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AIDS
Predicting virological decay in patients starting combination antiretroviral therapy
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| Abstract:          | Objective: Model trajectories of viral load measurements from time of starting combination antiretroviral therapy (cART), and use the model to predict whether patients will achieve suppressed viral load ( 200 copies/mL) within 6-months of starting cART. |
|                     | Design: Prospective cohort study including HIV-positive adults (UK Collaborative HIV Cohort Study). |
|                     | Methods: Eligible patients were antiretroviral-naïve and started cART after 1997. Random-effects models were used to estimate viral load trends. Patients were randomly selected to form a validation dataset with those remaining used to fit the model. We evaluated predictions of suppression using indices of diagnostic test performance. |
|                     | Results: Of 9562 eligible patients 6435 were used to fit the model and 3127 for validation. Mean log10 viral load trajectories declined rapidly for 2-weeks post-cART, moderately between 2-weeks and 3-months, and more slowly thereafter. Higher pre-treatment viral load predicted steeper declines, whilst older age, white ethnicity and boosted-PI/NNRTI-based cART-regimen predicted a steeper decline from 3-months onwards. Specificity of predictions and the diagnostic odds-ratio substantially improved when predictions were based on viral load measurements up to the 4-month visit compared to the 2 or 3-month visits. Diagnostic performance improved when suppression was defined by two consecutive suppressed viral loads compared to one. |
|                     | Conclusions: Viral load measurements can be used to predict if a patient will be suppressed by 6-months post-cART. Graphical presentations of this information could help clinicians decide the optimum time to switch treatment regimen during the first months of cART. |
Abstract

**Objective:** Model trajectories of viral load measurements from time of starting combination antiretroviral therapy (cART), and use the model to predict whether patients will achieve suppressed viral load ($\leq 200$ copies/mL) within 6-months of starting cART.

**Design:** Prospective cohort study including HIV-positive adults (UK Collaborative HIV Cohort Study).

**Methods:** Eligible patients were antiretroviral-naïve and started cART after 1997. Random-effects models were used to estimate viral load trends. Patients were randomly selected to form a validation dataset with those remaining used to fit the model. We evaluated predictions of suppression using indices of diagnostic test performance.

**Results:** Of 9562 eligible patients 6435 were used to fit the model and 3127 for validation. Mean log$_{10}$ viral load trajectories declined rapidly for 2-weeks post-cART, moderately between 2-weeks and 3-months, and more slowly thereafter. Higher pre-treatment viral load predicted steeper declines, whilst older age, white ethnicity and boosted-PI/NNRTI-based cART-regimen predicted a steeper decline from 3-months onwards. Specificity of predictions and the diagnostic odds-ratio substantially improved when predictions were based on viral load measurements up to the 4-month visit compared to the 2 or 3-month visits. Diagnostic performance improved when suppression was defined by two consecutive suppressed viral loads compared to one.

**Conclusions:** Viral load measurements can be used to predict if a patient will be suppressed by 6-months post-cART. Graphical presentations of this information could help clinicians decide the optimum time to switch treatment regimen during the first months of cART.
**Keywords:** combination antiretroviral therapy, CD4 cell count, HIV-1, predicted virological suppression, treatment switch, viral load
Predicting virological decay in patients starting combination antiretroviral therapy

Running head: Predicting viral decay on first-line cART

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Abstract

Objective: Model trajectories of viral load measurements from time of starting combination antiretroviral therapy (cART), and use the model to predict whether patients will achieve suppressed viral load (≤ 200 copies/mL) within 6-months of starting cART.

Design: Prospective cohort study including HIV-positive adults (UK Collaborative HIV Cohort Study).

Methods: Eligible patients were antiretroviral-naïve and started cART after 1997. Random-effects models were used to estimate viral load trends. Patients were randomly selected to form a validation dataset with those remaining used to fit the model. We evaluated predictions of suppression using indices of diagnostic test performance.

Results: Of 9562 eligible patients 6435 were used to fit the model and 3127 for validation. Mean log_{10} viral load trajectories declined rapidly for 2-weeks post-cART, moderately between 2-weeks and 3-months, and more slowly thereafter. Higher pre-treatment viral load predicted steeper declines, whilst older age, white ethnicity and boosted-PI/NNRTI-based cART-regimen predicted a steeper decline from 3-months onwards. Specificity of predictions and the diagnostic odds-ratio substantially improved when predictions were based on viral load measurements up to the 4-month visit compared to the 2 or 3-month visits. Diagnostic performance improved when suppression was defined by two consecutive suppressed viral loads compared to one.

Conclusions: Viral load measurements can be used to predict if a patient will be suppressed by 6-months post-cART. Graphical presentations of this information could help clinicians decide the optimum time to switch treatment regimen during the first months of cART.

Keywords: combination antiretroviral therapy, CD4 cell count, HIV-1, predicted virological suppression, treatment switch, viral load
Introduction

Combination antiretroviral therapy (cART) based on ≥3 antiretroviral drugs from at least two drug classes slows HIV replication and prevents transmission of HIV. Factors taken into consideration when selecting a patient’s first cART-regimen include: the presence/absence of genotypic resistance against specific antiretroviral drugs; potential side-effects; comorbidities; drug-interactions and patient preference[1]. Current guidelines recommend monitoring the effectiveness of first-line cART using routine viral load (VL) measurements (copies of HIV-1 RNA per millilitre of plasma)[1–3], at about 4-weeks after initiation of treatment and then every 3-months to confirm undetectable VL levels[1].

HIV-dynamic studies have improved our understanding of the process of virus elimination after initiation of cART[4–5]. During the first few weeks of treatment there is a rapid decline in VL, primarily due to the decay of productively infected cells[4,6–8]. The rate of decay becomes slower thereafter due to the release of HIV viruses by macrophages and other long-lived cells of the lymph nodes[4,5,8]. Finally, the decline levels off, probably due to reservoirs of long-lived cells still producing HIV virus[4]. In some cases the VL level may rise again, for example because of non-adherence to the cART regimen or emergence of resistant virus[4].

