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Long-term Outcomes in a Large Randomized Trial of HIV-1 Salvage Therapy: 96-week Results of AIDS Clinical Trials Group A5241 (OPTIONS)

--Manuscript Draft--

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Abstract:	Background: Short-term (48-week) results of the OPTIONS trial showed that nucleoside reverse transcriptase inhibitors (NRTIs) can be safely omitted from salvage therapy as long as the regimen has a cumulative activity of >2 active antiretroviral (ARV) medications. The long-term durability of this approach and outcomes in persons with more-extensive drug resistance are uncertain. Methods: Participants with virologic failure and anticipated ARV susceptibility received an optimized regimen and were randomized to Omit or Add NRTIs. A separate group with more resistance (cumulative activity ≤2 active agents) received an optimized regimen including NRTIs. Results: At week 96, among 360 participants randomized to Omit or Add NRTIs, 70% and 65% had HIV-1 RNA <200 copies/mL, respectively. Virologic failure was uncommon after week 48. Younger age and starting fewer new antiretroviral medications were associated with higher odds of virologic failure. In the Highly Resistant group, 53% had HIV-1 RNA <200 copies/mL at week 96.

	Conclusions: HIV-1 salvage therapy can safely omit NRTIs without compromising efficacy or durability of response as long as the new regimen has a cumulative activity of >2 active drugs. Younger people and those receiving fewer new ARVs require careful monitoring. Even among individuals with more-extensive resistance, most achieve virologic suppression.

JID Submission MS# JID-66190: Long-term Outcomes in a Large Randomized Trial of HIV-1 Salvage Therapy: 96-week Results of AIDS Clinical Trials Group A5241 (OPTIONS)

Dear Editor:

We would like to thank the reviewers for their thoughtful comments. Please see below our responses to the comments. We have also revised the manuscript in response to their suggestions. The revisions are marked in track changes and in bold text in the revised manuscript and in bold italics in the responses below. We have also included a "clean copy" of the revised manuscript in the resubmission.

We appreciate the opportunity to respond and believe the reviewers' suggestions have strengthened the manuscript.

Reviewers' Comments:

Reviewer #1:

Reviewer's Summary: This is an important study that informs the clinical management of HIV infected individuals. It is likely that the results described will have a lasting impact on treatment guidelines. The manuscript is well-written and well-organized. The discussion was appropriately circumspect given evolving "options" for ARV. Findings were placed in historical context of available therapy at the time, and in so doing, provide guidance for care providers moving forward.

Specific Comments from Reviewer 1:

1. Reviewer Comment: Line 182 (Results; Study Participants; outcomes). It is important to address the difference in number of deaths in the Add (n=11) and Omit (n=1) groups. Provide details about what appears to be an excess number of deaths in one group. Discuss attribution or lack thereof.

   Response: As noted in the revised Figure 1, which shows participant disposition, there were 11 deaths in the Add NRTIs group and 1 death in the Omit NRTIs group. However, one of the deaths in the Add NRTIs group (due to respiratory distress) occurred one day following randomization and before assigned study treatment was initiated. The timing of the remaining 10 deaths, in the Add NRTIs group was as follows: <24 weeks (3), 24-48 weeks (2), 48-72 weeks (2), and 72-96 weeks (3). Causes of death (with contributing factors) were heart failure (lymphoma) (1), cardiac disease (2), *E. coli* sepsis (liver failure, acute renal failure, hepatitis C) (1), cirrhosis (intra-abdominal bleed, hepatitis C) (1), *Listeria* meningitis (1), pneumonia (2), progressive multifocal leukoencephalopathy (1) and renal failure (immune reconstitution inflammatory syndrome, hepatitis, autoimmune enteropathy) (1). All 10 of these participants had initial virologic response to treatment. The one death in

the Omit NRTIs group was due to trauma and pneumonia. In no instance was the cause of death thought to be related to antiretroviral therapy nor was there a pattern in the causes of death in the Add NRTIs that suggested a common mechanism or specific etiology for the observed imbalance in the number of deaths.

