



# The effect of antiretroviral intensification with dolutegravir on residual virus replication in HIV-infected individuals: a randomised, placebo-controlled, double-blind trial

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## Summary

**Background** Whether ongoing virus replication occurs in HIV-infected individuals on antiretroviral therapy (ART) is unclear; therefore, whether residual virus replication is a barrier to achieving a cure for HIV is also unknown. We aimed to establish whether ART intensification with dolutegravir would reveal or affect residual virus replication in HIV-infected individuals on suppressive treatment.

**Methods** In this randomised, placebo-controlled, double-blind trial, we enrolled HIV-infected adults (aged 18 years and older) receiving combination ART (at least three agents) for at least 3 years from the Alfred Hospital and Melbourne Sexual Health Centre, Melbourne, VIC, Australia. Eligible participants had fewer than 50 copies per mL HIV-1 plasma RNA for more than 3 years and fewer than 20 copies per mL at screening and two CD4 counts higher than 350 cells per  $\mu$ L in the previous 24 months including screening. Participants were randomly assigned (1:1) to receive 50 mg oral dolutegravir or placebo once a day for 56 days in addition to background ART. Follow-up was done at days 1, 3, 7, 14, 28, 56, and 84. The primary outcome was the change from baseline in frequency of 2-long terminal repeat (2-LTR) circles in peripheral blood CD4 cells at day 7. This trial is registered with ClinicalTrials.gov, number NCT02500446.

**Findings** Between Sept 21, 2015, and Sept 19, 2016, 46 individuals were screened for inclusion. 40 were eligible for inclusion and were randomly assigned to the dolutegravir (n=21) or placebo group (n=19). All enrolled participants completed the study procedures and no individuals were lost to follow up. All participants were on suppressive ART with 12% receiving protease inhibitors and the others non-nucleoside reverse transcriptase inhibitors. Median 2-LTR circles fold-change from baseline to day 7 was  $-0.17$  (IQR  $-0.90$  to  $0.90$ ) in the dolutegravir group and  $-0.26$  ( $-1.00$  to  $1.17$ ) in the placebo group ( $p=0.17$ ). The addition of dolutegravir to pre-existing ART regimens was safe and there were no treatment discontinuations or treatment-related serious adverse events.

**Interpretation** Our findings show that in HIV-infected individuals on modern suppressive ART regimens, residual replication is rare, if at all present, and was not recorded in blood after dolutegravir intensification. Because tissue biopsies were not done we cannot exclude the possibility of residual virus replication in tissue. Strategies other than ART alone are needed to eliminate HIV persistence on treatment.

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## Introduction

Several mechanisms could explain the persistence of HIV in HIV-infected individuals on suppressive antiretroviral therapy (ART). These include long-lived latently infected CD4 cells,<sup>1</sup> clonal expansion of infected cells with an intact provirus,<sup>2</sup> limited penetration of antiretrovirals or cytotoxic T cells in tissue sites,<sup>3-5</sup> and residual virus replication (ie, when the virus continues to infect cells and produce new virions, resulting in ongoing rounds of replication at low levels).<sup>6,7</sup> Understanding the potential contribution of residual virus replication to HIV persistence on ART is important for several reasons. First, because interventions aimed at activating latency with latency reversal agents will not be effective in the presence of residual virus replication;

second, because residual virus replication might contribute to persisting immune activation, which has been associated with all-cause mortality;<sup>8</sup> and finally, because residual virus replication will require specific strategies to achieve an HIV cure or long-term remission without ART.

Whether residual virus replication occurs while individuals are on ART is unclear. Findings of several studies have shown that intensification of a suppressive ART regimen with either the integrase inhibitor raltegravir, or a boosted protease inhibitor, efavirenz or maraviroc, does not alter the frequency of latently infected cells or low-level viraemia.<sup>6,9-20</sup> However, in two randomised controlled studies in which ART was intensified with raltegravir, investigators reported an

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### Research in context

#### Evidence before this study

We searched PubMed in August, 2017, with the keywords: “ongoing replication AND HIV”, “residual replication AND HIV”, “ART intensification”, “HIV persistence”, “HIV latency”, “HIV reservoir”, “HIV cure”, and “dolutegravir”. Titles and abstracts were reviewed and assessed for relevance. In recent years, findings of numerous studies have shown that the long-term stability and proliferation of latently infected CD4 cells are key mechanisms of HIV persistence. By contrast, whether residual virus replication or ongoing infection of cells on antiretroviral therapy (ART) also contributes significantly to HIV persistence has remained much more controversial. Several studies have reported no viral evolution or emergence of drug resistance during suppressive ART, and findings of previous ART intensification studies showed no effect on measures of HIV persistence. However, two randomised controlled trials (Buzon, 2010, and Hatano, 2013) of raltegravir intensification showed significant increases in 2-long terminal repeated (2-LTR) circles soon after the addition of raltegravir versus placebo controls, suggesting that residual virus replication persists in some individuals on ART.

#### Added value of this study

The second-generation integrase inhibitor dolutegravir has not previously been investigated in ART intensification studies. We hypothesised that intensifying ART with dolutegravir would affect residual replication in individuals on long-term suppressive ART, similar to raltegravir. In contrast to previous intensification

studies, we did frequent sampling of 2-LTR circles in the first 14 days after intensification as we speculated that given the short half-life of 2-LTR circles, any effect on 2-LTR circles would be quick and short lived following intensification. A rapid increase and decrease in 2-LTR circles would be expected if dolutegravir had interrupted ongoing virus replication; however, the findings of our study were negative across multiple parameters and we recorded no significant differences between the placebo and dolutegravir group in the change from baseline of the frequency of 2-LTR at any timepoint. We also recorded no differences in the change from baseline in soluble and cell-associated measures of HIV persistence and immune activation. These results show that despite very careful mapping of early effects, adding dolutegravir to suppressive ART did not affect residual virus replication in blood in HIV-infected individuals on ART.