Clinicians may be tempted to increase monitoring or switch drug therapy during the phase of slow VL decline, even though this is predictable and the patient is likely to achieve viral suppression. Early treatment switching may be unnecessary and has disadvantages including that the new regimen may be less effective than the current one, a reduction in the number of available future treatment options, and the possibility of side-effects associated with the new
regimen. Conversely, delays in switching regimen after virologic failure has occurred could result in the accumulation of resistance mutations, immunologic decline and an increased risk of clinical events. Guidelines recommend that a switch of cART-regimen should be considered if a patient’s VL fails to fall to undetectable levels (<50 copies/mL) after 24 to 36-weeks of treatment[1,2].

In this article we model repeated measurements of VL from start of cART to the first suppressed VL. Among patients with ≥2 observed measurements, we use this model to predict a patient’s future post-cART VL measurements given their observed measurements up to 2,3 or 4-months post-cART. Based on these future measurements we predict whether patients will achieve a suppressed VL measurement within 26-weeks of start of cART, test the reliability of these predictions, and show how this information can be used to enhance decisions on when to switch first-line cART.

**Methods**

**Study patients**

The UK Collaborative HIV Cohort (UK-CHIC) study was initiated in 2001 and collates routine data on HIV-positive patients attending some of the largest clinical centres in the UK since 1st January 1996. The project was approved by a Multicentre Research Ethics committee and local ethics committees. Patients are included in the study provided they are HIV-positive, have attended one of the collaborating centres at any time since 1996 and are aged 16 years or over[9]. Analyses are based on data collected up to 31st December 2012.

Patients were eligible for analysis if they were antiretroviral-naïve, started cART after 1997, had at least one CD4 measurement within the period 90-days before to 6-days after starting
cART, at least one VL measurement within the period 90-days before to 0-days after starting cART, and at least two post-cART VL measurements observed within the first year of starting cART, where the first measurement was >200 copies/mL. Suppression was a priori defined as a single VL ≤200 copies/mL.

Statistical analyses

Because we were only concerned with modelling the viral decay phase from start of treatment to time of first suppression within the first year of cART, VL measurements after time of first suppression or first year of cART were censored. Patients may stop or switch treatment regimens due to toxicities, side-effects, suspected treatment nonresponse and other problems. Because stopping or switching treatment due to suspected treatment nonresponse could have biased our analyses and reasons for switching were sparsely recorded, we censored VL measurements after a patient stopped treatment for at least 7-days or switched treatment. For a minority of patients their first suppressed VL, included in the analysis, was below the detection limit and was replaced with the detection limit value.

VL measurements were $\log_{10}$-transformed in order to stabilize the variance and to meet normality assumptions of the residuals[10]. When modelling the relationship between $\log_{10}$-transformed VL and time we considered a fractional-polynomial of one and two degrees with powers -2, -1, -0.5, 0, 0.5, 1, 2, 3 (power zero is interpreted as a natural-log transformation)[11] and linear-spline models of one and two knots with the first knot at 2, 4 or 6-weeks and the second knot at 2, 3 or 4-months. We fitted random-effects models with the intercept and trajectory terms random at the patient-level, thus allowing VL trajectories to vary between patients. We compared the fractional-polynomials and linear-spline models
with respect to the Bayesian Information Criterion (BIC) and satisfaction of the model’s assumptions[12].

Patients were classified by their first-line cART-regimen (NNRTI-based, PI-based, boosted-PI, other), pre-treatment CD4 count (<25, 25 to 49, 50 to 99, 100 to 199, 200 to 349, 350 to 499, ≥500 cells/µL) and pre-treatment VL (<10000, 10000 to <100000, 100000 to <500000, ≥500,000 copies/mL). Patients with >1 measurement within the pre-treatment period were classified using the measurement closest to the start of cART.

We included covariates sex, age at start of cART, ethnicity, exposure, type of first-line cART-regimen, pre-treatment CD4 cell count and pre-treatment VL. For each covariate, interactions between the covariate and the intercept and trajectory terms were considered. We compared the BIC statistic of all models with up to 5 interactions.

Predictions of future VL measurements and the associated prediction error (the measure of uncertainty about those predictions) depend upon the fixed-effects coefficients and the variance parameters[13,14]. See Appendix for details about generation of these predictions and prediction error.

We validated the prediction model by randomly selecting patients to form a validation dataset. Because our aim was to predict suppression within the first 6-months of a patient starting (and continuing on) their first cART-regimen, to form the validation dataset we randomly selected 40% of those patients who did not switch or stop treatment either before their first suppressed VL or during the first 6-months since starting cART. The remaining patients (including those ineligible for random selection) formed the model-fitting dataset.
All patients in the model-fitting and validation datasets were used in the analysis to validate the prediction model. The model-fitting dataset was the training data for our prediction model. Using this model we predicted future VL measurements for patients of the validation dataset. For patients in the model-fitting dataset we used all of their observed VL measurements up to one year post-cART. And, for patients in the validation dataset we categorized VL measurements within specific clinic visits by rounding the measurement time to the nearest month (e.g. measurements at 2.7 and 3.12 months were categorized as observed at the 3-month visit). Observed VL measurements up to and including specified clinic visits were used to predict future measurements. We only predicted future measurements among patients who were not censored (due to suppression, treatment switching or dropout) at the follow-up prior to the time-interval being predicted.

Based on the predicted future VL trajectories we predicted whether each patient would achieve suppression (single predicted VL ≤ 200 copies/mL) within 6-months of starting cART. We also classified patients in the validation dataset according to whether they were observed to achieve suppression (single observed VL ≤ 200 copies/mL) within 6-months of starting cART. We evaluated prediction of suppression using common indices of diagnostic test performance: sensitivity, specificity, positive-predictive value, negative-predictive value, likelihood-ratio of a positive result, likelihood-ratio of a negative result and the diagnostic odds-ratio (DOR) [15]. We conducted four sensitivity analyses: (1) suppression defined by two consecutive VL measurements ≤200 copies/mL, (2) patients of the validation dataset randomly selected from all eligible patients, (3) VL measurements not censored after a patient stopped or switched treatment, and (4) among the first suppressed VL measurements we censored those measurements below the detection limit.
Following Taylor, Yu and Sandler[16], we derived prediction-graphs depicting patients’ predicted VL measurements (with 95% prediction intervals) up to 6-months post-cART, patients’ observed measurements from previous visits and their measurement from the current visit. Using this most recent measurement, a new graph can be produced, allowing real time monitoring of patients’ progression.