The cumulative probability of death following treatment initiation (with corresponding 95% confidence intervals) at week 96 in the Add NRTIs group and the Omit NRTIs group are 5.7% (3.1%, 10.3%) and 0.6% (0.1%, 4%), respectively. Because of the small number of events, the 95% confidence intervals on the cumulative probabilities of death are overlapping. The cumulative probability of death from treatment initiation through 96 weeks in the Highly Resistant group, who also received NRTIs, was similar to the randomized Add NRTIs group at 4% (1%, 15.1%). For context, as reported in the publication describing the week 48 results (Tashima KT et al, Annals of Internal Medicine, 2015), three deaths occurred during the pre-randomization screening period (median follow-up, 63 days) when all participants were continuing an NRTI-containing regimen; the incidence of death during this period was 4.2 per 100 person years (95% CI: 1.3, 12.9).

We are currently investigating whether there are differences in mitochondrial function in cells taken from the two groups; those investigations are underway and will be the topic of a separate report.

Because of the length of this clarifying response, we can either include all of the details in the Supplementary Materials or we can summarize the main points as follows in the revised manuscript. For the purposes of this response, we have revised the manuscript to include the explanations below. If the reviewer and editor would like us to include the more detailed response above, we would be happy to add it to the Supplementary Materials.

Revised text added to the Results (lines 184 to 191):
There were fewer deaths following treatment initiation in the Omit NRTIs group than in the Add NRTIs group (Figure 1): 1 and 10, respectively; cumulative probability of death through 96 weeks (95% CI): 0.6% (0.1%, 4%) and 5.7% (3.1%, 10.3%), respectively. Because of the small number of events, the 95% confidence intervals on the cumulative probabilities of death overlap. The cumulative probability of death through 96 weeks (2 deaths) in the Highly Resistant group (also receiving NRTIs) was 4% (1%, 15.1%). The causes and timing of death were heterogeneous and there was no pattern suggesting a common mechanism or specific etiology.

Revised text added to the Discussion (lines 321 to 327):

The number of deaths between treatment initiation and 96 weeks was lower in the Omit NRTIs group than in the Add NRTIs group but the 95% confidence intervals on the cumulative probability of death for this timeframe overlapped. The causes of death were heterogeneous and there was no pattern to suggest a common mechanism or specific etiology for the imbalance. Additional investigations of mitochondrial function and inflammation in the two groups are underway and will be the topic of a separate report.

2. Reviewer Comment: Line 223 (Results; Baseline Factors Associated with Virologic Failure in the Randomized Groups). It appears from the Supplementary Table that having 2 or 3 active NRTI had a significantly higher OR of virologic failure than having 1 active NRTI. This is confusing, and does not support the offered explanation that this reflects "the relative resistance to this class". Suggest clarification in text and in Table.

   Response: We were also struck by the finding that having 2 or 3 active NRTI was associated with a higher OR of virologic failure than having 1 active NRTI. This puzzling observation has also been noted in previous trials of second-line therapy, such as EARNEST, SECOND-LINE and ACTG A5273, and may reflect differential adherence to antiretroviral therapy. We have revised the explanation in the Discussion (lines 333 to 335) to make this point more clearly (new text in bold italics.)

   *"Several characteristics were associated with virologic failure in the randomized groups in OPTIONS . . . .As in **previous** studies of second-line therapy (EARNEST, SECOND-LINE, ACTG A5273), **in OPTIONS** having virus with less NRTI resistance at **time of regimen selection** was associated with **higher odds of** virologic failure, perhaps related to poorer adherence[9-11]."*

3. Reviewer Comment: Line 168 (Methods; Statistical Analysis). For analyses of baseline characteristics associated with virologic failure, was observed or imputed virologic failure used?
   Response: Observed virologic failure was used. The manuscript has been revised to clarify this point (line 168).

Additional Comments:

4. Reviewer Comment: Line 170: spelling of reparameterization
   Response: This misspelling has been corrected in the revised manuscript.
5. Reviewer Comment: A number of sentences use wording such as, "persons with drug resistance". Acknowledging that this is common practice, this reviewer favors re-wording to, "persons who have HIV-1 with antiretroviral drug resistance" (or a variation thereof).

Response: We have made this change in the revised manuscript.

Reviewer #2: The authors provide a clear and concise description of the 96-week results of the ACTG 5241 OPTIONS trial. The data are clearly and concisely presented. The manuscript is well written.

Response: We appreciate these positive comments.

