Background: Fat distribution, bone mineral density (BMD) and mitochondrial DNA (mtDNA) may improve, in the long-term, after switching from nucleoside reverse transcriptase inhibitors (NRTIs) to fixed-dose abacavir (ABC)/lamivudine (3TC) or tenofovir (TDF)/emtricitabine (FTC).

Methods: This was a prospective, randomized, open-label, multicentre substudy of the BICOMBO trial in which virologically suppressed patients had their NRTIs switched to ABC/3TC or TDF/FTC. Whole-body dual-energy X-ray absorptiometry (DXA) was used to measure limb, trunk and total body fat and total BMD. Lumbar and hip DXA scans were used to measure lumbar and hip BMD. Fat mass ratio (FMR; % trunk fat/% leg fat by whole-body DXA) was used to assess fat distribution. mtDNA was measured in peripheral blood mononuclear cells (PBMCs). Parameters of interest were measured at baseline, 48 and 96 weeks, and were compared between treatment groups.

Results: Of 56 patients included, 45 (20 ABC/3TC and 25 TDF/FTC) completed the substudy. After 96 weeks, ABC/3TC (+756 g, +12.1%) and TDF/FTC (+337 g, +7.6%) led to non-significantly different increases in limb fat (P=0.60). By contrast, trunk fat showed a significant increase (P=0.04) with ABC/3TC (+1,184 g, +10.6%) relative to TDF/FTC (-370 g, -4.2%). Median (IQR) FMR remained unchanged with ABC/3TC (-0.01 [-0.16–0.06]; P=0.23), but it decreased significantly with TDF/FTC (-0.13 [-0.30–0.00]; P=0.007). Total BMD and mtDNA significantly increased after 96 weeks, without differences between groups.

Conclusions: Switching from NRTIs to either ABC/3TC or TDF/FTC led to similar increases in limb fat, BMD and PBMC mtDNA after 96 weeks.

Introduction

Currently, most antiretroviral (ARV) therapy guidelines recommend a once-daily fixed-dose combination (FDC) of two nucleoside analogue reverse transcriptase inhibitors (NRTIs), consisting of either abacavir (ABC)/lamivudine (3TC) or tenofovir (TDF)/emtricitabine (FTC), plus a third drug [1–3]. These combinations offer convenience (one pill once daily) and good tolerability, favouring adherence and long-term efficacy [4,5].

One of the main advantages of these two FDCs compared with older NRTIs, mainly thymidine analogues stavudine (d4T) and zidovudine (AZT), is the low potential for causing mitochondrial toxicity [6]. Probably the most feared side effect of long-term ARV therapy is lipoatrophy, a subcutaneous fat loss in face, limbs and buttocks. Lipoatrophy is multifactorial [7,8], but a key factor is the ARV regimen used, mostly thymidine NRTIs [8,9]. Several studies in treatment-naive patients have reported significant increases in limb fat with TDF or ABC when compared with thymidine NRTIs [10–12]. There are also studies in which switching thymidine NRTIs to ABC [13,14], TDF [15–18] or any of them [19] has led to increases in limb and total fat. The STEAL study, switching NRTIs to either ABC/3TC or TDF/FTC,
has shown similar increases in limb and total fat at 96 weeks with both FDCs [20].

In order to establish a simple and objective measure to define lipodystrophy and improve its early recognition, the fat mass ratio (FMR) has been developed from dual-energy X-ray absorptiometry (DXA) measurements [21]. The FMR is defined as the ratio of the trunk fat mass percentage divided by the lower limb fat mass percentage. After comparing with values of the HIV-negative control population, a cutoff value of ≥1.5 has been proposed to define lipodystrophy [21,22]. The normal values of FMR in the Caucasian US population have been reported to be ≤1 [23]. Although FMR has not yet been used to evaluate the outcomes of body fat changes in randomized studies, it may be more informative of body fat distribution than just reporting limb or total fat changes.

There are conflicting data regarding the long-term bone effects of TDF-containing therapy. Studies in treatment-naive patients suggest that there is a small decrease in bone mineral density (BMD) in the first months of any ARV therapy that stabilizes later [10,24,25]. In the STEAL study, there were differential effects of ABC/3TC (increase) and TDF/FTC (decrease) in hip and spine T-scores after 96 weeks; however, BMD data was not reported in this study [20].