**Results**

Of 47201 patients included in UK-CHIC up to 31st December 2012, 24135 started cART before 1998 or before entering the study, or did not start cART. A further 5235 had no CD4 or VL measurements within the specified pre-treatment periods. Of the remaining patients, 1617 were suppressed before start of cART, 519 had zero post-cART VL measurements, 385 had one (unsuppressed) post-cART VL measurement, and for 5748 their first post-cART VL measurement was suppressed, leaving 9562 eligible for analyses. Table 1 presents patient characteristics according to pre-treatment VL. Most were men, approximately half were homosexual or bisexual, of white ethnicity and started on a NNRTI-based cART-regimen. Compared with patients with pre-treatment VL ≥10000 copies/mL, a higher proportion of patients with pre-treatment VL <10000 copies/mL were female, Black African, heterosexual and started on a boosted-PI cART-regimen. Median pre-treatment CD4 decreased with increasing pre-treatment VL.

A total of 7249 (76%) patients achieved at least one suppressed VL measurement within the first year of cART. Among these, the median time to first suppressed VL measurement was 2.76 [interquartile range (IQR) 1.91–3.91] months and the median number of VL measurements, up to and including the first suppressed measurement, was 4 [IQR 3–5]
measurements. Of the 2313 (24%) patients who did not achieve at least one suppressed VL, the median number of VL measurements was 3 [IQR 2–4].

Among the 9562 patients eligible for analysis, 1649 (17%) stopped their first-line cART-regimen (for at least 7-days) or switched to a second-line cART-regimen either before their first suppressed VL or during the first 6-months after starting cART. We randomly selected 3127 (40%) of the remaining 7913 patients to form the validation dataset. The 6435 patients not randomly selected (including the 1649 ineligible for random selection) formed the model-fitting dataset. Figure 1 shows how the patients eligible for analysis were assigned to the validation and model-fitting datasets. The patients’ characteristics in the model-fitting (Appendix-table 2) and validation (Appendix-table 3) datasets were similar.

Figure 2 shows mean log_{10} VL trajectories predicted by the best fitting model, a linear-spline with knots at 2-weeks and 3-months post-cART, in which mean log_{10} VL trajectories varied between patients with different pre-treatment VL group, age at start of cART, ethnic group and type of first-line cART-regimen. For all patient groups except those with pre-treatment VL <10,000 copies/mL, mean log_{10} VL trajectories declined rapidly between start of cART and 2-weeks post-cART, moderately between 2-weeks and 3-months and more slowly from 3-months onwards. Higher pre-treatment VL predicted a steeper decline in mean log_{10} VL for all three phases. For example, among patients with pre-treatment VL between 10,000 and <100,000 copies/mL estimated decline in mean log_{10} VL during phases 1,2 and 3 were respectively 3.58 [95% CI 3.52, 3.65], 0.39 [95% CI 0.36, 0.41] and 0.06 [95% CI 0.03, 0.08] log_{10} copies/mL per month, whilst among patients with pre-treatment VL ≥500,000 copies/mL the corresponding declines were 4.46 [95% CI 4.38, 4.54], 0.56 [95% CI 0.53, 0.59] and 0.15 [95% CI 0.12, 0.17] log_{10} copies/mL per month. For the first and
second phases there was little difference according to age and ethnic group, and the decline of
mean log_{10} VL was more gradual for PI-based regimen than for the other cART-regimen
groups. During the third-phase, older age at start of cART predicted a steeper decline, the
decline was steeper for White than non-White patients, and steeper for boosted-PI and
NNRTI-based regimens than for PI-based or other regimens.

Table 2 compares observed and predicted viral suppression within 6-months of start of cART
among patients in the validation dataset, based on observed VL measurements up to and
including the 2, 3 and 4-month visits. Because predictions were not generated for patients
who were censored on or before the specified visit or who did not have an observed
measurement at the specified visit, the number of patients in the validation dataset decreases
from the 2-month to the 4-month visit. Between the 2 and 4-month visits, specificity of the
predictions substantially improved whilst sensitivity of the predictions slightly decreased.
Diagnostic accuracy improved substantially, from DOR 5.25 [95% CI 4.09, 6.74] at 2-
months to 15.60 [10.77, 22.56] at 4-months.

Compared to suppression defined by a single VL ≤200 copies/mL, under the stricter
definition of suppression based on two consecutive VLs ≤200 copies/mL then, at each
specified visit, the number of patients at risk (i.e. not previously suppressed) was higher and
the percentage of patients observed and predicted to be suppressed was lower (Appendix-
table 4). Specificity and negative-predictive value were substantially higher under the stricter
definition of suppression. All indicators of diagnostic performance showed greater accuracy
of predicting suppression when suppression was defined by two consecutive VLs ≤200
copies/mL compared to a single VL ≤200 copies/mL.
The results of the remaining sensitivity analyses, where: the validation dataset was a random sample of all patients eligible for analysis (Appendix-table 5), measurements after stopping or switching treatment were not censored (Appendix-table 6) and first suppressed VLs below the detection limit were censored (Appendix-table 7), were similar to the results of the main analysis (Table 2).