#### Implications of all the available evidence

Our findings show that in HIV-infected individuals on current suppressive ART regimens, residual replication is rare, if at all present, and could not be detected in blood after dolutegravir intensification. Key differences in the uptake of antiretrovirals in the gastrointestinal tract where raltegravir has much higher concentrations compared with dolutegravir might explain why different findings were reported in previous studies of raltegravir intensification. We acknowledge that in the absence of tissue analyses, effects on residual virus replication in lymphoid tissue cannot be excluded by this study. Strategies other than ART alone are needed to eliminate HIV persistence while on treatment.

increase in 2-long terminal repeat (2-LTR) circles, which are short-lived by-products of unsuccessful integration, in about 30% of study participants within 2 weeks of intensification.<sup>6,7,15</sup> Integrase inhibitors block the integration of linear HIV DNA, which subsequently becomes circularised by host repair enzymes to form 2-LTR circles. Therefore, an increase in 2-LTR circles occurs when active replication is inhibited through blocking integration. 2-LTR circles have a short half-life, which could explain why an increase in 2-LTR circles was only recorded in studies that measured 2-LTR circles within 2 weeks of raltegravir intensification. In these two studies, the transient increase in 2-LTR was accompanied by a decrease in circulating levels of d-dimer<sup>7</sup> or decreases in measures of T-cell activation.<sup>6,15</sup> Results of two other studies did not show increases in 2-LTR circles following raltegravir intensification, but these findings might be because of the absence of sampling in the early phases of intensification.<sup>10,16</sup>

Several potential explanations exist for why residual viral replication on ART might occur. One hypothesis is that there is insufficient penetration of ART in tissue sites,<sup>21</sup> but the tissue concentration is at a sufficient level that does not allow for emergence of drug resistant variants.<sup>3</sup> The other possibility is that in tissue sites there is ongoing transmission of virus through cell-cell

transfer leading to infection of both activated and resting T cells.<sup>22</sup> Several studies have recorded no viral evolution in sequences isolated from CD4 cells collected from blood and tissue in individuals on ART,<sup>23,24</sup> which is consistent with minimal virus replication, although it is possible that these techniques are not sufficiently sensitive to identify very low levels of virus replication.

Raltegravir is the only integrase inhibitor that has been investigated in ART intensification trials.<sup>6,7,11,12,16</sup> Dolutegravir is a once-daily integrase inhibitor that is safe and highly potent. In randomised clinical trials, dolutegravir was non-inferior to raltegravir for the outcome of virological suppression after 48 weeks of treatment in a treatment-naive population<sup>25</sup> and superior to raltegravir in a treatment-experienced population that had not previously been exposed to integrase inhibitors.<sup>26</sup> To address whether ART intensification with dolutegravir inhibits residual virus replication, we compared the effect of intensification with dolutegravir or placebo in HIV-infected individuals on suppressive ART.

## Methods

### Study design and participants

In this investigator-initiated, randomised, placebo-controlled, double-blinded trial, HIV-infected adults receiving combination ART for at least 3 years with no

change in ART regimen in the previous 6 months were recruited at two sites in Melbourne, VIC, Australia: the Alfred Hospital and Melbourne Sexual Health Centre. Eligible participants had CD4 counts higher than 350 cells per  $\mu\text{L}$  and virological suppression for at least 3 years (plasma HIV RNA  $<50$  copies per mL for  $>3$  years and  $<20$  per mL at screening). We excluded individuals with hepatitis C co-infection, unstable liver disease, renal impairment (estimated glomerular filtration rate [eGFR]  $<50$  mL per min), gastrointestinal disorders that would affect the absorption of study treatment, current or previous use of any integrase inhibitor, current or previous use of any latency-reversing agent, current or recent use of immunomodulating treatment including chemotherapeutic agents, current use of drugs with significant interactions with dolutegravir, hospitalisation for acute illness within the previous 8 weeks, and pregnant or breastfeeding women. The study was approved by the Human Research Ethics Committee of the Alfred Hospital, Melbourne, VIC, Australia, and was done in accordance with the principles of the Declaration of Helsinki and the National Health and Medical Research Council of Australia, National Statement on Ethical Conduct in Human Research. All participants provided written informed consent before study initiation.

### Randomisation and masking

We randomly assigned study participants (1:1) to receive dolutegravir or placebo by use of computer-generated (Stata, version 13) randomisation lists with two or four participants per block. The allocation sequence was generated by a statistician independent of the study team. Within each block, half the participants were randomly assigned to receive dolutegravir and the other half to receive placebo of identical appearance. Randomisation was stratified by current use of a protease inhibitor as the increase in 2-LTR circles reported in previous studies occurred more often in participants receiving a protease inhibitor-based regimen.<sup>6,7,15</sup> Study site personnel received allocation codes from a central randomisation service hosted by the sponsor, which was also provided to the clinical trial pharmacy at the Alfred Hospital. The sponsor maintained logs of all study numbers, allocation codes, and allocation at the central randomisation service. All study site staff, study investigators, and study participants were masked to treatment assignment from enrolment until completion of statistical analyses for the primary and secondary outcome measures. To enable statistical comparisons under masked conditions, the study statistician was provided with a colour code to represent treatment assignment.

