1. Overview of inclusion criteria and exclusion criteria.

Reasons for not switching to DTG monotherapy	Total (N=6)
Moved away from treating hospital, N=1	Moved away from treating hospital, so was not able to comply with all scheduled study procedures.
Withdrew informed consent, N=1	Was satisfied with cART-regimen, so did not want to switch from cART to DTG monotherapy
Other, N=2	No show at scheduled visit for switch from cART to DTG monotherapy and following visits
Physician's decision, N=2	Diagnosis and surgery for prostate carcinoma during the cART period.
	In need of use of Mg^{2+}, so drug-drug interactions with DTG expected.

Table S2. Reasons for not switching to DTG monotherapy



	W0	W4	W12	W24	W30	W36	W40	W42	W48	W60	W64	W72	W76	W80	W84	W90	W96
DTG monotherapy																	
No at risk	98	98	96	93	88	86	80	79	78	59	33	24	18	14	11	4	1
Censored	0	1	2	5	0	5	1	1	18	25	9	5	4	3	7	3	1
VF	0	1	1	0	2	1	0	0	1	1	0	1	0	0	0	0	0
Concurrent Controls																	
No at risk	152	152	152	152	151	151	151	151	151	150	150	150	150	150	150	150	150
Censored	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VF	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0

Figure S3. Kaplan Meier curve of percentages virological suppression (HIV-RNA < 200 copies/mL) in the entire study population on DTG monotherapy versus Concurrent Controls. DTG=dolutegravir, VF=virological failure.