Predicting time to suppression

Figure 3 compares observed with predicted future VL measurements before and after 3-month visit, for patients who were selected to illustrate a range of VL patterns and predictions. The shaded areas denote 95% prediction intervals for each patient. Because patients had a small number of observed measurements the prediction intervals were wide. At the 3-month visit patient-A was not predicted to achieve suppression within 6-months of starting cART (left-hand graph). The new measurement (labelled +) was better than expected (below the predicted trajectory) and the updated graph predicted a steeper decline from 3 to 6-months (right-hand graph), although still not predicted to be suppressed by 6-months. Patient-B was predicted to be suppressed approximately 3-months post-cART (left-hand graph) and the new measurement agrees with the predicted trajectory, and so very little has changed in the updated prediction (right-hand graph). Based on these graphs, a clinician may decide that patients A and B should continue on their first-line cART-regimen, as they are predicted to decline steadily, and to next measure the patients’ VL at the 5-month visit to confirm that they have become suppressed. Patient-C was initially predicted to achieve suppression by 3-months post-cART and patient-D was predicted to steadily decline almost achieving suppression by 6-months. Their 3-month measurements were worse than expected (above the predicted trajectory) and the updated graphs show that they were less likely to be suppressed by 6-months, which is consistent with their future measurements. For patient-C a
clinician may decide at the 3-month visit to switch to second-line cART therapy as the patient’s trajectory is predicted to level off to above 200 copies/mL. For patient-D a clinician may decide to continue with the first-line cART therapy and to measure the patient’s VL at 4-months post-cART to confirm that the decline has slowed down. The clinician could then update the prediction-graph using the 4-month measurement and review the decision to maintain the first-line regimen.

Discussion

We fitted a flexible linear mixed-effects model to repeated VL measurements from the time of starting cART, and used this model to predict the effectiveness of the first cART-regimen in achieving VL suppression based on individual patients’ pre-treatment clinical information and post-cART VL measurements. Mean log_{10} VL trajectories declined rapidly between start of cART and 2-weeks post-cART, moderately between 2-weeks and 3-months and more slowly thereafter. Higher pre-treatment VL predicted a steeper decline in mean log_{10} VL for all three phases. During the third-phase, older age at start of cART predicted a steeper decline, the decline was steeper for White than non-White patients, and steeper for boosted-PI and NNRTI-based regimens than for PI-based or other regimens. The model’s predictive ability improved markedly when based on VL measurements up to the 4-month clinic visit compared to the 2 or 3-month visits. Patients’ current VL trajectory and future VL predictions can be graphically presented and used to assess if a patient is likely to become virologically suppressed within 6-months of start of treatment whilst on their current regimen.

Among the patients eligible for analysis 60% (5753) had a least one post-cART VL within the first 2-weeks since starting treatment and so we are confident that our data supports
estimation of a change in VL within the first 2-weeks. A key feature is that the model predicts future VL measurements using a series of observed measurements, making efficient use of all available data. Furthermore, the predictions can be updated as new measures are obtained, which further improves prediction accuracy.

This study has several limitations. Patients’ measurements were censored after the first occurrence of a suppressed VL measurement and so those patients who had a rapid decline in VL contribute only a few observations to the model. Our model cannot reliably predict suppression before 3-months post-cART, which occurred among 3187 (33%) of the patients eligible for inclusion in our analyses. Only a few patients were treated with integrase inhibitors, which are now more widely used. Our predictions were based on a small number of observed measurements: the prediction intervals were consequently wide. Some patients stopped taking treatment or switched to a second-line cART-regimen before their VL measurements had dropped below 200 copies/mL. Information on reasons for a change in treatment was not available. We censored all VL measurements that were observed after a patient stopped or switched treatment and, in a sensitivity analysis, inclusion of these censored measurements did not change our conclusions. Lastly, patients may have dropped out of the study due to reasons unrelated to virological response, or because of loss to follow-up or AIDS-related mortality. Random-effects models, as used in this study, are robust to dropout that is predictable from observed data (‘missing at random’) [17,18] but our estimates may have been biased by a dropout mechanism that is not predicted by observed VL measurements.

Several HIV-dynamic studies, modelling data from start of treatment up to 8 or 12-weeks post-treatment, have reported a rapid decline in weeks 1 to 3 and a slower decline
thereafter[19-28]. A HIV-dynamic study with 72-weeks of follow-up reported three phases of decreasing decay rates, where the transition from phases 1 to 2 was estimated at 16.1 days and from phases 2 to 3 at 15.7 weeks[29]. A cohort of cART-naïve and cART-experienced patients, with measurements at 2-weeks, 3, 6 and 9-months, modelled viral decay using a linear-spline with a single knot at 3-months[30].

Our finding that higher pre-treatment VL predicted steeper declines in mean log_{10} VL is broadly consistent with the literature[19,21,28,31]. Findings in some studies that trends did not differ by pre-treatment VL[20], or that higher pre-treatment VL predicted slower decline during phase-1[22,26], may be explained by differences in the potency of the treatment regimens and pre-treatment virus clearance-ratios and turnover rates of infected cells[21]. Although a few small studies (<225 patients) reported that VL trends did not differ by age or ethnicity[19,22,30], our findings that older age predicted steeper declines and that declines were steeper for White than non-White patients are consistent with reports that older age predicted a shorter time to suppression[32-37] and that White patients are more likely to become suppressed than non-Whites[37-43]. In keeping with our results Wu et al[21] reported a steeper decline for NNRTI-based regimens compared to a PI-based regimen.

Several studies have reported that declines in VL during weeks 1 to 3 predicted virological response at 8, 12 and 24-weeks[19,23,24,27] and that VL measurements at 4 and 8-weeks were strong predictors of virological response at 24-weeks[44,45]. However, our study is the first of which we are aware to use all available VL measurements to predict first suppression by 24-weeks.
We have shown that frequent VL monitoring can reliably predict by 4-months post-cART if a patient will be suppressed within 6-months of starting treatment. Presenting the observed and future predicted measurements in a graphical plot could aid clinicians in their decision whether to change cART regimens in patients not suppressed by 3-months post-cART. Possible actions might include: returning at 6-months post-cART to confirm VL suppression, returning in 1-month for next VL measurement to minimize any uncertainty, or switch to second-line therapy. We hope that the information provided in these prediction-graphs will provide reassurance in making robust decisions regarding future cART-regimens, and avoid unnecessary changes of regimen.

