Safety and feasibility of treatment simplification to atazanavir/ritonavir + lamivudine in HIV-infected patients on stable treatment with two nucleos(t)ide reverse transcriptase inhibitors + atazanavir/ritonavir with virological suppression (Atazanavir and Lamivudine for treatment Simplification, AtLaS pilot study)

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Objectives: To explore 48 week safety and efficacy of treatment simplification to atazanavir/ritonavir + lamivudine in HIV-infected patients with virological suppression on a stable atazanavir/ritonavir-based standard triple regimen.

Methods: This was a single-arm pilot study, enrolling 40 patients on atazanavir/ritonavir + two nucleos(t)ide reverse transcriptase inhibitors (NRTIs), without previous treatment failure, with HIV-RNA <50 copies/mL for >3 months and CD4 >200 cells/mm³. At baseline, patients were switched to 300/100 mg of atazanavir/ritonavir + 300 mg of lamivudine once daily. Laboratory parameters, atazanavir plasma levels, self-reported adherence, quality of life, neurocognitive performance, bone composition and body fat distribution were monitored. Virological failure was defined as HIV-RNA >50 copies/mL on two consecutive determinations or a single level >1000 copies/mL.

Results: After 48 weeks, 4/40 (10%) regimen discontinuations occurred: 1 death (brain haemorrhage), 1 study withdrawal (inadequate atazanavir plasma levels), 1 re-induction with two NRTIs due to pregnancy and 1 virological failure without development of resistance. Seven moderate to severe adverse events were recorded (including four renal colics, possibly treatment-related) in six patients. At week 48, increases in total (mean change +17 mg/dL, \(P=0.001\)), high-density lipoprotein ( HDL; +6 mg/dL, \(P<0.001\)) and low-density lipoprotein (LDL; +8 mg/dL, \(P=0.052\)) cholesterol were observed. The glomerular filtration rate improved (+7 mL/min/1.73 m², \(P<0.001\)), as did scores exploring self-reported physical and mental health (+11, \(P=0.009\) and +13, \(P<0.001\) on a 0–100 scale), neuropsychological performance (−1 pathological task, \(P=0.002\)) and total bone mineral density (+0.03 g/cm², \(P=0.026\)). There were no significant changes in CD4 cell count, bilirubin, atazanavir plasma levels, adherence and body fat distribution over time.

Conclusions: Simplification to atazanavir/ritonavir + lamivudine was apparently safe and associated with rare virological failure, without resistance selection. This strategy deserves further investigation in a randomized trial.

Keywords: switch, dual therapy, combined antiretroviral therapy, long-term tolerability

Introduction

The introduction of combination antiretroviral therapy (cART) has dramatically changed the natural history and prognosis of HIV infection. In high-income countries, treatment guidelines recommend as first-line therapy the use of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) with a third drug to be chosen among non-nucleoside reverse transcriptase inhibitors.
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(NNRTIs) or ritonavir-boosted protease inhibitors (PIs) or integrase inhibitors. Once started, lifelong treatment continuation is recommended. However, concerns about long-term toxicity and sustainability of cART have been raised; these have led to the evaluation of new, simpler therapeutic approaches that may limit the number of drugs (PI-based monotherapies or two-drug regimens), in order to improve tolerability while maintaining virological suppression. In particular, decreasing exposure to short- and long-term NRTI toxicity remains a relevant unmet clinical need.

Maintenance monotherapy with a ritonavir-boosted PI, which is a type of drug that shows a high genetic barrier to resistance, is the most widely investigated strategy. Lopinavir/ritonavir and darunavir/ritonavir monotherapies as simplification strategies have shown interesting results, although their non-inferiority to darunavir/ritonavir monotherapies as simplification strategies has not always been confirmed. Monotherapy with atazanavir/ritonavir for treatment simplification has been evaluated only in small single-arm studies. In two studies, simplification to atazanavir/ritonavir alone maintained viral suppression in most subjects. However, a third small pilot study was prematurely terminated because of unacceptably high rates of failure and another single-arm study showed that only 67% of patients maintained virological suppression ≤50 copies/mL after 48 weeks, with significant resistance selection.