### Procedures

Study participants received 50 mg oral dolutegravir or matching placebo tablets once a day for 56 days in addition to their usual ART regimen. Participants whose

ART regimen included efavirenz or nevirapine received 50 mg dolutegravir or placebo twice a day because of the effect of these antiretrovirals in lowering dolutegravir concentrations.<sup>27,28</sup> Follow-up study visits and blood draws were done at days 1, 3, 7, 14, 28, 56, and 84 after commencing study drug. At each visit, we assessed self-reported adherence to ART and study treatment. We also measured plasma concentrations of dolutegravir in dolutegravir recipients at day 7 to confirm adherence. Safety was actively assessed at all study visits by recording of all adverse events and serious adverse events. For all adverse events, we assessed the causal relation to study treatment and graded its severity according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 1.0). Safety assessment included clinical review, vital signs, clinical laboratory tests, and monitoring of liver chemistry stopping criteria at selected visits (days 14, 28, 56, and 84). Laboratory abnormalities constituted an adverse event if the abnormality developed after initiating study treatment or if associated with clinical signs or symptoms and required additional testing.

For virological assays, CD4 cells were isolated from peripheral blood mononuclear cells with a CD4 cell isolation kit and magnetic-activated cell sorting columns (Miltenyi Biotec, Teterow, Germany; purity  $>95\%$ ). Isolated CD4 cells were lysed and lysates stored at  $-80^\circ\text{C}$  until analysed. 2-LTR circles were analysed in triplicate from DNA extracted from cell lysates with quantification as described by Vandergeeten and colleagues.<sup>29</sup> Cell-associated total and integrated HIV DNA were analysed in triplicate employing DNA extracted from cell lysates using a two-step real-time PCR using primers and probes as previously described.<sup>29</sup>

For quantification of cell-associated unspliced HIV-RNA, RNA was extracted using the Qiagen AllPrep kit (Qiagen, Hilden, Germany). Cell-associated unspliced HIV-RNA was quantified with a semi-nested real-time quantitative PCR as previously described.<sup>30</sup> Primers used for first and second round amplified gag HIV-1 and RNA copy numbers were standardised to cellular equivalents with the 18S TaqMan gene Expression Assay (Applied Biosystems, Foster City, CA, USA). The lower limit of detection was one copy per well. For all samples, a non-RT control was included.

For all PCR assays, we recorded replicate measurements with no PCR signal as zero and measurements less than 1 copy but detected were recorded as 0.5. For the primary analysis, we used the mean of three (for all HIV DNA assays) and four (for cell-associated unspliced HIV RNA) replicates divided by the cell equivalents measured as CCR5 copy number and 18S expression respectively. For negative binomial regression analyses, individual's replicates were the outcome, and input cell number was used as an exposure variable.

We measured low-level viraemia with an ultrasensitive assay using published methods.<sup>31</sup> This assay has a lower

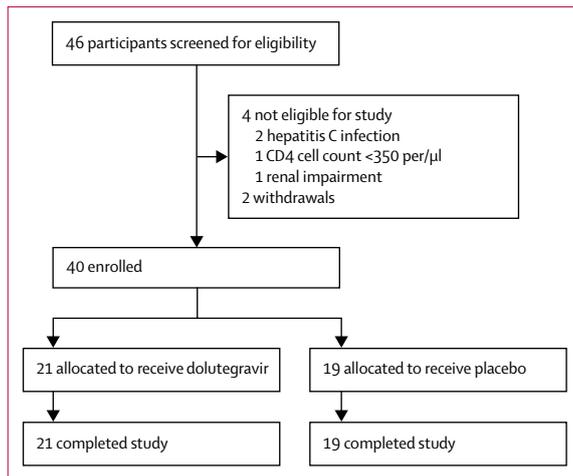


Figure 1: Trial profile

	Dolutegravir group (n=21)	Placebo group (n=19)
Age (years)	49.4 (10.8)	48.5 (8.0)
Sex		
Men	18 (86%)	19 (100%)
Women	3 (15%)*	0
Baseline CD4 count (cells per µL)†	721 (648–953)	664 (545–891)
Regimen		
Non-nucleoside reverse transcriptase inhibitor‡	18 (86%)	17 (89%)
Protease inhibitor§	3 (14%)	2 (11%)

Data are mean (SD), n (%), or median (IQR). \*Two cisgender, one transgender.  
 †Screening CD4 values were used for four individuals instead of baseline values.  
 ‡Non-nucleoside reverse transcriptase inhibitor regimens consisted of two nucleoside reverse transcriptase inhibitors combined with: efavirenz (n=17), nevirapine (n=10), or rilpivirine (n=8). §Protease inhibitor regimens consisted of two nucleoside reverse transcriptase inhibitors combined with: ritonavir-boosted atazanavir (n=1) or darunavir (n=2) or unboosted atazanavir (n=2).

**Table 1: Baseline characteristics**

limit of detection of 0.3 copies per mL of HIV RNA. For this study, we used 4–7 mL of plasma, depending on availability.

For immunological assays, we used flow cytometry and fluorescence activated cell sorting to quantify markers of T-cell activation and exhaustion. Briefly, peripheral blood mononuclear cells were thawed and surface-stained with Live/Dead fixable Dead cell aqua stain (Invitrogen, CA, USA) as per manufacturer’s instructions and then with anti-CD3 V450, anti-HLA-DR FITC, anti-CD38 PE, anti-PD-1 AlexaFluor647 (BD Biosciences, NJ, USA), anti-CD4 PE-Texas Red, and anti-CD8 Qdot805 (Invitrogen). Cells were then washed and fixed in 1% paraformaldehyde and data were obtained on a BD Fortessa flow cytometer (BD Biosciences). Lymphocytes were gated based on forward and side scatter and live CD3 cells were then gated. Expression of HLA-DR, CD38, and PD-1 was analysed in CD4 and CD8-positive CD3 cells.