Mitochondrial DNA (mtDNA) in peripheral blood mononuclear cells (PBMCs) has been associated with the degree of lipoatrophy [26] and the improvement of lipoatrophy after switching NRTIs [15,16,27]. However, we are not aware of any comparison on mtDNA between ABC/3TC and TDF/FTC.

We have compared the effects of switching from NRTIs to either ABC/3TC or TDF/FTC on body fat distribution, BMD and mtDNA in a subgroup of patients from the BICOMBO trial.

Methods

Subjects and design
The BICOMBO trial is a prospective, randomized, open-label, multicentre study in which patients with suppressed HIV-1 infection on a 3TC-containing regimen were offered to change the NRTI backbone to one of two FDCs: ABC/3TC 600/300 mg once daily or TDF/FTC 300/200 mg once daily, without modifying the rest of the treatment. The details of the global study are described elsewhere [28].

The ToxBICOMBO substudy was planned prior to the initiation of the BICOMBO trial. This substudy aimed to analyse changes in body fat distribution, BMD and mtDNA in a subgroup of consecutive patients from three sites (Hospital Universitari Vall d’Hebron, Barcelona, Spain; Hospital Clinic i Provincial, Barcelona, Spain; and Hospital Universitari de Bellvitge, L’Hospitalet de Llobregat, Barcelona, Spain). Patients were enrolled between June 2005 and February 2006 and were assessed using the same time points and visits as in the general study. The primary study end point was the median absolute and percentage change in limb fat mass measured by DXA at week 96. Secondary end points were median changes in total and trunk fat mass, FMR, BMD and mtDNA at weeks 48 and 96.

At baseline and at weeks 48 and 96, whole body and lumbar and hip DXA scans (Lunar DPXL, Madison, WI, USA) were performed to assess body and bone composition. FMR was calculated as the ratio of the percentage of trunk fat mass divided by the percentage of lower limbs fat mass [21]. Bone data (total body, hip and lumbar spine) were expressed as BMD (g/cm²) and T-scores. Scans were centrally performed and read by a single radiologist (SV-S) unaware of the ARV treatment received.

In two of the sites (Hospital Universitari Vall d’Hebron and Hospital Universitari de Bellvitge) participating in the substudy, additional blood samples were obtained at each visit to determine mtDNA in PBMCs. Relative quantification of mtDNA versus nuclear DNA (nDNA) was performed by real-time PCR in an ABIPRISM 7700 sequence detector (Applied Biosystems, Foster City, CA, USA) as described previously [16]. All samples were tested in duplicate and the mean value was used. The relative quantification of mtDNA versus nDNA was calculated using the formula 2-EXP (CtmtDNA-CtnDNA), where CtmtDNA and CtnDNA are the threshold cycles for the mitochondrial and nuclear targets, respectively. Cells were preserved at -80°C until analysed.

The protocol was approved by the Ethics Committee at each participating site and specific written informed consent for the substudy was obtained from all patients.

Statistical analysis
A sample size of 25 evaluable patients in each group was necessary to achieve 80% power to detect a difference from -200 g to +200 g, with an sd of 500 g, in mean limb fat at 96 weeks with a significance level of 0.05 using a two-sided test.

For quantitative variables, the medians and IQR (25th to 75th percentiles) were used as measures of central tendency and dispersion. The number of patients in each category and the corresponding percentages were given for qualitative variables. The changes from baseline were compared with the Wilcoxon signed-rank tests and the McNemar or χ² test for qualitative variables, with the continuity correction for the χ² when a subgroup included five or fewer subjects. Comparisons between quantitative non-paired variables were performed with the Mann–Whitney U test. Correlations were analysed by Spearman’s rank test. All statistical tests were two-tailed and performed at a level of statistical significance.
Body changes after switch to ABC/3TC or TDF/FTC