In summary, we have shown how a series of VL measurements can be utilized to predict future VL measurements, and how this information can be presented graphically. Future work could extend models to allow for informative dropout and develop a web-based tool[46], where a clinician inputs the information into a web-based calculator and the tool outputs a prediction-graph.
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Conflicts of interest

There are no conflicts of interest.
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Table 1. Characteristics of the 9562 eligible patients

|                              | Pre-treatment HIV-1 RNA (copies/mL) | Pre-treatment HIV-1 RNA (log_{10} copies/ml) | Pre-treatment CD4 cell count (cells/μL) |
|------------------------------|-------------------------------------|---------------------------------------------|---------------------------------------|
|                              | <10k<sup>b</sup>                  | 10k to <100k                   | 100k to <500k                  | ≥500k                  |
| Number of patients           | 756                                 | 3372                           | 3825                                | 1609                                |
| Median (IQR)<sup>a</sup> age (years) | 36 (31-42)                       | 37 (31-43)                     | 37 (32-44)                           | 38 (33-45)                           |
| Male %                       | 56                                  | 74                            | 79                                   | 79                                   |
| Risk group %                 |                                     |                               |                                      |                                      |
| Homo/bisexual                | 35                                  | 55                            | 61                                   | 59                                   |
| IDU                          | 4                                   | 2                             | 2                                    | 2                                    |
| Heterosexual                  | 55                                 | 37                            | 32                                   | 35                                   |
| Other/not known              | 6                                   | 5                             | 4                                    | 4                                    |
| Ethnicity %                  |                                     |                               |                                      |                                      |
| White                        | 40                                 | 57                            | 61                                   | 62                                   |
| Black African                | 43                                 | 27                            | 23                                   | 25                                   |
| Other                        | 14                                 | 14                            | 14                                   | 12                                   |
| Not known                    | 3                                  | 2                             | 2                                    | 1                                    |
| First-line cART-regimen %    |                                     |                               |                                      |                                      |
| NNRTI-based                   | 52                                 | 63                            | 67                                   | 63                                   |
| PI-based                      | 8                                  | 5                             | 5                                    | 5                                    |
| Boosted-PI                    | 33                                 | 27                            | 23                                   | 27                                   |
| Other                        | 7                                  | 5                             | 5                                    | 5                                    |
| Median (IQR) pre-treatment HIV-1 RNA (log_{10} copies/ml) | 3.43                             | 4.67                          | 5.32                                 | 5.88                                 |
| Median (IQR) pre-treatment CD4 cell count (cells/μL) | (2.86-3.78)                       | (4.43-4.86)                   | (5.15-5.50)                          | (5.71-6.00)                          |
|                              | 272                                 | 236                           | 180                                  | 114                                  |
|                              | (180-400)                          | (159-320)                     | (84-270)                             | (42-218)                             |

<sup>a</sup> IQR: Inter-quartile range; <sup>b</sup> k: A thousand.
Table 2. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit

|                      | 2-month visit | 3-month visit | 4-month visit |
|----------------------|---------------|---------------|---------------|
| No. patients$ ^5    | 1927          | 1127          | 698           |
| Observed suppressed  | 81%           | 69%           | 51%           |
| Predicted suppressed | 80%           | 67%           | 51%           |
| Sensitivity [95% CI] | 86% [84%, 88%] | 81% [79%, 84%] | 80% [76%, 85%] |
| Specificity [95% CI] | 46% [41%, 51%] | 63% [58%, 68%] | 79% [75%, 83%] |
| PPV [95% CI]         | 87% [85%, 89%] | 83% [80%, 86%] | 80% [76%, 84%] |
| NPV [95% CI]         | 44% [39%, 49%] | 60% [55%, 65%] | 79% [75%, 84%] |
| LR+ [95% CI]         | 1.60 [1.45, 1.76] | 2.21 [1.92, 2.55] | 3.86 [3.12, 4.78] |
| LR– [95% CI]         | 0.30 [0.26, 0.36] | 0.30 [0.25, 0.35] | 0.25 [0.20, 0.31] |
| DOR [95% CI]         | 5.25 [4.09, 6.74] | 7.49 [5.65, 9.93] | 15.60 [10.77, 22.56] |

$ ^5 $ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.
Fig. 1. A flowchart depicting assignment of the patients eligible for analysis to the validation and model-fitting datasets.
Fig. 2. Predicted mean \( \log_{10} \) HIV-1 RNA trajectories within the first year of starting combination antiretroviral therapy (cART) according to (A) baseline viral load groups, (B) age at start of cART, (C) ethnic group and (D) type of cART-regimen. The solid black line in each graph denotes the predicted mean \( \log_{10} \) HIV-1 RNA trajectory for the reference patient: white male, aged 35 years at start of cART, homosexual or bisexual, first-line cART-regimen includes a NNRTI, pre-treatment CD4 count between 200 and 349 cells/\( \mu \)L and pre-treatment viral load between 100,000 and < 500,000 copies/mL.
Before 3-month visit

After 3-month visit

- : Observed log_{10} HIV-1 RNA measurement from the analysis dataset
+ : Observed log_{10} HIV-1 RNA measurement at 3-month clinic visit
Δ : Future observed log_{10} HIV-1 RNA measurement from the validation dataset
--- : Log_{10} 200 copies/mL
Fig. 3. Prediction graphs of 4 selected patients based on observations measured before 3-month visit (left-hand column) and on observations measured after 3-month visit (right-hand column). The solid line is the patient’s predicted log_{10} HIV-1 RNA trajectory with 95% uncertainty intervals (shaded regions). The dashed line indicates the cut-off for suppression (200 copies/mL).
Appendix

We used the parameter estimates from our final random-effects model (Appendix-table 1) to generate predictions of future viral load (VL) measurements and the associated prediction error. Following Taylor and Law, we describe how these predictions were generated for patient i [1].

Suppose patient i has $n_i$ observed VL measurements $Y_i = (Y_{i1}, \ldots, Y_{ij}, \ldots, Y_{in_i})$, where $Y_{ij}$ is the log10 VL measurement observed at measurement time-point $j$. The random-effects model is

$$Y_i = X_i \beta + Z_i u_i + e_i,$$

with fixed-effects coefficients $\beta$ and design matrix $X_i$, random-effects coefficients $u_i$ and design matrix $Z_i$ and level-1 residuals $e_i$. The random effects $u_i$ and residuals $e_i$ are independently, normally distributed with zero means and covariances $G$ and $\sigma^2 I_{n_i}$.