Plasma concentrations of inflammatory biomarkers were measured by use of a D-dimer Human ELISA Kit, CD14 Human ELISA Kit and Human interleukin-6 (IL-6) ELISA Kit (all Life Technologies, CA, USA). High-sensitivity C-reactive protein (hsCRP) was analysed by a standard clinical assay at the Alfred Hospital. Cryopreserved plasma samples collected during the study were thawed and diluted as per manufacturer’s protocol for each biomarker tested. We used standards from the kits to establish the concentration of the samples.

Dolutegravir concentration in plasma was measured by Pharmaceutical Product Development (LLC, Middleton, WI, USA). Briefly, a 25 µL matrix aliquot was fortified with dolutegravir-d5 internal standard working solution. Analytes were isolated through protein precipitation. The extract was diluted and the final diluted extract analysed using positive ion electrospray. A linear, 1/concentration weighted, least-squares regression algorithm was used to quantitate unknown samples.

**Outcomes**

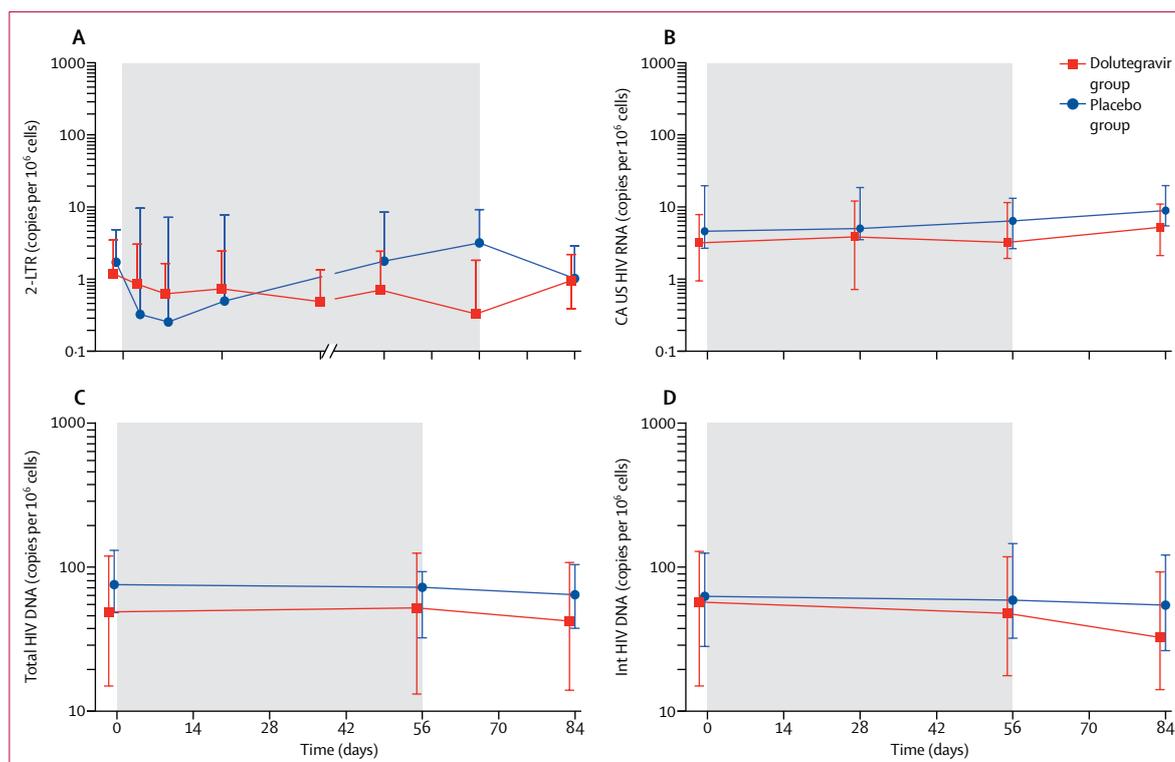
The primary outcome measure was the change from baseline of the frequency of 2-LTR in peripheral blood CD4 cells after 7 days of ART intensification with dolutegravir or 7 days of placebo. Secondary virological outcome measures were change from baseline in the frequency of 2-LTR in CD4 cells at other timepoints during follow-up, low-level HIV viraemia as measured by a quantitative ultrasensitive assay with a lower limit of detection of one copy per mL, cell-associated unspliced HIV RNA in peripheral blood CD4 cells, and total and integrated HIV DNA in peripheral blood CD4 cells.

Secondary immunological outcome measures were change from baseline in T-cell activation and exhaustion as measured by expression of HLA-DR, CD38, and PD-1 in populations of CD4 cells and CD8 cells with flow cytometry. Soluble markers of immune activation were measured in plasma by use of ELISA for IL-6, hsCRP, d-dimer, and soluble CD14. Safety was a secondary outcome measure and assessed by the incidence and severity of adverse events.

**Statistical analyses**

We designed the study to detect a three-fold increase in 2-LTR levels with 80% power at a 0.05 significance level. Based on our previous work with a droplet digital assay,<sup>32</sup> we assumed a mean and SD of 2-LTR circles in HIV-infected individuals on ART of 10.8 and 18.3 copies per 10<sup>6</sup> CD4 cells, respectively. On the basis of these estimates, the study required 17 fully evaluable participants in each group. To account for study withdrawals we aimed to enrol up to 40 participants, 20 in each group.