Finally, we have revised a paragraph (lines 256 to 258) in the manusript to make the information regarding treatment-emergent etravirine resistance parallel to the data on treatment-emergent darunavir resistance (the revisions are marked using track changes). Lines 351-355 of the Discussion have also been revised accordingly.

To remain within the 3500 word count limit after addition of text to incorporate the reviewers' suggestions, we have shortened other parts of the manuscript.

Thank you for your consideration of these responses and the revised manuscript.

Rajesh Gandhi, MD

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Long-term Outcomes in a Large Randomized Trial of HIV-1 Salvage Therapy: 96-week Results of AIDS Clinical Trials Group A5241 (OPTIONS)

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**These authors contributed equally.*

Running title: Long-term Results of HIV Salvage Therapy

Word counts: Abstract: 200 words. Text: 34978 words.

Summary: HIV-1 salvage therapy can safely omit nucleoside reverse transcriptase inhibitors without compromising efficacy or durability of response as long as the new regimen has a cumulative activity of 2 or more active drugs.

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Footnotes

Conflicts of Interest: RTG's institution has received educational grants from Gilead, Viiv, Janssen, Theratechnologies and Merck; RTG has served on scientific advisory boards for Merck, Gilead and Theratechnologies. KTT's institution receives research grants from ViiV, Janssen, Merck and Gilead, and she has served as an advisor for Gilead Sciences. JJE is an ad hoc consultant to Merck, Gilead Sciences, Janssen and ViiV Healthcare and receives contract support from Gilead Sciences, Janssen and ViiV Healthcare for work unrelated to this study. CJF receives grants to his institution for research and education from Gilead, ViiV, Janssen, Merck, Amgen, Cytodyn and is on the speakers bureau for Clinical Care Options. The views expressed are those of the authors and do not necessarily represent the views of the NIH or Department of Health and Human Services.

Sources of funding: This study received grant support from the National Institute of Allergy and Infectious Diseases: AI-68634 (Statistical and Data Management Center), AI-68636 (ACTG). Boehringer Ingelheim, Janssen, Merck, ViiV Healthcare, AbbVie, and Roche provided study medications. Monogram Biosciences provided resistance and tropism tests. Merck provided additional funding to support the costs of integrase genotypic resistance testing for the trial. Dr. Fichtenbaum received support to conduct this study from NIAID UM1-AI-069501 (Case CTU; Cincinnati CRS).

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ABSTRACT

Background: Short-term (48-week) results of the OPTIONS trial showed that nucleoside reverse transcriptase inhibitors (NRTIs) can be safely omitted from salvage therapy as long as the regimen has a cumulative activity of >2 active antiretroviral (ARV) medications. The long-term durability of this approach and outcomes in persons who have~~with~~ more-extensive HIV-1 drug resistance are uncertain.

Methods: Participants with virologic failure and anticipated ARV susceptibility received an optimized regimen and were randomized to Omit or Add NRTIs. A separate group with more resistance (cumulative activity ≤2 active agents) received an optimized regimen including NRTIs.

Results: At week 96, among 360 participants randomized to Omit or Add NRTIs, 70% and 65% had HIV-1 RNA <200 copies/mL, respectively. Virologic failure was uncommon after week 48. Younger age and starting fewer new antiretroviral medications were associated with higher odds of virologic failure. In the Highly Resistant group, 53% had HIV-1 RNA <200 copies/mL at week 96.

Conclusions: HIV-1 salvage therapy can safely omit NRTIs without compromising efficacy or durability of response as long as the new regimen has a cumulative activity of >2 active drugs. Younger people and those receiving fewer new ARVs require careful monitoring. Even among individuals with more-extensive resistance, most achieve virologic suppression.

Keywords: HIV-1, antiretroviral therapy, treatment-experienced participants, randomized controlled trial, salvage therapy, drug resistance.

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INTRODUCTION

In people with HIV-1 infection (PWH) who have virologic failure on antiretroviral therapy (ART), guidelines recommend starting at least two, and preferably three, new active antiretroviral medications[1]. The question of whether nucleoside reverse transcriptase inhibitors (NRTIs) should be included in a new regimen when other active agents are available was addressed in the OPTIONS trial (AIDS Clinical Trials Group (ACTG) ~~study~~ A5241)[2]. In this study, participants who were failing protease inhibitor (PI)-based therapy but whose virus was sensitive to a new regimen with a cumulative activity of >2 active agents were randomized to ~~either~~ add ~~NRTIs to~~ or omit NRTIs from their new regimen. At week 48, the Omit NRTIs group was not inferior to the Add NRTIs group for the primary outcome of regimen failure[2].