Nonetheless, atazanavir/ritonavir has shown good tolerability with limited impact on lipid and glucose metabolism. It can be administered once daily and its efficacy in treatment-naive subjects is well established. Atazanavir also has a peculiar resistance profile, with low potential for cross-resistance with other PIs, thus not compromising subsequent treatment options in case of virological failure. For these reasons, its combination with other agents in dual therapies could be of potential interest. Lamivudine is an NRTI administered once daily with optimal tolerability. Despite a low genetic barrier to resistance, the rapid development of the M184V mutation, conferring a high level of lamivudine resistance in vitro, has been associated with slower development or prevention of thymidine analogue mutations and increased susceptibility to tenofovir, zidovudine and other NRTIs.

On these bases, a combination of atazanavir/ritonavir and lamivudine could be a suitable option for treatment simplification in selected patients but it has not yet been investigated. With respect to boosted PI monotherapies, the inclusion of lamivudine could theoretically offer the advantage of better pharmacological pressure in sanctuary sites (e.g. CNS, male and female genital tract), where atazanavir penetration can be poor. With respect to NRTI-sparing regimens with a boosted PI combined with an integrase inhibitor (usually raltegravir) or a CCR5 antagonist, the combination of atazanavir/ritonavir + lamivudine could have the advantages of once-daily administration, fewer drug interactions (in the case of raltegravir with atazanavir), activity against all strains (CCR5 antagonists are active only against R5 virus) and lower costs, especially today with the generic formulation of lamivudine.

The aim of our study was to evaluate the safety and feasibility of treatment simplification to atazanavir/ritonavir + lamivudine in patients with optimal virological control. Changes in metabolic parameters, pharmacokinetics and cognitive performance were also explored. We report the main study outcomes at 48 weeks.

Patients and methods

Patients
This was a single-centre, open-label, single-arm simplification pilot study (registered at www.clinicaltrials.gov, number NCT00885482). The protocol was approved by the local Ethics Committee and all patients signed an informed consent form before participation.

Eligible patients were HIV-1-infected subjects aged ≥18 years, treated with a stable regimen including two NRTIs + atazanavir/ritonavir for at least 6 months, with at least two HIV-RNA levels <50 copies/mL on two consecutive determinations at least 3 months apart, with CD4 cell count >200 cells/mm³ for at least 6 months and without a history of AIDS-related events in the year before screening.

Exclusion criteria included pregnancy, breastfeeding status or plans for a pregnancy in the short term, positive serum hepatitis B virus surface antigen, a history of major toxicity to any of the study drugs, receiving concomitant treatment with antacids or proton-pump inhibitors or any other drug known to interact with the study medications or any condition which, in the opinion of the investigator, might have influenced the probability of maintaining the assigned medications during the study period. Patients who previously experienced virological failure to anti-retroviral therapy, those previously exposed to monotherapies or dual therapies including lamivudine and/or PIs, those with grade 3–4 laboratory abnormalities (except for lipid levels) or having atazanavir plasma concentration at screening below pre-defined efficacy thresholds (150 ng/mL at 24 ± 2 h or 230 ng/mL at 12 ± 2 h after last self-reported drug intake) were also excluded.

Study design and procedures

In this pilot study, enrolment of 40 patients was planned. At baseline, eligible patients were switched to 300 mg of lamivudine and 300/100 mg of atazanavir/ritonavir once daily. All subjects were instructed to take the prescribed drugs with meals. Follow-up visits were scheduled at weeks 4, 12, 24, 36 and 48. Routine physical examination and laboratory tests, including CD4 cell counts and HIV-1 RNA levels (Versant HIV-1 RNA 1.0 (kPCR), Siemens Diagnostics, quantification limit 37 copies/mL), were performed at baseline and at each follow-up visit. Glomerular filtration rate (GFR) was estimated by the modification of diet in renal disease (MDRD) formula at each visit.

Determination of atazanavir plasma levels

Therapeutic drug monitoring was routinely performed at each study visit for atazanavir and ritonavir. Patients were instructed not to take the morning antiretroviral dose, and drug levels were measured by a validated HPLC-UV method (limit of quantification 50 ng/mL). The times of the last drug intake and of blood sampling were recorded for each patient: only drug levels obtained at the mid-dosing interval (C12, 12 ± 2 h after the last self-reported drug intake) or at the trough (C24, 24 ± 2 h after the last self-reported drug intake) were evaluated and classified as therapeutic according to the thresholds described above.