We used repeated-measures ANOVA as the protocol-defined primary analysis to address whether changes from baseline were significantly different in the dolutegravir group versus the placebo group. This analysis allowed us to include data from all intervening timepoints in any



**Figure 2: Cell-associated virological markers during ART intensification**

Median (IQR) concentrations of 2-LTR circles (A), cell-associated unspliced HIV RNA (B), total HIV DNA (C) and integrated HIV DNA (D) in CD4 T cells during ART intensification with dolutegravir or placebo. The grey zone indicates the period of dolutegravir and placebo administration. For 2-LTR circles shown in (A), the lower error bars for the IQR go below zero and are therefore not displayed on the  $\log_{10}$  y-axis. ART=antiretroviral therapy. 2-LTR=2-long terminal repeat. CA US HIV RNA=cell-associated unspliced HIV RNA

analysed timespan. We also employed student's *t* test or rank sum test, depending on data distribution, to compare changes from baseline to specific timepoints across study groups. Finally, because of the exploratory nature of the study, we further analysed all virological measures using ANCOVA and a negative binomial regression model as recently described.<sup>33</sup> In this model, the number of HIV copies (2-LTR, cell-associated unspliced HIV RNA, HIV DNA or plasma HIV RNA) was included as the outcome variable and the input quantity for the PCR analysis (total RNA, total DNA, or plasma volume) as an exposure variable. Importantly, this model accounted for variation in the amount of input RNA, DNA, or plasma volume such that specimens with higher input quantity provided more weight than specimens with lower input quantity; this is relevant when measuring rare events as in this study. Additional details of the model can be found elsewhere.<sup>33</sup> All analyses were done with STATA (version 14.2).

This trial is registered with ClinicalTrials.gov, number NCT02500446.

#### Role of the funding source

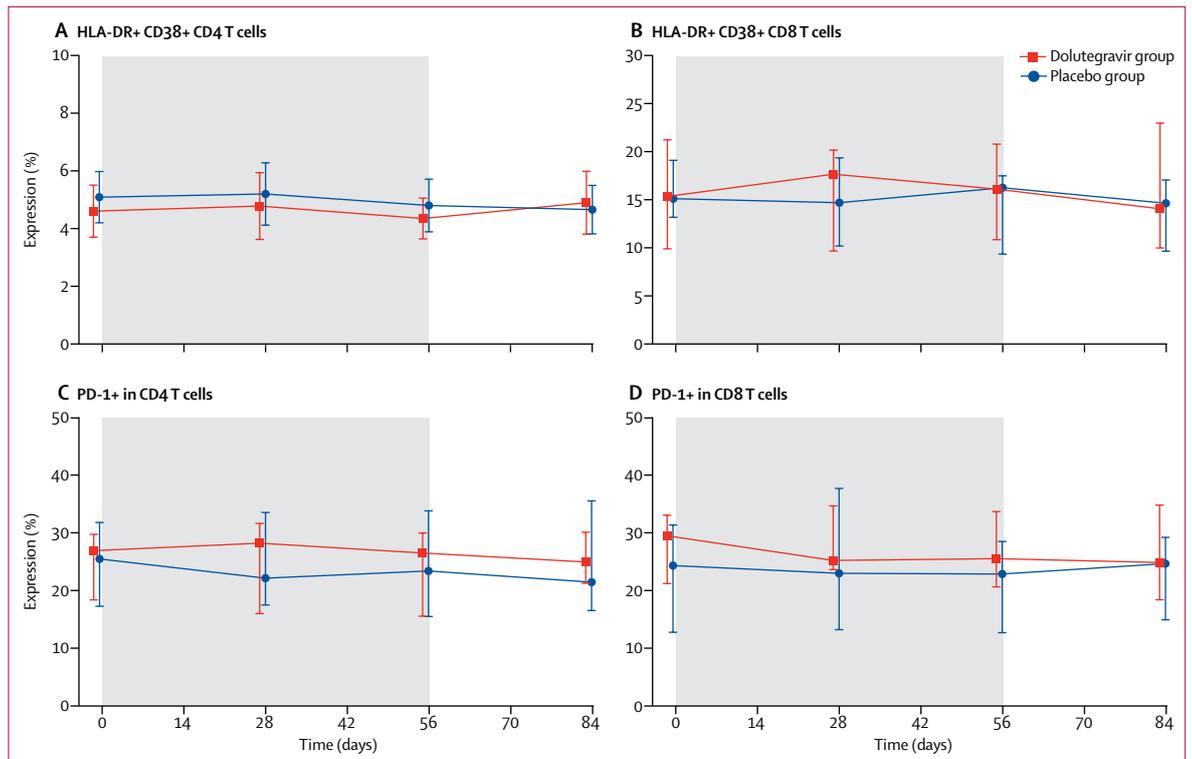
The funder did not have any role in study design, data collection, data analysis, data interpretation, manuscript writing, or in the decision to submit the paper for

publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Results

Between Sept 21, 2015, and Sept 19, 2016, 46 individuals were screened for inclusion of which 40 were enrolled and randomly assigned to the dolutegravir ( $n=21$ ) or placebo group ( $n=19$ ; figure 1). All enrolled participants completed the study procedures and no individuals were lost to follow-up. Baseline characteristics were similar between groups (table 1). ART regimens at enrolment for three individuals in the dolutegravir group and two in the placebo group were protease inhibitor-based, with all other participants receiving non-nucleoside reverse transcriptase inhibitor-based regimens.