We wish to predict $n_i^F$ future log10 VL measurements $Y_i^F$ at pre-specified time-points. Let $X_i^F$ and $Z_i^F$ denote the fixed-effects and random-effects design matrices corresponding to these future time-points. To generate the predictions we require the following components: $\Omega_i = Z_i G (Z_i)^T + \sigma^2 I_{n_i}$, $\Lambda_i = Z_i^F G (Z_i)^T$ and $\Omega_i^F = Z_i^F G (Z_i^F)^T + \sigma^2 I_{n_i^F}$. The prediction of future measurements $Y_i^F$ given the observed measurements $Y_i$ is

$$Y_i^F = X_i^F \beta + \Lambda_i (\Omega_i)^{-1} (Y_i - X_i \beta)$$

and the prediction error is

$$\Omega_i^F - \Lambda_i^F (\Omega_i)^{-1} (\Lambda_i^F)^T + (X_i^F - \Lambda_i^F (\Omega_i)^{-1} X_i) var(\beta) (X_i^F - \Lambda_i^F (\Omega_i)^{-1} X_i)^T.$$
where \( \text{var}(\beta) \) represents the covariance matrix of the fixed-effects coefficients \( \beta \) and is obtained from the fitted random-effects model.

Our approximation for the prediction error accounted for uncertainty in the estimation of the fixed effects coefficients and variance parameters, but not the estimation of the variances and covariances between the observed and future measurements[1]. Provided the sample size is reasonably large then this uncertainty can be ignored[1,2].
### Appendix-table 1: Coefficients of the final model

| Fixed effects                  | Estimates reported on the log_{10} scale [95% confidence interval] |
|-------------------------------|---------------------------------------------------------------|
| Reference group               |                                                               |
| Time\(^a\): 0 to 2 weeks     | -4.18 [-4.24, -4.12]                                          |
| Time: 2 weeks to 3 months     | -0.49 [-0.51, -0.47]                                          |
| Time: 3 to 12 months          | -0.10 [-0.08, -0.13]                                          |
| Compared to the reference group |                                                               |
| IDU                           | 0.03 [-0.02, 0.07]                                            |
| Heterosexual                  | -0.01 [-0.03, 0.02]                                           |
| Other risk group              | 0.01 [-0.03, 0.04]                                            |
| CD4\(^c\) < 25                | 0.12 [0.09, 0.15]                                             |
| CD4 25 to 49                  | 0.11 [0.07, 0.14]                                             |
| CD4 50 to 99                  | 0.10 [0.08, 0.12]                                             |
| CD4 100 to 199                | 0.05 [0.03, 0.07]                                             |
| CD4 350 to 500                | -0.02 [-0.04, 0.01]                                           |
| CD4 \(\geq\) 500             | 0.003 [-0.03, 0.04]                                           |
| VL\(^d\) \(<\) 10000         | -1.48 [-1.51, -1.45]                                          |
| Time: 0 to 2 weeks            | 1.91 [1.78, 2.04]                                             |
| Time: 2 weeks to 3 months     | 0.35 [0.31, 0.39]                                             |
| Time: 3 to 12 months          | -0.02 [-0.06, 0.02]                                           |
| VL 10000 to \(<\) 100000     | -0.60 [-0.62, -0.58]                                          |
| Time: 0 to 2 weeks            | 0.60 [0.53, 0.67]                                             |
| Time: 2 weeks to 3 months     | 0.06 [0.03, 0.08]                                             |
| Time: 3 to 12 months          | 0.04 [0.01, 0.07]                                             |
| VL \(\geq\) 500000           | 0.50 [0.48, 0.52]                                             |
| Time: 0 to 2 weeks            | -0.32 [-0.41, -0.24]                                          |
| Time: 2 weeks to 3 months     | -0.08 [-0.11, -0.06]                                          |
| Time: 3 to 12 months          | -0.05 [-0.09, -0.02]                                          |
| Age at start of cART          | -0.001 [0.00, 0.002]                                          |
| Time: 0 to 2 weeks            | -0.0004 [-0.004, 0.003]                                       |
| Time: 2 weeks to 3 months     | -0.003 [-0.004, -0.002]                                       |
| Time: 3 to 12 months          | -0.001 [-0.002, 0.0005]                                       |
| Black African                 | -0.04 [-0.06, -0.01]                                          |
| Time: 0 to 2 weeks            | -0.06 [-0.13, 0.01]                                           |
| Time: 2 weeks to 3 months     | 0.09 [0.06, 0.11]                                             |
| Time: 3 to 12 months          | 0.04 [0.02, 0.07]                                             |
| Other ethnicity               | -0.02 [-0.04, 0.001]                                          |
| Time: 0 to 2 weeks            | -0.08 [-0.17, 0.004]                                          |
| Time: 2 weeks to 3 months     | 0.03 [-0.002, 0.06]                                           |
| Time: 3 to 12 months          | 0.04 [0.004, 0.08]                                            |
Appendix-table 1 continued: Coefficients of the final model

