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Residual viremia in HIV-infected patients who continue a two-drug or switch to a three-drug integrase strand transfer inhibitor based regimen

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In this randomized, single-centre, open-label, 96-week, superiority, controlled trial of 50 HIV-infected patients with HIV-RNA less than 50 copies/ml on a two-drug regimen based on dolutegravir as well as one reverse transcriptase inhibitor (RTI), switching to a single-tablet regimen of cobicistat, elvitregravir, emtricitabine along with tenofovir alafenamide did not appear to mitigate the burden of residual viremia, both at week 48 and at week 96. The immunological changes observed during follow-up and the safety of the two regimens were similar.

High-genetic barrier integrase strand transfer inhibitors (InSTIs) have made it possible to achieve and maintain virologic suppression also with two-drug regimens (2DRs) [1–3].

Although its origin and full implications have not been fully clarified, residual viremia below 50 copies/ml has been associated with a higher risk of virologic failure in retrospective studies [4–6].

We hypothesized that, despite its lower genetic barrier, switching to a single-tablet regimen of cobicistat/elvitregravir/emtricitabine/tenofovir alafenamide (E/C/F/TAF), in patients virologically suppressed on a 2DR based on dolutegravir (DTG) as well as one RTI, could reduce the exposure to residual viremia.

Here, we present the results of a randomized, single-centre, open-label, 96-week, superiority, controlled trial (Be-One Study, NCT03493568). The study has been approved by the Ethics Committee of the San Raffaele Scientific Institute, conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent.

Patients with HIV-RNA less than 50 copies/ml for at least 6 months while receiving DTG as well as one RTI, were randomized 1 : 1 to continue the ongoing treatment (2DR, control am) or to switch to E/C/F/TAF. Those with documented resistance to NRTIs or InSTIs were excluded. Viral load was measured by standard Abbott Real-Time PCR (Abbott Molecular Inc., Des Plaines, Illinois, USA).

Participants were evaluated at screening, baseline, week 4, week 24 and then every 24 weeks until week 96.

The Abbott Real-Time PCR assay provides three possible outputs: a quantitative result for HIV-RNA values more than 40 copies/mL, a qualitative result (target not detected, TND) when no HIV-RNA can be detected by the assay and a semiquantitative result for any HIV-RNA detectable below 40 copies/ml.

For the purpose of this study, we defined residual viremia as any detectable HIV-RNA below 50 copies/mL by Abbott Real-Time PCR assay. As a consequence, the absence of residual viremia was defined by a TND result at viral load determination.

The primary study endpoint was the proportion of participant with no residual viremia (i.e. TND) through 48 weeks of follow-up (i.e. TND result at all measurement dates). Secondary endpoints were virologic failure and changes in immunologic and metabolic parameters in the two arms through 96 weeks of follow-up.

Virologic failure was defined as a two consecutive HIV-RNA values at least 50 copies/ml.

A sample size of 50 patients per arm allowed to detect, with 80% power and $\alpha = 0.05$, a 25% increase in the primary endpoint in the E/C/F/TAF arm, assuming a 35% proportion in the control arm.

The primary efficacy analyses were performed on the intent-to-treat (ITT) population, including all randomized patients who took at least one dose of the study drug. The analyses on the secondary endpoints were performed on the per-protocol population, defined as ITT patients who completed the study protocol with no protocol violations.

A futility analysis was performed on the primary efficacy endpoint, when 50% of patients ($n = 50$) had been enrolled and had completed 24-week follow-up.

The primary efficacy analyses estimated the difference between the proportions of TND through 48 and 96 weeks of the two arms (E/C/F/TAF minus 2DR); the corresponding 95% confidence interval (95% CIs) were two-sided following the superiority study design.

Comparisons between study arms of other endpoints were conducted using Chi-square or Fisher's exact test or Wilcoxon rank-sum test, as appropriate.

Analyses were performed using SAS version 9.4 (SAS Inc., Cary, North Carolina, USA).