There was no significant difference between dolutegravir and placebo in change from baseline of 2-LTR circles in peripheral blood CD4 cells as tested by repeated-measures ANOVA over 7 days ( $p=0.17$ ; figure 2A). Median 2-LTR circles fold-change from baseline to day 7 was  $-0.17$  (IQR  $-0.90$  to  $0.90$ ) in the dolutegravir group and  $-0.26$  ( $-1.00$  to  $1.17$ ) in the placebo group. In addition, we found no significant difference in the exploratory analyses of 2-LTR circles comparing baseline



**Figure 3: T-cell activation and exhaustion during ART intensification**

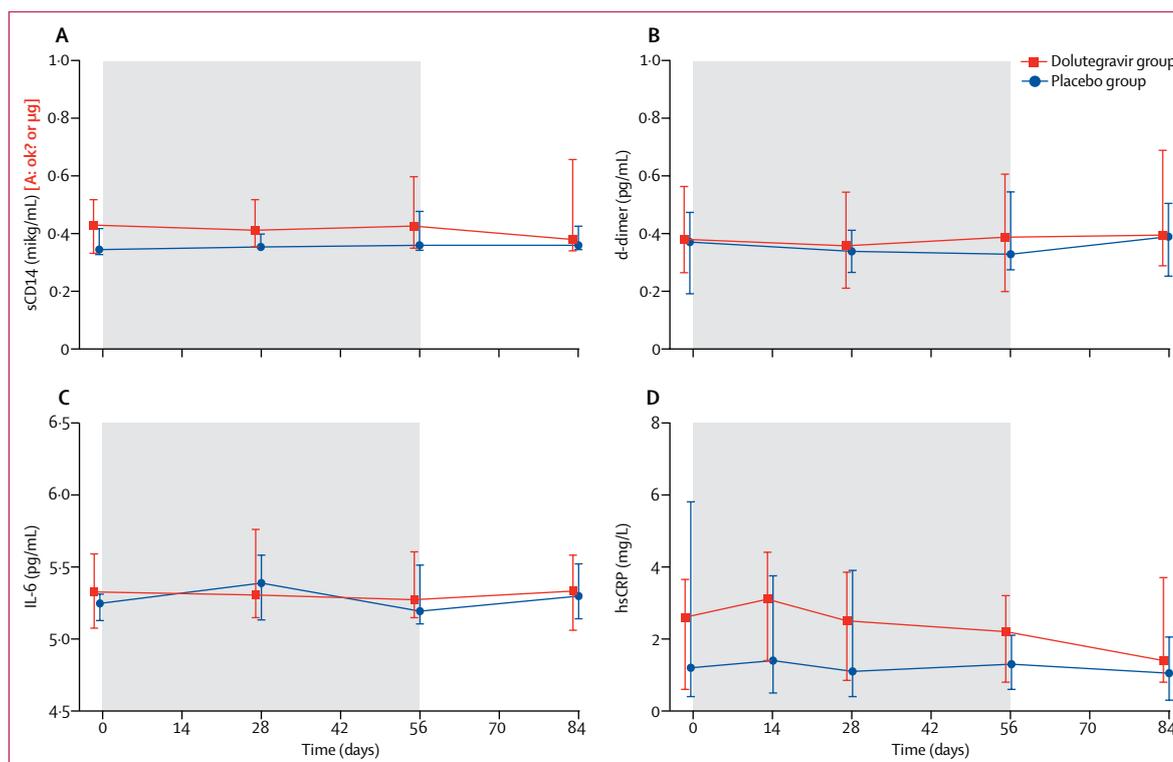
The percentage of T cells from blood co-expressing CD38 and HLA-DR measured by flow cytometry during ART intensification with dolutegravir or placebo is shown for CD4-positive T cells (A) and CD8 cells (B). The percentage of T cells expressing the exhaustion marker PD-1 is shown for CD4 cells (C) and CD8 cells (D). The grey zone indicates the period of dolutegravir or placebo administration. The graphs display mean levels with 95% CIs. ART=antiretroviral therapy. HLA-DR=human leucocyte antigen D related. PD-1=programmed death-1.

to day 7 by ANCOVA, whereas we noted an increase in the placebo group compared with the dolutegravir group ( $p=0.06$ ). In the analysis with a negative binomial regression model that adjusted for input quantity, we noted a significant reduction in 2-LTR from baseline to day 7 in the dolutegravir group compared with the placebo group ( $p<0.0001$ ). However, when the intervening timepoints at days 1 and 3 were included in the analysis, this change was no longer significant. These three statistical methods did not identify a significant increase in 2-LTR circles in the dolutegravir group relative to placebo at any of the timepoints from baseline to day 84. Finally, there was no change from baseline to day 7 in either group in the proportion of samples with undetectable levels of 2-LTR, nor was there any difference across the two groups at day 7.

Additional virological analyses of cell-associated unspliced HIV RNA, total HIV DNA, integrated HIV DNA, and plasma HIV RNA (as measured by the single copy assay) by repeated measures ANOVA showed no significant difference in the change from baseline to any timepoint between the dolutegravir and placebo group (figure 2). When employing negative binomial regression analysis, we found no other significant changes in relation to baseline for cell-associated unspliced

HIV-RNA and total HIV DNA, but for integrated HIV DNA we noted reductions in the dolutegravir group relative to placebo from baseline to day 84 ( $p=0.014$ ), and across all timepoints ( $p=0.012$ ). In a post-hoc analysis, we asked if there were changes between timepoints on study and identified significant increases in cell-associated unspliced HIV-RNA in the dolutegravir group compared with the placebo group from day 28 to day 84 ( $p=0.014$ ) and day 56 to day 84 ( $p=0.022$ ). Binomial regression analysis also identified reductions in plasma HIV RNA in the dolutegravir group comparing day 28 to 56 ( $p=0.045$ ) and day 56 to 84 ( $p=0.007$ ).