Adherence and quality of life

Adherence and quality of life measures were evaluated with a previously validated self-reported questionnaire administered at each visit. The global self-reported adherence, satisfaction and belief in therapy, and patient-reported physical and mental health scores were indicated on a visual analogue scale of 0 (worst level) to 100 (best level). A symptom score was built summing self-reported scores (from 0 for ‘not at all’ to 4 for ‘very much’) for 19 listed symptoms identified based on previously published studies and clinical experience.
Neuropsychological assessment

At baseline and at the 48 week visit, all patients underwent a Mini Mental State Examination to assess general cognitive status, and a comprehensive neuropsychological battery exploring memory (immediate and delayed recall of Rey’s words, delayed recall of Rey’s figure, forward digit span, forward spatial span), attention and executive functions (Stroop test, trail-making test B, backward digit span, backward spatial span, drawings and multiple features target cancellation), visual–spatial and constructual functions (Rey’s figure copy), speed of mental processing (Wechsler Adult Intelligence Scale (WAIS) digit symbol), language (letter fluency) and logical reasoning skill (Raven’s matrices). For the Rey’s words memory task, at the follow-up visit we adopted a ‘parallel form’ in order to limit the possible learning effect. For other tasks, parallel forms were not available. The scores obtained on each task were adjusted for age, gender and education on the basis of normative data available for the Italian population. We also evaluated the total number of pathological scores (i.e. those below the normative cut-off) for each patient, in order to obtain a global score of neuropsychological performance of the whole study battery. Finally, depression was evaluated using the self-reported Zung Depression Scale.

Evaluation of bone composition, bone metabolism and body fat distribution

At baseline and at week 48, whole body, lumbar and hip dual-energy X-ray absorptiometry scans (Hologic Inc., Waltham, MA, USA) were performed. Bone composition was evaluated using total, L2–L4 lumbar column, femoral neck and total hip bone mineral density (BMD) and the Z-score. Moreover, at the same timepoints, the following plasma bone metabolism biomarkers were measured: 25(OH) vitamin D, parathyroid hormone (PTH) and osteocalcin. Body fat distribution was assessed by measuring total, limb and trunk fat and limb/trunk fat ratio.

Definitions

Treatment failure was defined as any of the following events: virological failure, discontinuation of any study drug or reintroduction of standard three-drug regimens, modification of standard drug dosages, occurrence of treatment-limiting toxicities, withdrawal of consent, loss to follow-up, progression to AIDS or death.

Virological failure was defined as the first of two consecutive HIV-RNA levels >50 copies/mL or a single level >1000 copies/mL. In the case of virological failure, genotypic resistance testing for NRTI, NNRTI and PI resistance mutations was performed.

A trial stopping rule was planned by assuming that an acceptable total virological failure rate should not have exceeded 12.5% (five patients). Therefore, study termination was planned if more than five virological failures were observed. A similar stopping rule had been used in a pilot trial investigating atazanavir monotherapy. Clinical and laboratory adverse events occurring during the study were classified according to the Division of AIDS tables for grading adverse events.

Study endpoints

The primary study endpoint was the proportion of patients who were free of treatment failure during 48 weeks at the intention-to-treat analysis (with patients discontinuing treatment counted as failures). Secondary endpoints were the proportion of patients with virological failure (intention-to-treat analysis) and changes in CD4 cell count, metabolic parameters, atazanavir plasma concentration, self-reported adherence and quality of life, as well as neurocognitive function during the 48 weeks of study using on-treatment analysis.

Statistical analysis

Descriptive statistics were used to evaluate the main study outcomes and serious adverse events. At each timepoint, longitudinal differences from baseline were assessed using Student’s t-test for paired samples and serious adverse events. At each timepoint, longitudinal differences from baseline were assessed using Student’s t-test for paired samples for the following parameters: routine biochemical and viroimmunological laboratory tests, adherence and quality of life measures, results of the neurocognitive tests (global performance and scores at each test), markers of bone metabolism and BMD, and body fat distribution. A two-tailed P value <0.05 was considered to be statistically significant. The changes in atazanavir plasma levels were evaluated by calculating geometric means, the geometric mean ratio between follow-up and baseline concentration and the 95% CI at each timepoint. Only subjects with sampling times allowing comparison with concentrations obtained at baseline (C12 or C24) at study visits were included in this analysis. The changes in pharmacokinetic parameters were considered significant if the CI for the geometric mean ratio did not cross the value of 1. All analyses were performed using the SPSS version 13.0 software package (SPSS, Chicago, IL, USA).