Fifty-one patients were randomized; 50 received at least one dose of study drug, 25 allocated to the E/C/F/TAF arm and 25 to the 2DR arm. At enrolment, 45 (90%) were receiving lamivudine and five (10%) were receiving rilpivirine, together with dolutegravir, since 1.27 (0.79–2.00) years.

Baseline characteristics are illustrated in detail in Table 1.

The trial was terminated by the investigators when the futility analysis determined a low probability that the final analysis would demonstrate superiority of E/C/F/TAF over 2DR arm (conditional power of 2% indicating that the trial was likely to be futile).

Throughout the first 48 weeks of follow-up, 12 out of 25 (48%) patients in the E/C/F/TAF arm and 19 out of 25 (76%) patients in the 2DR arm [difference E/C/F/TAF–DTG: −28.0% (95% CI: −57.8% to 1.8%; $P = 0.079$)] showed TND results at every measurement. Throughout 96 weeks of follow-up, the proportion of patients showing TND results at every measurement was 24% (6/25) in the E/C/F/TAF arm and 64% (16/25) in the 2DR arm (difference: −40.0%; 95% CI: −65.2 to −14.8; $P = 0.01$).

Reasons for study withdrawal were virologic failure (one in each arm, with no emergence of drug resistance in both cases), diarrhoea and weight gain in the E/C/F/TAF arm and consent withdrawal and central nervous system toxicity in the 2DR arm.

The immunological changes observed during follow-up and the safety of the two regimens were in general similar; details are reported in supplementary materials, http://links.lww.com/QAD/C98.

The results of this study suggest that intensifying treatment with a single tablet of E/C/F/TAF in patients receiving an effective 2DR of DTG along with one RTI, does not mitigate the burden of residual viremia. Despite

Table 1. Baseline patients' characteristics according to study arm.

	E/C/F/TAF ($n = 25$)	DTG + 1 RTI ($n = 25$)
Age (years)	54.1 (51.3–59.2)	48.6 (40.7–49.8)
Male sex	23 (92%)	21 (84%)
Italian origin	25 (100%)	24 (96%)
C3 stage at CDC classification	3 (12%)	2 (8%)
Years since first HIV diagnosis	14.3 (7.5–21)	7.4 (5.3–12.7)
Years since ART start	10.9 (4.2–16.8)	6.7 (4.5–10.9)
Months between HIV diagnosis and ART start	24.9 (6.7–81.6)	4.9 (2.3–29.8)
NRTI-included in the baseline ART regimen		
lamivudine	22 (88%)	23 (92%)
rilpivirine	3 (12%)	2 (8%)
Years since start of the 2-drugs regimen	1.18 (0.87–2.30)	1.34 (0.67–1.99)
On lipid-lowering treatment	15 (60%)	7 (28%)
Target not detected (TND) at viral load determination	18 (72%)	21 (84%)
$CD4^+$ T-lymphocytes (cells/μl)	763 (593–962)	727 (605–863)
$CD4^+$ T-lymphocytes (%)	37.3 (31.2–39.8)	35.2 (30–40.8)
$CD8^+$ T-lymphocytes (cells/μl)	850 (594–1079)	826 (768–1050)
CD8+ T-lymphocytes (%)	37.3 (28.2–46.3)	37.7 (34.9–45.8)
$CD4^+/CD8^+$ T-lymphocytes ratio	0.98 (0.71–1.38)	0.96 (0.66–1.15)
CD38-HLA-DR+ T-lymphocytes (%)	25.1 (22–32.55)	24.1 (20–27.9)
D-dimer (μg/ml)	0.27 (0.27–0.36)	0.27 (0.27–0.3)
IL-6 (pg/ml)	1.83 (1.83–1.83)	1.83 (1.83–1.83)
C-reactive protein (mg/l)	2.3 (0.7–6.1)	0.9 (0.5–2.1)
ALT (U/l)	28 (21–36)	28 (23–35)
AST (U/l)	25 (21–31)	27 (23–32)
Total cholesterol (mg/dl)	179 (151–192)	179 (157–203)
HDL-cholesterol (mg/dl)	43 (39–56)	52 (43–64)
LDL-cholesterol (mg/dl)	112.5 (88.5–132.5)	117 (97–134)
Total/HDL-cholesterol ratio	3.69 (2.98–4.68)	3.13 (2.77–3.87)
Triglycerides (mg/dl)	123 (78–167)	85 (73–105)
Glucose (mg/dl)	92 (82–104)	80 (75–90)
Creatinine (mg/dl)	1.1 (0.96–1.17)	1.05 (0.98–1.13)
Urine glucose (mg/dl)	0 (0–0)	0 (0–0)
Urine protein (mg/dl)	0 (0–10)	0 (0–10)
Weight (kg)	76 (71–86)	74 (68–80)
BMI (kg/m^2)	25.5 (23.7–29.7)	23.9 (21.8–25.4)
Abdominal circumference (cm)	75 (68–82)	72 (64–78)