The initial report of the OPTIONS trial findings focused on week 48 results (~~the time point for the~~ primary outcome) leaving important questions unanswered, such as the long-term durability of the two strategies. In addition, the impact of NRTIs on metabolic outcomes and ~~the effect of NRTIs on~~ quality-of-life were not described. Now, we report on the virologic responses through 96 weeks (~~the~~ end of study follow-up) and factors associated with virologic failure. In participants who experienced virologic failure ~~during follow-up~~, we describe the frequency and type of treatment-emergent drug resistance. Because of the importance of safety and tolerability with long-term ART, we present the metabolic, renal and self-reported quality-of-life outcomes.

In addition to the two groups that were randomized to omit or add NRTIs, OPTIONS included a third, non-randomized, group with more drug resistance (sensitive only to a regimen with a cumulative phenotypic susceptibility score of ≤2 active agents as opposed to >2 in the randomized groups). Based on ~~their~~ treatment history and resistance testing, the participants in this group were treated with a combination of active and partially-active agents that included NRTIs. Here, for the first time we report the outcomes following treatment in these individuals with highly drug-resistant HIV-1.

METHODS

The OPTIONS design, eligibility criteria and procedures were previously described[2]. OPTIONS (NCT00537394) was an open-label, Phase III, partially randomized strategy trial in ~~HIV-1-infected~~ treatment-experienced ~~participants~~ PWH (failing PI-based regimen with triple-class experience or drug resistance [non-nucleoside reverse transcriptase inhibitors (NNRTIs), NRTIs and PIs]) that used a continuous phenotype susceptibility score (cPSS) to select an optimized antiretroviral (ARV) regimen. The cPSS is the sum of the predicted activity of ARVs (excluding NRTIs) in each study regimen[3]. An optimized regimen was the combination of ARVs with the highest cPSS that was acceptable to the participant and local study investigators. Optimized regimens and NRTIs were recommended based upon treatment history, viral resistance and co-receptor tropism test results (PhenoSense GT® and Trofile®, respectively; Monogram Biosciences). Participants who had previously received enfuvirtide or an integrase strand transfer inhibitor (INSTI) were presumed to be resistant to these agents. Participants with cPSS

>2 were randomly assigned to receive their optimized regimen only (Omit NRTIs group) or to add NRTIs (Add NRTIs group) to their optimized regimen, stratified by INSTI experience and choice of maraviroc-containing study regimen. A separate group of participants with cPSS ≤2 (Highly Resistant group) were directly assigned to receive an optimized regimen and add NRTIs. Optimized regimens, consisting of medications available at the time of the trial, were composed of 3 or 4 of the following: ritonavir-boosted darunavir or tipranavir, raltegravir, etravirine, maraviroc or enfuvirtide. All participants were in the U.S. and provided informed consent in compliance with ~~guidelines of the~~ U.S. Department of Health and Human Services guidelines.

Procedures and outcomes

Study evaluations occurred before entry, at entry, weeks 1, 4, 8, 12, 16, and 24, and every 12 weeks thereafter through week 96. The primary efficacy outcome was regimen failure, which was a composite outcome of first confirmed virologic failure or discontinuation of NRTI assignment. Virologic failure was defined as any one of the following (with confirmation on repeat measurement): <1 $\log_{10}$ copies/mL HIV-1 RNA decrease from baseline to week 12; virologic rebound to >200 copies/mL after suppression to <200 copies/mL; lack of suppression to <200 copies/mL by week 24; or HIV -1 RNA ≥200 copies/mL at or after week 48. Following intention-to-treat principles, participants who experienced virologic failure or who discontinued their assigned NRTI strategy (primary study endpoint) continued to be followed through 96 weeks to be evaluated for secondary outcomes. Secondary outcomes included change in CD4 cell

count from baseline; occurrence of newly acquired HIV-1 drug resistance or tropism shift between baseline and confirmed virologic failure; change in lipids from baseline; change in cardiovascular risk score from baseline; and change in quality of life (QoL) scores from baseline. Fasting lipids were collected at entry, weeks 24, 48 and 96. Quality of life was assessed at baseline, weeks 8, 24 and every 24 weeks thereafter using the general health score, which uses a visual analog scale that ranges from 0 (worst possible health) to 100 (perfect health). Cardiovascular risk was assessed using the Framingham risk score as this study was completed in 2011 prior to the introduction of newer guidelines for assessing risk.