There was a minor increase in PD-1 expression in CD4 cells from baseline to day 56 in the dolutegravir group compared with placebo ( $p=0.03$ ), whereas there was no difference in PD-1 expression in CD8 cells in the change from baseline to any timepoint during study therapy. There were no significant differences in the change from baseline between study groups in other T-cell markers of immune activation (ie, expression of CD38, HLA-DR, or both), in neither CD4 nor CD8 cell subsets (figure 3). We recorded no difference between study groups in the change from baseline to any timepoint in plasma levels of sCD14, d-dimer, IL-6 or hsCRP (figure 4). When analysing changes in parameters between timepoints



**Figure 4: Soluble biomarkers of inflammation and coagulation during ART intensification**  
 Concentrations of sCD14 (A), d-dimer (B), IL-6 (C), and hs-CRP (D) during ART intensification with dolutegravir or placebo shown as the median (IQR). The grey zone indicates the period of dolutegravir or placebo administration. ART=antiretroviral therapy. sCD14=soluble CD14. IL-6=interleukin-6. hs-CRP=high sensitivity C-reactive protein.

following study drug, in the dolutegravir group there was a modest but statistically significant reduction in PD-1 expression on CD8 cells when comparing day 56 with day 84 ( $p=0.018$ ), which was not seen in CD4 cells or in the placebo group.

The addition of dolutegravir to pre-existing ART regimens was safe; no patients discontinued treatment or reported treatment-related serious adverse events. One individual receiving dolutegravir had a serious adverse event unrelated to study drug; this was a short hospital admission to manage cellulitis. All clinical and laboratory treatment-related adverse events were mild (grade 1) and included gastrointestinal and CNS effects (table 2). Dolutegravir trough concentrations at day 7 confirmed adherence to study treatment. Dolutegravir concentrations ranged from  $0.773 \mu\text{g/mL}$  to  $5.670 \mu\text{g/mL}$  in 19 participants tested with a mean of  $3.08 \mu\text{g/mL}$ . This is consistent with therapeutic concentrations recorded in a phase 3 clinical trial of 399 participants receiving 50 mg dolutegravir daily who had mean trough dolutegravir concentration of  $1.18 \mu\text{g/mL}$ .<sup>25</sup>

## Discussion

This is the first study to compare ART intensification with dolutegravir in a placebo-controlled randomised setting to assess the effect on residual virus replication

	Dolutegravir group (n=21)	Placebo group (n=19)
Clinical events	21† (100%)	14 (67%)
Vivid dreams	..	2 (10%)
Diarrhoea	..	1 (5%)
Disassociation	..	2
Nausea or vomiting	..	1 (5%)
Fatigue	1 (5%)	1 (5%)
Oesophageal pain	..	1 (5%)
Dizziness	..	2 (10%)
Abdominal bloating	..	1 (5%)
Anxiety	1 (5%)	..
Dry mouth	1 (5%)	..
Rash	1 (5%)	..
Laboratory events‡	3 (14%)	7 (33%)
Increased creatinine	1 (5%)	..
Increased alanine aminotransferase	3 (14%)	..
Increased aspartate aminotransferase	..	1 (5%)
Treatment unrelated adverse events§	..	..

Data are n (%). \*Grading according to Division of AIDS Table for Grading the Severity of Adult and Paediatric Adverse Events (version 1.0). All events were grade 1. There were no adverse events leading to discontinuation. †One patient had a serious adverse event: admitted to hospital for intravenous antibiotics for cellulitis to the right first toe; onset at day 11 and resolved by day 49. ‡Laboratory events considered related to study drug if onset of abnormality after, and while still receiving, study drug. All events graded mild (grade 1). §Same grading system as for treatment-related adverse events.

**Table 2: Treatment-related adverse events\***

while taking ART. Overall, in the a-priori analyses, we recorded no difference in the change in concentrations of 2-LTR circles between participants in the dolutegravir or placebo group. In addition, there were no consistent differences detected between the two groups for changes in other virological analyses including cell-associated unspliced HIV RNA, total and integrated HIV DNA, and ultra-sensitive plasma HIV RNA. We noted no consistent differences in changes in markers of T-cell activation and exhaustion or in plasma markers of inflammation. As such, intensification with dolutegravir did not reveal or affect virus replication in blood in individuals taking virally suppressive ART.