Values are median (Q1-Q3) or frequency (%), as appropriate.

the small numbers enrolled in this study, it is reasonable to exclude that E/C/F/TAF is superior to the two-drug DTG-based regimens.

The present study is unique and thus it cannot be directly compared with others. However, our findings are consistent with those from other randomized trials of simplification of 3DRs with 2DRs based on DTG plus one RTI [7–9]. Altogether, these findings suggest that 2DRs based on a high-genetic barrier InSTI along with one RTI are as potent as more conventional antiretroviral regimens also in controlling the burden of residual viremia.

Although the use of TND result from a Real-Time PCR assay cannot be considered the golden standard to assess the absence of residual viremia below 50 copies/ml, this assay has some distinctive advantages: first, it is widely used in clinical practice, and thus available in most centres; second, it has been associated with a relevant clinical outcome (i.e. virologic failure) in a number of retrospective studies [4–6]; and third, it allows comparisons between different randomized trials.

In conclusion, in patients with HV-RNA less than 50 copies/ml on a stable two-drug regimen based on DTG along with one RTI, the switch to E/C/F/TAF did not appear to mitigate the burden of residual viremia.

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Author's contribution: Nicola Gianotti conceived the study, followed the patients, analysed the results, drafted and finalized the manuscript.

Laura Galli conceived the study, performed the statistical analyses, drafted and finalized the manuscript.

Andrea Poli managed data (including data cleaning), contributed to the statistical analyses and contributed to the writing of the manuscript.

Liviana Della Torre, Concetta Vinci, Elisabetta Carini, Silvia Nozza, Vincenzo Spagnuolo, Adriano Lazzarin, Camilla Muccini followed the patients and contributed to the writing of the manuscript.

Andrea Galli managed sample banking and contributed to the writing of the manuscript.

Antonella Castagna conceived the study, followed the patients, analysed the results and contributed to the writing of the manuscript.

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Conflicts of interest

Nicola Gianotti has been an advisor for Gilead Sciences, Janssen-Cilag, ViiV Healthcare and Merck Sharp & Dohme and has received speakers' honoraria from Gilead Sciences, ViiV Healthcare, Janssen-Cilag, Bristol-Myers Squibb and Merck Sharp & Dohme.

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Vincenzo Spagnuolo has received consultancy payments and speaking fee from Gilead, ViiV Healthcare and Janssen-Cilag.

The other authors have no potential conflict of interest to declare.

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Hypophosphatemia in people with HIV: no benefit when switching from tenofovir disoproxil fumarate to tenfovir alafenamide

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Treatment with tenofovir disoproxil fumarate (TDF) has been associated with hypophosphatemia mainly because of injury of the renal proximal tubulus. Studies on the impact of tenfovir alafenamide (TAF) on phosphate homeostasis in people with HIV (PWH) are limited. Prompted by a patient with phosphate wasting under tenofovir but no other evidence for tubular dysfunction, a retrospective cohort analysis with 102 PWH revealed that hypophosphatemia remained largely unchanged after switching from TDF to TAF.