Statistical Analysis

Calculating percentages of participants with HIV-1 RNA below ~~thresholds~~ limits ~~(e.g. 50 copies/mL)~~ used two methods: ~~. In the~~ observed analysis~~, percentages were calculated among~~ included only participants with an observed RNA result ~~at week 96;. In the~~ imputed analysis~~, percentages were calculated among~~included all ~~enrolled~~ participants, and missing ~~RNA~~ values were ~~at week 96 were~~ imputed as greater than ~~threshold~~limit.

Cumulative probability of regimen or virologic failure by 96 weeks was estimated using a stratified Kaplan–Meier estimator. Stratum-specific estimates by ~~treatment~~ group used inverse variance weights. Confidence Intervals (CIs) used log(–log)-transformed Greenwood-estimated variance. Participants ~~discontinuing study~~ without regimen failure were censored at last visit. Non-inferiority was ~~was~~ concluded if the upper 95%

confidence bound of the treatment difference ~~in cumulative probability of regimen failure~~ was <15%.

Secondary outcomes ~~over time were assessed using~~ used marginal modeling with generalized estimating equations incorporating~~. For continuous outcomes , a linear regression model with~~ equicorrelation ~~for within-participant correlation~~structure ~~was used~~for continuous outcomes and ~~. For dichotomous outcomes, a logit link assuming a Bernoulli variance structure and~~ independence correlation ~~structure was used~~and logit link for dichotomous outcomes. Non-linear time trends were included as suggested by goodness of fit using Quasi-AIC.

~~Baseline characteristics were tested for A~~association of baseline characteristics with **observed** virologic failure in the randomized groups ~~using~~ used ~~multivariable~~ logistic regression, ~~incorporating~~ a stepwise covariate selection process, **reparameterization** ~~reparameteization~~ of ~~select~~ covariates exhibiting ~~evidence of~~ non-linearity in the logit, and testing for all 2-way statistical interactions in the main ~~-~~effects model.

RESULTS

Study Participants

A total of 413 participants enrolled. Three hundred-sixty participants with ~~a continuous phenotypic susceptibility score (~~cPSS~~)~~ of >2 were randomized to receive ~~either~~ an optimized regimen without NRTIs (Omit NRTIs group, n=179) or an optimized regimen

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that added NRTIs (Add NRTIs group, n=181). An additional 53 participants who had highly resistant virus received an optimized regimen with a cumulative activity of 2 or fewer active agents (cPSS ≤2) and added NRTIs (Highly Resistant group). Table 1 summarizes baseline characteristics. Figure 1 shows participant disposition: 159 in the Omit NRTIs group (89%), 158 in the Add NRTIs group (87%), and 44 in the Highly Resistant group (83%) completed the study with a week 96 visit.

There were fewer deaths following treatment initiation in the Omit NRTIs group than in the Add NRTIs group (Figure 1): 1 and 10, respectively; cumulative probability of death through 96 weeks (95% CI): 0.6% (0.1%, 4%) and 5.7% (3.1%, 10.3%), respectively. Because of the small number of events, the 95% confidence intervals on the cumulative probabilities of death overlap. The cumulative probability of death through 96 weeks (2 deaths) in the Highly Resistant group (also receiving NRTIs) was 4% (1%, 15.1%). The causes and timing of death were heterogeneous and there was no pattern suggesting a common mechanism or specific etiology.

Regimen and Virologic Failure

At week 96, 70% of all 179 participants in the Omit NRTIs group and 65% of all 181 participants in the Add NRTIs group had HIV-1 RNA <200 copies/mL; while 61% and 59% had HIV-1 RNA <50 copies/mL, respectively (Supplementary Table 1). Among the 158 participants in each randomized group who had a week 96 HIV-1 RNA value (observed

analysis), 79% in the Omit NRTIs group and 75% in the Add NRTIs group had HIV-1 RNA <200 copies/mL; while 69% and 68% had HIV-1 RNA <50 copies/mL, respectively.