Results

Patient characteristics at baseline

All the planned 40 patients were enrolled. The main baseline characteristics of the study population are summarized in Table 1. Overall, patients started their last cART regimen a median of 2.6 years before the study baseline, had had undetectable HIV-RNA for a median time of 44 months and showed a median CD4 cell count of 598 cells/mm³. At baseline, the majority of patients (97.5%) discontinued tenofovir from the NRTI backbone.

Main study outcomes: treatment and virological failure

According to the definition of the primary study endpoint, there were 4/40 (10%) treatment failures during the 48 study weeks. Details regarding these events are described in Table S1 (available as Supplementary data at JAC Online).

Only one case of protocol-defined virological failure, which occurred at week 48 (viral load 4947 copies/mL), was observed. In this patient, genotypic resistance testing showed no resistance mutations.
resistance-associated mutation; plasma atazanavir and ritonavir levels were undetectable despite the patient's self-reported good adherence. One month thereafter, viral load was undetectable without any treatment change.

One patient switched back to a standard three-drug atazanavir-based regimen at week 4 due to pregnancy, with optimal virological control. Another patient experienced treatment failure at week 21 due to severe adverse events (brain haemorrhage with subsequent myocardial infarction and death; see below for more detailed comments). Finally, a fourth patient was withdrawn from the study at week 36 despite persistent virological suppression since atazanavir plasma levels were undetectable at two previous determinations; however, he remained on the same two-drug regimen and subsequent viral load was confirmed as <37 copies/mL.

Five additional patients had single virological blips, three at week 36 (72, 90 and 150 copies/mL) and two at week 48 (70 and 82 copies/mL); all these subjects performed an unscheduled visit within 1 month and in all cases the viral load returned to <50 copies/mL. All these patients successfully completed the study without treatment modifications.

Clinical and laboratory adverse events

A total of seven clinical adverse events (three grade 4, one grade 3 and three grade 2) were observed in six patients. As described in Table 51, a 63-year-old female patient with a history of head trauma and hypertension was diagnosed with a brain haemorrhage (grade 4) at week 21 and subsequently died from acute myocardial infarction (grade 4); after carefully reviewing her medical records these events were not considered as drug-related. One hospitalization for hypertensive crisis (grade 4, not considered drug-related) occurred. Four episodes of renal colics (one grade 3, the others grade 2) occurred in four different patients (two between weeks 4 and 12, one between weeks 24 and 36, and one soon after week 36). None of these subjects had a previous history of urolithiasis. As previously described in a case report, in three of these patients atazanavir plasma levels seemed to increase transiently after baseline and urinary stones analysed in one subject showed an atazanavir level ranging from 7.9% to 15.8%. All colics occurred in the summer season and no lithiasis recurrence was observed during the 48 weeks of observation.

No treatment-limiting or grade 4 laboratory toxicities were observed. A total of five and three patients experienced a grade 3 increase in total cholesterol and low-density lipoprotein (LDL) cholesterol, respectively; these alterations were mostly transient and resolved without treatment modifications. Only one patient showed transient elevation in triglycerides and one in amylases. Of the 24 patients not already showing a grade 3 elevation in total bilirubin at baseline, 17 experienced a transient grade 3 increase during follow-up, but none discontinued the regimen for this reason.

Changes in CD4 cell count, blood lipids, bilirubin and renal function

Changes in immunological and lipid parameters, bilirubin and renal function between baseline and week 48 are reported in Table 2.

No significant changes in the CD4 cell counts and total and unconjugated bilirubin levels were observed.

At week 48, a significant increase in total cholesterol and high-density lipoprotein (HDL) cholesterol was observed, together with a trend towards a significant increase in LDL cholesterol. However, total cholesterol/HDL cholesterol and HDL cholesterol/LDL cholesterol ratios did not show any modification throughout the study (mean changes at 48 weeks −0.16 [standard deviation (SD) 0.88], P=0.287, and +0.04 (SD 0.13), P=0.086, respectively). Triglycerides did not show any modification after treatment simplification.

Renal function, estimated with the MDRD formula, significantly improved at week 48.

Among other chemistry parameters, no significant differences in liver function tests, blood glucose, phosphorus and amylase levels were observed at week 48 compared with baseline (data not shown).