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=45)</th>
<th>TDF/FTC (n=25)</th>
<th>ABC/3TC (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>42 (36–46)</td>
<td>42 (36–52)</td>
<td>41 (36–46)</td>
<td>0.610</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>33 (73)</td>
<td>20 (80)</td>
<td>13 (65)</td>
<td>0.320</td>
</tr>
<tr>
<td>Previous NRTI*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZT, n (%)</td>
<td>11 (24)</td>
<td>7 (28)</td>
<td>4 (20)</td>
<td>0.729</td>
</tr>
<tr>
<td>d4T, n (%)</td>
<td>7 (16)</td>
<td>6 (24)</td>
<td>1 (5)</td>
<td>0.112</td>
</tr>
<tr>
<td>ddl, n (%)</td>
<td>16 (36)</td>
<td>5 (20)</td>
<td>11 (55)</td>
<td>0.027</td>
</tr>
<tr>
<td>TDF, n (%)</td>
<td>9 (20)</td>
<td>7 (28)</td>
<td>2 (10)</td>
<td>0.260</td>
</tr>
<tr>
<td>ABC, n (%)</td>
<td>2 (4)</td>
<td>0</td>
<td>2 (10)</td>
<td>0.192</td>
</tr>
<tr>
<td>Third drug</td>
<td></td>
<td></td>
<td></td>
<td>0.642</td>
</tr>
<tr>
<td>NNRTI, n (%)</td>
<td>40 (89)</td>
<td>23 (92)</td>
<td>17 (85)</td>
<td></td>
</tr>
<tr>
<td>PI, n (%)</td>
<td>5 (11)</td>
<td>2 (8)</td>
<td>3 (15)</td>
<td></td>
</tr>
<tr>
<td>CD4+ T-cell count, cells/µl</td>
<td>468 (333–728)</td>
<td>441 (333–725)</td>
<td>482 (323–732)</td>
<td>0.732</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.9 (60.1–78.8)</td>
<td>67.5 (59.8–76.1)</td>
<td>71.0 (65.0–80.0)</td>
<td>0.335</td>
</tr>
<tr>
<td>Limb fat, g</td>
<td>5,082 (3,386–6,434)</td>
<td>4,271 (2,861–6,148)</td>
<td>5,509 (3,774–7,734)</td>
<td>0.091</td>
</tr>
<tr>
<td>Limb fat, %</td>
<td>17 (13–25)</td>
<td>16 (13–23)</td>
<td>21 (12–31)</td>
<td>0.394</td>
</tr>
<tr>
<td>Trunk fat, g</td>
<td>9,063 (6,746–12,774)</td>
<td>8,485 (6,664–12,704)</td>
<td>11,034 (6,611–13,925)</td>
<td>0.465</td>
</tr>
<tr>
<td>Trunk fat, %</td>
<td>29 (22–36)</td>
<td>27 (20–33)</td>
<td>30 (22–42)</td>
<td>0.272</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>14,619 (10,498–19,071)</td>
<td>12,434 (10,291–18,722)</td>
<td>15,523 (11,717–22,065)</td>
<td>0.263</td>
</tr>
<tr>
<td>Fat mass ratio</td>
<td>1.73 (1.19–2.39)</td>
<td>1.99 (1.29–2.39)</td>
<td>1.83 (1.13–2.40)</td>
<td>0.214</td>
</tr>
<tr>
<td>Total BMD, g/cm²</td>
<td>0.99 (0.90–1.15)</td>
<td>0.93 (0.84–1.14)</td>
<td>1.06 (0.96–1.21)</td>
<td>0.015</td>
</tr>
<tr>
<td>mtDNA PBMC</td>
<td>59 (38–85)</td>
<td>66 (53–86)</td>
<td>53 (34–76)</td>
<td>0.280</td>
</tr>
</tbody>
</table>

Values are expressed as median (IQR) unless indicated otherwise. *At study enrolment, other than lamivudine (3TC). ABC/3TC, abacavir/lamivudine fixed-dose combination; AZT, zidovudine; BMD, bone mineral density; ddl, didanosine; d4T, stavudine; mtDNA, mitochondrial DNA; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PBMC, peripheral blood mononuclear cell; PI, protease inhibitor; TDF/FTC, tenofovir/emtricitabine fixed-dose combination.