| Fixed effects                | Estimates reported on the log_{10} scale [95% confidence interval] |
|------------------------------|---------------------------------------------------------------------|
| **Unknown ethnicity**        |                                                                     |
| Constant                     | -0.01 [-0.06, 0.05]                                                 |
| Time: 0 to 2 weeks           | 0.25 [0.03, 0.47]                                                   |
| Time: 2 weeks to 3 months    | 0.07 [-0.002, 0.14]                                                 |
| Time: 3 to 12 months         | 0.03 [-0.06, 0.11]                                                  |
| **PI-based regimen**         |                                                                     |
| Constant                     | -0.02 [-0.05, 0.01]                                                 |
| Time: 0 to 2 weeks           | 0.37 [0.22, 0.51]                                                   |
| Time: 2 weeks to 3 months    | 0.09 [0.04, 0.14]                                                   |
| Time: 3 to 12 months         | 0.08 [0.04, 0.12]                                                   |
| **Boosted PI-based Regimen** |                                                                     |
| Constant                     | 0.02 [0.002, 0.03]                                                  |
| Time: 0 to 2 weeks           | 0.31 [0.24, 0.38]                                                   |
| Time: 2 weeks to 3 months    | -0.05 [-0.07, -0.02]                                                |
| Time: 3 to 12 months         | -0.03 [-0.06, -0.001]                                               |
| **Other regimen**            |                                                                     |
| Constant                     | -0.05 [-0.08, -0.02]                                                |
| Time: 0 to 2 weeks           | 0.22 [0.08, 0.35]                                                   |
| Time: 2 weeks to 3 months    | 0.06 [0.01, 0.10]                                                   |
| Time: 3 to 12 months         | 0.04 [-0.01, 0.09]                                                  |
| **Random effects**           | Variance or covariance [95% confidence interval]                    |
| Individual level             |                                                                     |
| Constant                     | 0.036 [0.033, 0.039]                                                |
| 0 to 2 weeks                 | 0.624 [0.566, 0.689]                                                |
| 2 weeks to 3 months          | 0.067 [0.061, 0.073]                                                |
| 3 to 12 months               | 0.039 [0.034, 0.044]                                                |
| Constant, 0 to 2 weeks       | 0.034 [0.024, 0.043]                                                |
| Constant, 2 weeks to 3 months| -0.008 [-0.012, -0.005]                                             |
| Constant, 3 to 12 months     | -0.005 [-0.009, -0.001]                                             |
| 0 to 2 weeks, 2 weeks to 3 months | 0.041 [0.026, 0.056]                       |
| 0 to 2 weeks, 3 to 12 months | -0.070 [-0.087, -0.054]                                             |
| 2 weeks to 3 months, 3 to 12 months | 0.002 [-0.003, 0.006]                     |
| Measurement level            |                                                                     |
| Constant                     | 0.159 [0.155, 0.162]                                                |

*Reference patient: white male, aged 35 years at start of cART, homosexual or bisexual, first-line cART-regimen includes a NNRTI, pre-treatment CD4 count between 200 and 349 cells/µL and pre-treatment viral load between 100,000 and < 500,000 copies/mL.  
*b Time since start of cART.  
*c Pre-treatment CD4 cell count (cells/µL).  
*d Pre-treatment viral load (copies/mL).
Appendix-table 2. Characteristics of the 6435 patients from the model-fitting dataset

| Pre-treatment HIV-1 RNA (copies/mL) | <10k | 10k to <100k | 100k to <500k | ≥500k |
|------------------------------------|------|---------------|---------------|------|
| Number of patients                 | 520  | 2227          | 2599          | 1089 |
| Median (IQR)$^a$ age (years)       | 37 (31-43) | 37 (31-43) | 37 (32-43) | 38 (32-45) |
| Male %                             | 56   | 73            | 78            | 79   |
| Risk group %                       |      |               |               |      |
| Homo/bisexual                      | 34   | 54            | 61            | 59   |
| IDU                                | 4    | 3             | 2             | 2    |
| Heterosexual                       | 56   | 38            | 33            | 34   |
| Other/not known                    | 6    | 5             | 4             | 5    |
| Ethnicity %                        |      |               |               |      |
| White                              | 40   | 56            | 60            | 62   |
| Black African                      | 43   | 27            | 23            | 24   |
| Other                              | 14   | 15            | 14            | 13   |
| Not known                          | 3    | 2             | 2             | 2    |
| First-line cART-regimen %          |      |               |               |      |
| NNRTI                              | 54   | 63            | 66            | 63   |
| PI                                 | 8    | 6             | 5             | 5    |
| Boosted-PI                         | 32   | 26            | 24            | 27   |
| Other                              | 6    | 5             | 5             | 6    |
| Median (IQR) pre-treatment         | 3.41 | 4.67          | 5.32          | 5.88 |
| HIV-1 RNA (log$_{10}$ copies/ml)   | (2.81-3.79) | (4.43-4.86) | (5.16-5.51) | (5.71-6.01) |
| Median (IQR) pre-treatment         | 270  | 231           | 180           | 110  |
| CD4 cell count (cells/µL)          | (165-400) | (150-320) | (80-268) | (43-207) |

$^a$ IQR: Inter-quartile range; $^b$ k: A thousand
Appendix Table 3. Characteristics of the 3127 patients from the validation dataset.

| Pre-treatment HIV-1 RNA (copies/mL) | <10k | 10k to <100k | 100k to <500k | ≥500k |
|-------------------------------------|------|--------------|---------------|-------|
| Number of patients                  | 236  | 1145         | 1226          | 520   |
| Median (IQR)* age (years)           | 36 (31-41) | 36 (31-43) | 38 (32-44) | 39 (33-45) |
| Male %                              | 58   | 75           | 82            | 81    |
| Risk group %                        |      |              |               |       |
| Homo/bisexual                       | 38   | 58           | 63            | 59    |
| IDU                                 | 3    | 2            | 2             | 2     |
| Heterosexual                        | 51   | 36           | 31            | 36    |
| Other/not known                     | 7    | 5            | 4             | 3     |
| Ethnicity %                         |      |              |               |       |
| White                               | 41   | 58           | 61            | 62    |
| Black African                       | 44   | 28           | 23            | 26    |
| Other                               | 14   | 13           | 15            | 12    |
| Not known                           | 1    | 1            | 2             | 1     |
| First-line cART-regimen %           |      |              |               |       |
| NNRTI                               | 50   | 63           | 69            | 63    |
| PI                                  | 6    | 5            | 5             | 5     |
| Boosted-PI                          | 36   | 28           | 22            | 28    |
| Other                               | 8    | 4            | 4             | 4     |
| Median (IQR) pre-treatment          | 3.51 | 4.68         | 5.31          | 5.86  |
| HIV-1 RNA (log_{10} copies/ml)      | (2.94-3.78) | (4.43-4.87) | (5.15-5.48) | (5.71-5.99) |
| Median (IQR) pre-treatment          | 276  | 242          | 187           | 122   |
| CD4 cell count (cells/µL)           | (196-391) | (168-321) | (90-277) | (40-242) |

a IQR: Inter-quartile range; b k: A thousand
Appendix Table 4. Sensitivity analysis regarding observed and predicted suppression defined respectively by two consecutive observed and predicted viral load measurements ≤200 copies/mL. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit.