Atazanavir plasma levels

Considering the defined mid-dosing interval and trough periods, 28 subjects had an evaluable atazanavir plasma concentration measured at baseline (the remaining 12 patients had samples...
Table 3. Changes in bone composition, bone metabolism biomarkers and body fat distribution after 48 weeks

<table>
<thead>
<tr>
<th></th>
<th>Baseline value</th>
<th>Change after 48 weeks</th>
<th>Percentage change in BMD after 48 weeks</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>74 (15)</td>
<td>0 (4)</td>
<td>—</td>
<td>0.965</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 (4)</td>
<td>0 (1)</td>
<td>—</td>
<td>0.990</td>
</tr>
<tr>
<td>Bone composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>total BMD, g/cm²</td>
<td>1.03 (0.09)</td>
<td>+0.03 (0.06)</td>
<td>+2.04 (5.7)</td>
<td>0.026</td>
</tr>
<tr>
<td>total Z-score</td>
<td>−0.60 (0.92)</td>
<td>+0.25 (0.65)</td>
<td>—</td>
<td>0.028</td>
</tr>
<tr>
<td>femoral neck BMD, g/cm²</td>
<td>0.79 (0.14)</td>
<td>+0.01 (0.28)</td>
<td>+0.75 (3.5)</td>
<td>0.262</td>
</tr>
<tr>
<td>femoral neck Z-score</td>
<td>−0.32 (1.02)</td>
<td>+0.14 (0.24)</td>
<td>—</td>
<td>0.002</td>
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<tr>
<td>total hip BMD, g/cm²</td>
<td>0.92 (0.14)</td>
<td>0 (0.07)</td>
<td>+0.02 (6.8)</td>
<td>0.864</td>
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<tr>
<td>total hip Z-score</td>
<td>−0.16 (0.96)</td>
<td>+0.10 (0.31)</td>
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<tr>
<td>L2–L4 column BMD, g/cm²</td>
<td>0.99 (0.17)</td>
<td>+0.01 (0.03)</td>
<td>+0.91 (0.9)</td>
<td>0.064</td>
</tr>
<tr>
<td>L2–L4 column Z-score</td>
<td>−0.59 (1.53)</td>
<td>+0.18 (0.46)</td>
<td>—</td>
<td>0.022</td>
</tr>
<tr>
<td>Bone metabolism biomarkers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vitamin D, ng/mL</td>
<td>27.20 (8.25)</td>
<td>−3.68 (9.51)</td>
<td>—</td>
<td>0.024</td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>53.71 (17.31)</td>
<td>−3.54 (18.13)</td>
<td>—</td>
<td>0.243</td>
</tr>
<tr>
<td>osteocalcin, ng/mL</td>
<td>34.07 (13.05)</td>
<td>−12.76 (14.81)</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body fat distribution</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>total fat, g</td>
<td>21488 (8014)</td>
<td>−327 (2)</td>
<td>—</td>
<td>0.307</td>
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<tr>
<td>limb fat, g</td>
<td>8451 (3114)</td>
<td>−33 (793)</td>
<td>—</td>
<td>0.804</td>
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<tr>
<td>trunk fat, g</td>
<td>12001 (5409)</td>
<td>−378 (1542)</td>
<td>—</td>
<td>0.150</td>
</tr>
<tr>
<td>limb/trunk fat ratio</td>
<td>1.10 (0.30)</td>
<td>+0.03 (0.09)</td>
<td>—</td>
<td>0.027</td>
</tr>
</tbody>
</table>

BMI, body mass index. Values are expressed as mean (SD). Bold values represent statistically significant P values.

Adherence and quality of life
At week 48 after treatment simplification, no changes were observed in self-reported adherence, satisfaction and belief in therapy (Table S3, available as Supplementary data at JAC Online). Significant improvements in total patient-reported physical (mean change +11, SD 23, P = 0.009) and mental (mean change +13, SD 17.0, P<0.001) health scores were found at week 48 compared with baseline. No significant changes were found in patient-reported symptoms.

Neurocognitive examination
Whereas all patients underwent neuropsychological evaluation at baseline, only 35 completed the 48 week re-evaluation (Table S4, available as Supplementary data at JAC Online); of the remaining 5 patients, 2 refused re-evaluation and 3 were off study at week 48.