Moderate-to-severe hypophosphatemia is observed in about 4–31% of people with HIV (PWH) using antiretroviral therapy [1–4]. Its pathogenesis may differ among patients and proposed risk factors are the HIV infection itself, antiretroviral drugs and vitamin D deficiency [5,6]. Among antiretroviral drugs, tenofovir disoproxil fumarate (TDF) has been associated with hypophosphatemia most frequently, and TDF is associated with increased parathyroid hormone (PTH) and alafenamide phosphaturia [6,7]. Tenofovir alafenamid (TAF) produces higher levels of intracellular TFV diphosphate at substantially reduced oral doses of tenofovir equivalents and is associated with a lower risk of renal proximal tubular dysfunction. However, little is known about the contribution of TAF to hypophosphatemia in PWH.

Our study was prompted by a patient who started with TDF/FTC/Efavirenz (EFV) in June 2007 and switched to TAF/FTC/Cobicistat (C)/Elvitegravir (EVG) in April 2017. His HIV-RNA values were persistently below the limit of detection since 8 months after treatment start and his most recent $CD4^+$ T-cell count was 593 cells/μl (29%). His phosphate levels and ratio of maximal reabsorption capacity [(TmP/glomerular filtration rate (GFR)] before antiretroviral therapy (ART) were within the normal range. Phosphate rapidly declined to mean 0.66 mml/l (range 0.48–0.92 mmol/l) while on the TDF-containing regimen (Fig. 1a). After switching to TAF/FTC/C/EVG, phosphate levels declined further (mean value 0.53 mmol/l, range 0.34–0.68 mmol/l). In June 2020, the patient had vitamin D deficiency but his parathyroid hormone levels were within the normal range. In September 2020, TmP/GFR was reduced (1.4 mg/dl) indicating renal phosphate wasting. Urine albumin–creatinine ratio was normal and there was no evidence for glucosuria or albuminuria. The patient never complained about signs and symptoms for phosphate deficiency, had no other comorbid conditions, and took no concomitant medication. We finally switched our patient to dolutegravir (DTG)/lamivudinde (FTC) after which his serum phosphate and renal tubular reabsorption of phosphate remained reduced. Again, absence of glucosuria or increased urinary α1-microglobulin and a normal albumin–creatinine ratio suggested normal global proximal tubular function.

We retrospectively studied a cohort of 74 male and 28 female patients with HIV, mean age 53.3 years (range 30–82 years), and mean time since HIV diagnosis of 15.3 years (range 1–33 years), who in between May 2016 and June 2019 had switched from TDF/FTC to TAF/FTC and for whom we had three successive phosphate measurements during 6 months before and 9 months after the switch. Hypophosphatemia was categorized as none (no phosphate measurement <0.82 mmol/l), sporadic (1 of 3 measurements <0.82 mmol/l), confirmed (2 of 3 measurements <0.82 mmol/l), and persistent (3 of 3 measurements <0.82 mmol/l). The antiretroviral drug regimens consisted of boosted protease inhibitors ($n = 29$), integrase inhibitors ($n = 53$), nonnucleoside reverse transcriptase inhibitors ($n = 16$), or combinations ($n = 4$) at the time of switch. Mean $CD4^+$ cell counts were 632 cells/μl (range 80–1544 cells/μl), 96% had a HIV-RNA below 400 copies/ml, and 90% below 50 HIV-RNA copies/ml.

As expected, 28.4% of PWH had confirmed or persistent hypophosphatemia when receiving TDF, and this proportion did not change after switching to TAF (Fig. 1b). Mean levels of serum phosphate did not differ between the two groups (TDF vs. TAF: 0.91 vs. 0.92 mmol/l, $P = 0.579$). However, four PWH fell two categories from confirmed to absent hypophosphatemia and two newly developed persistent hypophosphatemia on TAF. Overall, $n = 25$ (24.5%) PWH improved in their hypophosphatemia category, $n = 15$ (14.7%) PWH deteriorated, and $n = 62$ (60.8%) remained unchanged.