The strengths of this study lie in the prospective design with use of randomisation, placebo-control, and double blinding, and in its predefined frequent analyses of 2-LTR changes within the first 14 days after commencing study treatment. The negative findings are consistent with some studies of treatment intensification with raltegravir<sup>10-12,16</sup> and many other antiretrovirals such as efavirenz, maraviroc, and some boosted protease inhibitors.<sup>9,13,17-20</sup> The lack of change in markers of immune activation is consistent with an intervention that did not identify, or interfere with, residual viral replication as a reduction in immune activation may have been expected if residual replication was reduced. Persistent immune activation during chronic HIV infection has been shown to correlate with increased all-cause mortality<sup>1</sup> and, therefore, therapeutic approaches to reduce excess immune activation are of high interest. Our data suggest that intensifying suppressive ART with dolutegravir does not reduce measures of immune activation and offers no advantages over standard ART in this context. However, it is possible that persistent or episodic virus production from an infected cell that persists on ART could stimulate immune activation in the absence of ongoing infection of new target cells. The addition of dolutegravir would only disrupt infection of new uninfected cells. Finally, this study does not provide evidence that dolutegravir can decrease the replenishment of the reservoir or hasten reservoir decay as was suggested by a previous study of raltegravir intensification.<sup>6</sup>

The discrepancy between our findings in this study and two previous clinical trials showing an increase in 2-LTR circles during raltegravir intensification<sup>6,7</sup> might be explained by the population studied or key pharmacokinetic differences between raltegravir and dolutegravir. In the previous studies that reported a significant effect of raltegravir leading to an increase in 2-LTR circles, protease-inhibitor-based regimens in the study participants were more common, ranging from 30%<sup>6</sup> to 68%<sup>7</sup> in the raltegravir groups. The increase in 2-LTR circles in both of these studies was more common in participants taking protease inhibitors.<sup>6,7</sup> In our study, although we aimed to stratify based on protease inhibitor use, the number of participants taking protease inhibitors was only 12.5%, which probably

reflects the change in preferred ART regimens that are non-protease inhibitor based over the past 5 years in Australia and other high-income countries. Other minor differences include the methods used to quantify 2-LTR circles. In the study by Hatano and colleagues,<sup>7</sup> droplet digital PCR was used, which allows for a greater range in detection of a specific target compared to real-time PCR, which was used in this study and the study by Buzon and colleagues.<sup>6</sup> Concentrations of 2-LTR in HIV-infected individuals on ART are highly variable and are often below the limit of detection of the assay,<sup>6,7,34,35</sup> as noted in this study. The degree of variation in 2-LTR concentrations in this study was not higher than anticipated in the prestudy sample size calculation and the study thus retained statistical power to detect a three times difference in the change in 2-LTR between study groups.

Concentrations of dolutegravir in rectal tissue are estimated to be 17% of that in plasma<sup>36</sup> as compared with raltegravir where concentrations in the gastrointestinal tract can be more than 600 times higher than in plasma.<sup>37</sup> This difference might be important because if residual viral replication is occurring in the gastrointestinal tract where most virus persists,<sup>38</sup> then the higher concentration of raltegravir in this site could allow for a greater effect. A head-to-head comparative study of intensification with raltegravir and dolutegravir as well as assessment of rectal tissue would be needed to answer this question. Of note, no difference has been reported in the frequency of cell-associated HIV RNA and DNA in gut-associated lymphoid tissue from people with HIV receiving either raltegravir or dolutegravir.<sup>39</sup> Although this finding suggests a similar frequency of HIV-infected cells in gut-associated lymphoid tissue on both integrase inhibitors, a cross-sectional study of the HIV reservoir would not be able to address the issue of residual virus replication. Finally, there might also be different concentrations of raltegravir and dolutegravir in lymph node tissue, but data to support such a comparison are not available.

In post-hoc analyses, we identified significant but non-consistent decreases in integrated HIV DNA and ultrasensitive plasma HIV RNA in the dolutegravir group that might suggest a reduction in the frequency of infected cells. However, we interpret these changes cautiously and believe they should not be assigned too much significance in view of the modest changes and the many statistical comparisons made. Notably, these changes were only significant when a binomial linear regression analysis was used. This approach might arguably be the more accurate method, as this is the only statistical analysis method that accounted for input cell number and plasma volume. However, this approach was used only in post-hoc, not a-priori, analyses.

Our study has several limitations. First, the study was powered to detect a three-fold difference in the change in 2-LTR across study groups and, therefore, would not have identified minor changes below this threshold if they

occurred. Second, only five individuals on a protease-inhibitor-containing ART regimen were included in the study and, therefore, we were unable to specifically address the question of residual replication among individuals on such therapy, who in previous studies were more likely to have evidence of residual virus replication.<sup>6,7</sup> Finally, as the study did not include collection of lymphoid or gastrointestinal tissue samples, all analyses were based on peripheral blood CD4 cells. Consequently, an effect on residual virus replication in lymphoid tissue cannot be ruled out by this study, but we were unable to detect changes in peripheral blood.

In conclusion, we found no evidence that intensifying ART with dolutegravir leads to increases in the concentration of 2-LTR in peripheral blood CD4 cells in HIV-infected individuals. Dolutegravir intensification did not lead to any changes in cell-based measures of HIV persistence, in T-cell activation, or in plasma concentrations of inflammatory biomarkers. We conclude that in a randomised, placebo-controlled trial the addition of dolutegravir did not reveal or affect residual virus replication in blood in HIV-infected individuals on ART.

#### Contributors

SRL, TAR, JHE, and JHM conceived and designed the study and developed the study protocol. JA contributed to developing the protocol. SRL, TAR, and JHM provided clinical oversight of the study. AR, AD, ST, and JJC coordinated trial sample processing and laboratory procedures. TAR, SJK, TSc, and JHM enrolled patients in the study and oversaw follow-up. AR, AD, ST, and JJC did virological and immunological analyses. SP and VM oversaw ultrasensitive measurement of plasma HIV RNA. TSp did biostatistical analyses. SRL, TAR, TSp, and JHM interpreted data. The manuscript was drafted by TAR, JHM, and SRL. All authors reviewed and provided input to the manuscript and approved the final version.

#### Declaration of interests

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