At the end of the study period, no patient showed a worsened performance in any task. Indeed, the total number of pathologic performances was reduced [from a mean of 3.68 (SD 2.91) at baseline to 2.56 (SD 2.59) at week 48, P = 0.002]. Significant improvements were observed in tasks exploring memory (immediate recall of Rey’s words, 32.3 versus 35.4, P = 0.029), attention and executive functions [Stroop test (errors), 2.6 versus 1.0, P = 0.010; trail-making test B (time), 147.1 versus 118.6, P = 0.002; trail-making test B (errors), 1.1 versus 0.45, P = 0.002; backward spatial span, 4.1 versus 4.8, P<0.001]. No significant changes were observed in the depression score.

Changes in bone composition, bone metabolism markers and body fat distribution
Changes in bone composition, bone metabolism markers and body fat distribution are shown in Table 3. Evaluating bone composition in the total population, significant increases in total BMD, total Z-score, femoral neck Z-score and L2-L4 column Z-score were observed. Among bone metabolism biomarkers, 25(OH)vitamin D and osteocalcin showed a significant decrease while no modifications were observed for PTH levels.

In the total population no significant changes in weight, BMI, total fat, limb fat and trunk fat occurred after 48 weeks. However, a significant increase in limb/trunk fat ratio was observed.

Discussion
In this pilot study we evaluated safety and efficacy of treatment simplification to a dual therapy with atazanavir/ritonavir + lamivudine in virologically suppressed patients. Overall, a treatment failure rate of 10% at 48 weeks was observed; only one case (2.5%) of virological failure (without development of drug resistance) occurred. Moreover, few transient virological blips were observed with this regimen during the whole follow-up time. No decrease in CD4 cell count occurred after removing one drug from the standard cART regimen.

Taken together, these results suggest the potential for good efficacy of the investigated simplification strategy and encourage its investigation in larger randomized studies. However, it should be emphasized that simplification to a dual therapy could be
Atazanavir/ritonavir and lamivudine simplification

risky since it could promote reactivation of viral replication with treatment failure and possible development of resistance. For these reasons, in trials exploring simplification strategies, selection criteria of eligible patients should be carefully evaluated: our results were obtained in a population with no history of virological failure to the prescribed drugs and with successful virological suppression for a long time. Patients with these characteristics are probably those at lower risk of failure after simplification to regimens with a reduced number of drugs. This subgroup of patients is not negligible because the three-drug regimens used in recent years have allowed achievement and maintenance of virological suppression in the majority of patients.

Exploring safety, an unexpectedly high incidence (10%) of urolithiasis occurred in our population. Since these adverse events could be possibly related to atazanavir, 

caution should be used when interpreting safety data of this simplification strategy. It could be hypothesized that tenofovir discontinuation represented a trigger factor for urolithiasis in our patients, increasing plasma levels of atazanavir and consequently its urinary excretion. 

It should be considered reassuring that atazanavir-associated urolithiasis has been rarely reported in studies and post-marketing pharmacovigilance, even in patients treated with atazanavir monotherapy or atazanavir-based CART regimens not including tenofovir. 

However, since a contributory role of this treatment strategy cannot be excluded at the moment, it seems prudent to recommend adequate hydration in these subjects, particularly when exposed to a hot climate.

A brain haemorrhage with subsequent myocardial infarction and death was also observed. This serious adverse event occurred in a patient with high cardiovascular risk who was affected by hypertension. No data are available to support a causative association with the prescribed antiretroviral drugs. Indeed, recent data from large observational cohorts suggest that atazanavir/ritonavir is not associated with increased risk of myocardial infarction or cerebrovascular events. 

Among the other clinical adverse events observed, none was interpreted as drug-related and no treatment-limiting alteration of laboratory tests occurred. Overall, a limited number of severe clinical adverse events and grade 3 laboratory toxicities were observed.

At week 48, total cholesterol, HDL cholesterol and LDL cholesterol were increased when compared with baseline without any change in triglyceride levels, confirming previously published preliminary results. 

The increase in LDL cholesterol and total cholesterol was adequately counterbalanced by the concomitant increase in HDL cholesterol. However, since the total cholesterol/HDL cholesterol and HDL cholesterol/LDL cholesterol ratios remained unchanged this lipid effect might not be detrimental to the patients’ cardiovascular risk. Recently, a lipid-lowering effect of tenofovir has been suggested; 

our findings further confirm this hypothesis, since in our population nearly all patients discontinued tenofovir from their previous NRTI backbone.

After treatment simplification, renal function progressively improved at each study visit, possibly due to the discontinuation of tenofovir. This result suggests that the investigated strategy could be particularly interesting in patients at risk of deterioration of renal function (e.g. ageing patients).