of 0.05. SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

A total of 56 patients were included in the study. Of them, 11 patients did not complete the 96 weeks: 7 were lost to follow-up, 2 withdrew consent, 1 changed ARV due to virological failure and 1 patient died due to non-medication-related cause. There were no significant differences in baseline characteristics between patients who did and did not complete 96 weeks of follow-up and losses were balanced between groups. In total, 45 patients had 96 weeks data and were analysed. Of them, 25 were randomized to TDF/FTC and 20 to ABC/3TC. Patient baseline characteristics are described in Table 1. Patients had a median limb fat of 5,082 g and a median FMR of 1.73, consistent with baseline lipodystrophy. All but five patients (three randomized to ABC/3TC and two to TDF/FTC) had received thymidine analogues at some point in their ARV therapies. There were no significant differences in the baseline characteristics of patients who did and patients who did not receive thymidine analogues (data not shown). There were some significant differences between groups at baseline: a higher proportion of patients randomized to ABC/3TC had been previously receiving didanosine (ddl) and had higher BMD than patients randomized to TDF/FTC (Table 1).

Body fat distribution

No significant differences were seen in body fat composition during the first 48 weeks, either within or between groups. After 96 weeks, limb fat increased a median of 337 g (+7.6%) in the TDF/FTC arm (P=0.40) and 756 g (+12.1%) in the ABC/3TC arm (P=0.020), without significant differences (P=0.126) between groups (Figure 1A). Regarding trunk fat, a median decrease of 370 g (-4.2%) in the TDF/FTC group (P=0.580) and an increase of 1,779 g (+12.1%) in the ABC/3TC group (P=0.044) was seen, this difference between groups being significant in percentage fat (P=0.044) and nearly significant (P=0.075) in trunk fat mass (Figure 1B). Similar results to trunk fat were observed in total fat, with a median decrease of 130 g (-0.7%) in the TDF/FTC group (P=0.126) and an increase of 1,848 g (+10.6%) in the ABC/3TC group (P=0.08) was seen, this difference between groups being significant in percentage fat (P=0.044) and nearly significant (P=0.075) in trunk fat mass (Figure 1B).

Changes in limb fat were more pronounced in those patients previously receiving a thymidine analogue (AZT [n=11] or d4T [n=7]) compared with those not receiving these drugs, irrespective of randomization group, both at 48 weeks (P=0.044) and 96 weeks (P=0.039). In patients with previous AZT or d4T use, a significant increase in limb fat was seen after 48 weeks (455 g, +11.7%; P=0.022) and after 96 weeks (587 g, +14.5%; P=0.014), without significant differences in
trunk or total fat. No significant limb fat differences were seen after 48 weeks (-19 g, -0.4%) or 96 weeks (205 g, +3.7%) in those patients previously receiving TDF, ABC or ddi (Figure 2).

Regarding FMR, there was a significant decrease with TDF/FTC (-0.13 [-0.30–0.00]; P=0.007) and a non-significant decrease with ABC/3TC (-0.01 [-0.16–0.06]; P=0.227) after 96 weeks, independent of the prior NRTIs used. Differences between groups were not significant (P=0.139).

Bone mineral density

When analysing total BMD there were no significant differences after 48 weeks within or between treatment groups. After 96 weeks, increases in total BMD were seen both with TDF/FTC (0.21 g/cm² [0.00–0.28]; P<0.001, an increase of 23.5% compared with baseline; Table 2) and ABC/3TC (0.08 g/cm² [0.01–0.22]; P=0.001, an increase of 7.9% compared to baseline; Table 2). However, small but significant decreases in hip BMD (-0.01 g/cm² [-0.03–0.18]; P=0.008) and hip T-score (-0.16 SD [-0.31–0.04]; P=0.007) were seen within the TDF/FTC arm, although these were non-significant when compared with the ABC/3TC arm. No changes were seen in lumbar spine.