The cumulative probability for regimen failure (virologic failure or discontinuation of NRTI assignment) at week 96 was 33.6% in the Omit NRTIs group and 31.3% in the Add NRTIs group. The upper bound of the 95% CI on the difference in regimen failure between randomized groups (Omit – Add) was 11.5% and, thus, non-inferiority of Omit versus Add NRTIs (compared to the pre-specified bound of 15%) was achieved. Most regimen failures were due to virologic failure (46 of 60 in the Omit NRTIs group, 50 of 57 in the Add NRTIs group). By week 96, the estimated cumulative probability for virologic failure was 28.2% in the Omit NRTIs group and 30.2% in the Add NRTIs group (Figure 2); the upper 95% confidence bound on the difference between groups was 7.4% and, thus, the Omit NRTIs group can be concluded to be non-inferior to the Add NRTIs group at a lower non-inferiority threshold of 10%. Most virologic failures occurred in the first 48 weekss ~~s of the trial~~: only 15 of 104 (14%) virologic failures occurred in the randomized groups after week 48.

In the Highly Resistant group, at week 96, 53% (of 53 participants) had HIV-1 RNA <200 copies/mL and 47% had HIV-1 RNA <50 copies/mL. Among 43 participants who had a week 96 HIV-1 RNA value (observed analysis), 65% had HIV-1 RNA <200 copies/mL and 58% had HIV-1 RNA <50 copies/mL.

Change in CD4 Cell Count

At week 96, the mean (95% CI) CD4 cell count was 391 (357-425)/mm^3 for the Omit NRTIs group and 428 (383-473)/mm^3 for the Add NRTIs group. Mean increases in CD4 cell count from baseline to week 96 were 143 (115-170) and 174 (145-203)/mm^3, respectively. In the Highly Resistant group, the mean CD4 cell count at week 96 was 307/mm^3 and the mean increase from baseline to week 96 was 133/mm^3.

Baseline Factors Associated with Virologic Failure in the Randomized Groups

The following factors were significantly and independently associated with virologic failure in the randomized groups: age, number of active NRTIs chosen prior to randomization (regardless of treatment arm), total number of new antiretrovirals started following randomization, and quality--of--life (QoL) score (Supplementary Table 2). Younger participants (age 16-46 years) had significantly higher odds of virologic failure compared to older participants (age 47-69 years) (adjusted odds ratio (AOR) 4.4, 95%CI (2.4, 8.2). The number of active NRTI in the regimen chosen prior to randomization (reflecting the extent of resistance to this class) was associated with virologic failure; in general, having 1 active NRTI was associated with the lowest odds of virologic failure (Supplementary Table 2). Participants who started fewer new antiretrovirals had higher odds of virologic failure (1-2 new medications vs. 4-6, AOR 6.9, (2.0, 24.0); 3 vs. 4-6, AOR 3.0, (1.4, 6.5)). QoL score was not significantly associated with virologic failure in the Omit NRTIs group; however, in the Add NRTIs group, the AOR of virologic failure was higher in participants with low baseline QoL scores (quartile 1: 0-60 points) versus high QoL scores (quartiles 3 and 4: 76-100 points): AOR 5.1 (2.0, 13.2); or

medium (quartile 2: 61-75 points) versus high scores: AOR 3.4 (1.2, 9.3).

Tropism Changes at Virologic Failure

A total of 177 randomized participants had R5 virus at screening; most received a regimen containing maraviroc (71% in the Add NRTIs group; 69% in the Omit NRTIs group). Within the R5 subgroup, 27 of 89 participants in the Add NRTIs group (30%) and 22 of 88 participants in the Omit NRTIs (25%) experienced virologic failure. At virologic failure, ~~most participants still had circulating R5 virus:~~ only 5 of 45 (11%) who had a tropism result had non-R5 virus.

Treatment-emergent Resistance among Participants with Virologic Failure

Among the 131 participants across all three groups who experienced virologic failure, 9 did not have resistance test results. For the 122 participants with results, we assessed changes in HIV-1 sensitivity to NRTIs, NNRTIs and PIs using phenotypic testing (Monogram PhenoSense GT®) for the randomized groups and changes in INSTI resistance using genotyping (for participants who received raltegravir) for all groups. For phenotypic testing, a drug was considered susceptible if the individual's net assessment from the report was either "partially sensitive" or "sensitive". The findings are summarized by antiretroviral class.