Despite the discontinuation of tenofovir in most patients, atazanavir plasma levels did not seem to increase during follow-up, as would be expected on the basis of previous drug interaction data. 

However, no definite conclusions can be drawn about pharmacokinetics because of the limited number of patients with evaluable paired data. In patients developing urolithiasis, a transient increase in atazanavir concentration was indeed observed. Moreover, since this was not a formal drug-drug interaction study, inadequate self-reporting of drug intake time or adherence issues could have influenced the results. Nonetheless, bilirubin levels, which are considered a surrogate marker of atazanavir exposure, also did not show any modification throughout the study.

A concern of simplification to monotherapies or dual therapies is the potential for inadequate drug activity in sanctuary sites, especially the CNS. In particular, atazanavir has shown poor CSF penetration, 

which could be suboptimal during maintenance dual therapy, thus potentially leading to neurocognitive dysfunction. This prompted an in-depth neuropsychological evaluation in our protocol. After 48 weeks, no patient showed a worsening of cognitive function; indeed, we observed an improvement in global neurocognitive performance, especially in tasks exploring memory, attention and executive functions. Although this finding does not seem to raise concern about the possible deterioration of cognitive abilities, caution is needed when interpreting such results. It is possible that >48 weeks would be needed to detect cognitive dysfunction after treatment simplification. Moreover, a learning effect could not be fully excluded even if the tests were re-administered after almost 1 year. Extended follow-up is ongoing to explore long-term neurocognitive consequences of this treatment simplification strategy.

Antiretroviral agents have shown potential detrimental effects on bone composition.

Most data are available for tenofovir, but PIs can also influence bone mineralization. In our population, in which most patients discontinued tenofovir, a significant improvement in several bone composition parameters was demonstrated after baseline. Therefore, reducing the burden of drugs can have beneficial effects on bone mineralization, thus potentially reducing the risk of developing fractures in the long term. Since a comparator arm was not included in this pilot study, it is not possible to verify whether the same advantages would have been obtained by switching tenofovir to abacavir and maintaining a three-drug regimen. However, abacavir could also have an impact on bone metabolism and there are concerns about increased cardiovascular risk during treatment with this drug.

Exploring bone metabolism biomarkers, a significant decrease in osteocalcin occurred. Although this result may suggest a reduction in the bone remodelling process, a more precise evaluation of bone biomarkers, especially markers of bone resorption, could be required in order to investigate more accurately the effects of this simplification strategy on bone remodelling. After treatment simplification, which consisted of tenofovir removal in most patients, we observed a decrease in 25(OH)vitamin D levels. Since tenofovir does not have a known effect on vitamin D metabolism, this finding is difficult to interpret. In accordance with our findings, a previous study reported an association between treatment with tenofovir and higher 25(OH)vitamin D levels. 

This finding could be the consequence of tenofovir-induced proximal renal tubular dysfunction (affecting the activity of 1α-hydroxylase and thus promoting the accumulation of 25(OH)vitamin D) or of tenofovir-induced phosphaturia/hypophosphataemia (with
subsequent up-regulation of vitamin D). Further studies are needed to clarify this finding.

Tenofovir has been shown to have a lower impact on fat redistribution when compared with other NRTIs, especially thymidine analogues. However, it is not known whether the effect of this drug can be considered negligible. In our population no significant modifications of total, limb and trunk fat were observed, but the significant increase in limb/trunk fat ratio suggests that tenofovir might have some influence on fat distribution. A longer follow-up may be needed to adequately explore the extent to which tenofovir can affect body fat.

In conclusion, in this pilot study simplification to a dual regimen with atazanavir/ritonavir + lamivudine proved to be capable of maintaining virological suppression at 48 weeks. Moreover, improvements in renal function and bone composition related to tenofovir discontinuation are additional observations favouring this treatment strategy. On the other hand, the relevance of the observed high incidence of urolithiasis and of the increase in lipid levels needs to be further clarified. The efficacy and safety of this simplification strategy deserve to be addressed in a large multicentre, randomized trial. One is currently ongoing in Italy (www.clinicaltrials.gov, number NCT01599364).

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Supplementary data
Tables S1 to S4 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
16 Pulido F, Serrano O, Rivero A et al. Atazanavir/ritonavir monotherapy for maintenance of virologic suppression: 48 week primary analysis of