Regarding prior NRTI treatment, significant differences were observed in BMD after 48 weeks between the 34 patients previously receiving AZT, d4T or ddi and...
Body changes after switch to ABC/3TC or TDF/FTC

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the 11 patients with prior use of TDF or ABC (P=0.011), with small decreases with the former (-0.001 g/cm², -0.1%; P=0.189) and increases with the latter (0.02 g/cm², +1.5%; P=0.037). After 96 weeks, these differences, depending on prior NRTI groups, disappeared (P=0.575): observing an increase in BMD of 0.19 g/cm² (+19.1%; P<0.001) in patients with prior AZT, d4T or ddI use (without significant differences between randomization arms) and an increase of 0.01 g/cm² (+0.9%; P=0.169) in patients previously taking TDF or ABC.

Mitochondrial DNA

mtDNA in PBMCs was assessed in a subgroup of 26 patients (16 with TDF/FTC and 10 with ABC/3TC). There were no significant differences in mtDNA after 48 weeks within or between treatment groups. After 96 weeks, there were significant increases in mtDNA in the TDF/FTC arm (142 [60–184]; P=0.008) and in the ABC/3TC arm (148 [105–241]; P=0.008), with no significant differences between treatment arms (Table 2). There was no significant correlation between changes in mtDNA and changes in body fat composition (data not shown).

Discussion

With two FDCs that have similar efficacy as switch strategies [19,20,28], the decision of choosing one or the other could be influenced by other factors, such as changes in body fat composition or BMD.

In this study, patients had a median limb fat of 5.1 kg at baseline, higher than the mean content in lipoatrophic HIV-infected patients (3.1 kg) but lower than in non-HIV-infected adults (7.1 kg) [29]. FMR (1.73) was higher than the mean values in non-HIV-infected adults (≤1) [23] and in HIV-infected adults without lipodystrophy (<1.5) [21]. After 96 weeks,
both groups showed a limb fat increase, without significant differences between drugs (median increase of 337 g and 756 g with TDF/FTC and ABC/3TC, respectively). These changes are similar to those observed in prior switch studies from thymidine analogues to ABC or TDF [13-20,29]. A significant increase in limb and total fat and a nearly significant increase in trunk fat were seen within the ABC/3TC group. Higher increases in body fat with ABC/3TC compared with TDF/FTC, although without reaching statistical significance, have been seen in two recent studies: in treatment-naive patients (limb fat 1.7 versus 1.1 kg [P=0.12] in intention-to-treat analysis and 2.1 versus 1.2 kg [P=0.02] in as-treated analysis) of the ACTG 5224s study [24] and in the STEAL study [20], which compared both FDCs as switch strategies (limb fat 0.53 versus 0.42 kg and total fat 1.42 versus 1.12 kg; both P=0.46).

It is noteworthy that no significant changes were seen during the first year and fat recuperation took place in the second year. However, in patients previously receiving a thymidine analogue, significant increases in limb fat were seen as soon as 48 weeks after the switch and these differences, compared with patients previously receiving ABC, TDF or ddI, were seen at 48 and 96 weeks.

When using FMR as an objective measure of lipodystrophy there were no differences between FDC after 96 weeks. However, there is a significant decrease in FMR with TDF/FTC, as limb fat increases and trunk fat decreases. By contrast, there are no changes in FMR with ABC/3TC, as both limb fat and trunk fat increases. Theoretically, the aforementioned TDF/FTC fat change profile would be more desirable from the metabolic point of view. However, this is the first time, to our knowledge, that FMR has been used to compare body fat changes between drugs in a randomized clinical trial and larger studies with longer follow-up would be necessary to confirm this finding and its implications.

No changes in total BMD were seen in the first 48 weeks. After 96 weeks, slight although significant increases in total BMD were observed with both treatment groups. However, small decreases in hip BMD and T-score were seen with TDF/FTC, although nonsignificant when compared with ABC/3TC. In patients who had not previously received TDF or ABC, a decrease was seen during the first year, irrespective of randomization group, with significant increases during the second year, whereas patients previously receiving TDF or ABC did not show significant changes after 96 weeks. In prior studies, discordant results have been reported regarding BMD. Some studies have shown no significant changes in BMD after switching from a thymidine analogue to TDF or ABC [18,19]. In the STEAL study [20], significant differences were seen after 96 weeks in lumbar and hip T-scores (decreases with TDF/FTC and increases with ABC/3TC), but BMD data were not available. In treatment-naive patients, an initial decrease is seen with all NRTI-based treatments, which is more pronounced with TDF, stabilizing or even improving thereafter [10,24,25].