|                      | 2-month visit | 3-month visit | 4-month visit |
|----------------------|---------------|---------------|---------------|
| No. patients\$       | 2787          | 2224          | 1782          |
| Observed suppressed  | 57%           | 51%           | 43%           |
| Predicted suppressed | 59%           | 51%           | 42%           |
| Sensitivity [95% CI] | 90% [89%, 92%]| 93% [92%, 95%]| 96% [94%, 97%]|
| Specificity [95% CI]| 82% [80%, 84%]| 93% [92%, 95%]| 96% [94%, 97%]|
| PPV [95% CI]         | 87% [85%, 89%]| 93% [92%, 95%]| 94% [92%, 96%]|
| NPV [95% CI]         | 86% [84%, 88%]| 93% [91%, 94%]| 97% [96%, 98%]|
| LR+ [95% CI]         | 5.03 [4.45, 5.68]| 13.37 [10.76, 16.61]| 21.40 [16.13, 28.39]|
| LR– [95% CI]         | 0.12 [0.10, 0.14]| 0.07 [0.06, 0.09]| 0.04 [0.03, 0.06]|
| DOR [95% CI]         | 42.16          | 183.15        | 481.66        |

\$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR– is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.
Appendix Table 5. Sensitivity analysis regarding the validation dataset was a random sample of the entire analysis dataset. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit.

| No. patients$^5$ | 2-month visit | 3-month visit | 4-month visit |
|------------------|---------------|---------------|---------------|
| Observed suppressed | 1486          | 872           | 532           |
| Predicted suppressed | 79%           | 66%           | 50%           |
| Sensitivity [95% CI] | 85% [83%, 87%] | 81% [77%, 84%] | 77% [72%, 82%] |
| Specificity [95% CI] | 45% [39%, 50%] | 63% [57%, 69%] | 76% [71%, 81%] |
| PPV [95% CI] | 84% [82%, 86%] | 81% [78%, 85%] | 76% [71%, 81%] |
| NPV [95% CI] | 47% [41%, 52%] | 62% [56%, 67%] | 78% [73%, 83%] |
| LR+ [95% CI] | 1.54 [1.40, 1.71] | 2.18 [1.86, 2.54] | 3.26 [2.60, 4.08] |
| LR− [95% CI] | 0.33 [0.27, 0.39] | 0.31 [0.26, 0.37] | 0.30 [0.24, 0.38] |
| DOR [95% CI] | 4.71 [3.60, 6.18] | 7.07 [5.16, 9.69] | 10.84 [7.25, 16.20] |

$^5$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR− is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.
Appendix Table 6. Sensitivity analysis regarding observations were not censored after the end of first-line cART. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit.

|                         | 2-month visit | 3-month visit | 4-month visit |
|-------------------------|---------------|---------------|---------------|
| **No. patients** \(^a\) | 1933          | 1134          | 707           |
| **Observed suppressed** | 81%           | 68%           | 50%           |
| **Predicted suppressed**| 75%           | 65%           | 51%           |
| **Sensitivity [95% CI]** | 82% [80%, 83%]| 79% [76%, 82%]| 79% [75%, 83%]|
| **Specificity [95% CI]** | 52% [47%, 57%]| 65% [60%, 70%]| 79% [74%, 83%]|
| **PPV [95% CI]**        | 88% [86%, 89%]| 83% [80%, 86%]| 79% [75%, 83%]|
| **NPV [95% CI]**        | 41% [36%, 45%]| 59% [54%, 64%]| 79% [75%, 83%]|
| **LR+ [95% CI]**        | 1.70 [1.53, 1.90]| 2.26 [1.96, 2.62]| 3.70 [3.01, 4.55]|
| **LR− [95% CI]**        | 0.35 [0.31, 0.41]| 0.32 [0.28, 0.38]| 0.26 [0.21, 0.33]|
| **DOR [95% CI]**        | 4.82 [3.79, 6.12]| 7.01 [5.31, 9.25]| 14.02 [9.77, 20.13]|

\(^a\) Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR− is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.
Appendix Table 7: Sensitivity analysis regarding censoring of first suppressed measurements below the detection of limit. Validation of the model for predicting future suppression by 6 months since start of treatment given observations up to a specified visit

|                | 2-month visit | 3-month visit | 4-month visit |
|----------------|---------------|---------------|---------------|
| No. patients$ | 1237          | 652           | 393           |
| Observed suppressed | 77%          | 61%           | 40%           |
| Predicted suppressed | 77%          | 63%           | 45%           |
| Sensitivity [95% CI] | 83% [81%, 86%] | 77% [73%, 82%] | 78% [71%, 84%] |
| Specificity [95% CI] | 46% [40%, 52%] | 61% [55%, 67%] | 77% [72%, 83%] |
| PPV [95% CI]       | 84% [81%, 86%] | 76% [72%, 80%] | 70% [63%, 77%] |
| NPV [95% CI]       | 44% [39%, 50%] | 63% [57%, 69%] | 84% [79%, 89%] |
| LR+ [95% CI]       | 1.53 [1.37, 1.71] | 1.99 [1.69, 2.35] | 3.45 [2.69, 4.44] |
| LR− [95% CI]       | 0.37 [0.30, 0.45] | 0.37 [0.30, 0.45] | 0.29 [0.21, 0.39] |
| DOR [95% CI]       | 4.16 [3.11, 5.55] | 5.41 [3.83, 7.64] | 12.07 [7.44, 19.59] |

$ Number of patients not suppressed at the specified visit and with at least one future measurement.

Abbreviations: CI is confidence interval; PPV is positive predictive value; NPV is negative predictive value; LR+ is likelihood ratio of a positive result; LR− is likelihood ratio of a negative result; DOR is diagnostic odds-ratio.
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1. Taylor JMG and Law N. Does the covariance structure matter in longitudinal modelling for the prediction of future CD4 counts? Stat Med 1998; 17: 2381–2394.

2. Diggle PJ, Heagerty P, Liang KY, and Zeger SL. Analysis of Longitudinal Data, 2nd edition. Oxford: Oxford University Press; 2002.
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Important Note: Once you electronically sign this form, you will not be able to make any additional changes to it.

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