The usefulness of mtDNA determination in PBMCs as a marker of mitochondrial toxicity is controversial, with some studies finding [27,30] and others not finding [15,31] correlation between mtDNA levels and ARV switch. In our study, no changes were seen in the first 48 weeks, but significant increases in PBMC mtDNA were seen with both treatment groups after 96 weeks, without differences between them. This mtDNA recovery has also been seen in other studies, being related to lipoatrophy reversal in some cases [32] and, as in our study, without correlation with body fat in others [14]. This lack of correlation could be explained by organ-specific drug toxicities, meaning that mitochondrial changes should be measured in the affected tissues and not in PBMCs [9,33].

Some limitations of our study must be highlighted. The first is the relatively low number of patients included. From the initial 56 patients, 11 did not complete the follow-up, although their baseline characteristics did not differ from the analysed patients and losses were balanced between treatment arms. Secondly, some patients were taking TDF or ABC before the switch, as the inclusion criteria for the study was a 3TC-containing regimen without other limitations. We have performed analyses excluding these patients observing benefits in fat, bone and mtDNA after the switch, but with a lower statistical power. We also do not have data on duration of prior exposure to thymidine analogues. Although this can be a confounding factor, baseline characteristics were well-balanced between treatment groups and the randomized design of the study minimizes the risk of selection bias. Another limitation is that an abdominal computed tomographic scan was not performed, which means that differentiation between changes in subcutaneous or visceral fat in the abdomen could not be made.

Most of the studies analysing body composition in HIV patients limit their follow-up to 48 weeks. A key issue is whether fat recovery persists over time after treatment switch or it achieves a plateau, as suggested in the few studies with a longer follow-up [14,20,34]. As seen in our study, significant changes can take place in body fat distribution, BMD and mtDNA after the first year with both FDCs, especially in those patients who were previously receiving thymidine analogues. Longer follow-up is warranted to obtain valuable information about the reversibility of some side effects, especially in fat and bone.
Acknowledgements

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AC, EM, JMG and ER conceived the study, participated in its design, coordination and data analysis, and drafted the manuscript. SVS performed and analysed DXA scans. DP, ML, PB, MC, MM, AI and VF recruited patients, carried out the study protocol and supervised data integrity and analysis.

Disclosure statement

AC has received honoraria, speakers’ fees and/or funds for research from Bristol–Myers Squibb, Abbott, Boehringer–Ingelheim, Gilead, Janssen–Cilag, MSD, ViVi, Roche Farma and GlaxoSmithKline. EM has received honoraria, speakers’ fees, consultant fees and/or funds for research from Abbott, Boehringer–Ingelheim, Bristol–Myers Squibb, Gilead Sciences, GlaxoSmithKline, MSD, Theratechnologies, Tibotec and ViVi Healthcare. DP has received research grants and/or honoraria for advisory boards and/or conferences from Boehringer–Ingelheim, GlaxoSmithKline, ViVi, Pfizer, Bristol–Myers Squibb, Abbott, Gilead, Janssen and Merck. VF has received honoraria, speakers’ fees and/or funds for research from Bristol–Myers Squibb, Abbott, Boehringer–Ingelheim, Gilead, Janssen–Cilag, MSD, ViVi, Roche Farma and GlaxoSmithKline. JMG has received honoraria, speakers’ fees, consultant fees and/or funds for research from Abbott, Boehringer–Ingelheim, Bristol–Myers Squibb, Gilead Sciences, GlaxoSmithKline, MSD, Pfizer, Theratechnologies, Tibotec and ViVi Healthcare. ER has received honoraria, speakers’ fees, consultant fees and/or funds for research from Bristol–Myers Squibb, Abbott, Boehringer–Ingelheim, Gilead, Janssen–Cilag, MSD, Pfizer, ViVi, Roche Farma, Schering Plough and GlaxoSmithKline. ML, MC, PB, AI, SVS and MM declare no competing interests.

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