NRTI: Treatment-emergent phenotypic resistance to NRTIs at ~~time of~~ virologic failure was uncommon. For example, for tenofovir, in the Add NRTIs group, 6 participants (11%) with virologic failure had an increase in fold-change resistance and 2 (4%) had

reversion to less resistance; in the Omit NRTIs group, 2 participants (4%) had an increase in resistance and 5 (10%) had reversion to less resistance.

NNRTI: Eighty-two percent of randomized participants received an ARV regimen containing etravirine. **Of the 82 etravirine-exposed participants who experienced virologic failure and had resistance data, 13 (16%) developed treatment-emergent etravirine resistance.** ~~A total of 88 of the 104 randomized participants (85%) who had virologic failure had prior exposure to etravirine. By the time of virologic failure, 13 participants (24%) in the Add NRTIs group and 9 (18%) participants in the Omit NRTIs group had an increase in resistance to etravirine compared to baseline.~~

PI: Eighty-six percent of participants in the randomized groups ~~who had~~with virologic failure received ritonavir-boosted darunavir. Treatment-emergent darunavir resistance was rare: Of the 89 darunavir-exposed participants with virologic failure, only 3 (3.4%) developed treatment-emergent darunavir resistance.

INSTI: Among the 131 participants with virologic failure, 116 received raltegravir; of these, 104 had integrase genotyping completed at baseline and 104 had testing completed at time of virologic failure. At baseline, 4 participants (all in the Highly Resistant group) had ≥1 major primary integrase resistance mutation (~~as defined by the Stanford HIV Drug Resistance Database Version 8.2;~~ Supplementary Table 3)[4]; 15 participants had ≥1 major accessory integrase resistance mutation; and 88 participants

had no mutations. At time of virologic failure, 24 participants had ≥1 major primary or major accessory mutation; 11 participants had both major primary and major accessory mutations (8 of these were in the Highly Resistant group), 4 participants had 1 major primary mutation (1 in Highly Resistant group), and 9 participants had ≥1 major accessory mutations (none from the Highly Resistant group). The rate of treatment-emergent major primary integrase resistance among participants who did not have such a mutation at baseline was 11% (11/100).

Effect of NRTIs on Metabolic and Renal Outcomes

We examined the effect of NRTIs on lipids by comparing the randomized groups. There was a ~~significantly~~ greater increase in total cholesterol from baseline in the Omit NRTIs group compared to the Add NRTIs group (Omit NRTIs group estimated changes 17 mg/dL higher than Add NRTIs group; p=0.0007), non-HDL cholesterol from fasting samples (Omit NRTIs estimated changes 17 mg/dL higher than Add NRTIs group p= 0.0013), and LDL cholesterol (Omit NRTIs estimated changes 13 mg/dL higher than Add NRTIs group, p=0.0026). Ninety-five percent of participants in the Add NRTIs group received tenofovir disoproxil fumarate (TDF), which ~~is known to decrease~~decreases lipids[5, 6].

We also assessed the Framingham Risk Score (FRS) ~~of participants~~ in the randomized groups (FRS was the most widely-used cardiovascular risk prediction tool at the time of the study). The Omit NRTIs group had increasingly higher proportions (39% at week 24, 43% at week 48, 46% at week 96) of participants with moderate-to-high (>10%) risk

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scores compared to the Add NRTIs group (38% at week 24, 40% at week 48, 43% at week 96) (p=0.04 for treatment-by-time interaction), perhaps related to differences in lipids between the groups.

~~Due to concerns that NRTIs may affect renal function, w~~We examined changes in estimated creatinine clearance among participants in the randomized groups. There was ~~a~~ greater decline in creatinine clearance from baseline in the Add NRTIs group than in the Omit NRTIs group at week 96: mean -2.7% vs. +1.7% (p=0.037).

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Quality-of-Life Scores

In all three groups, the mean QoL score significantly increased from baseline to week 96 (Figure 3). There were no significant differences between randomized treatment groups in change in QoL from baseline over 96 weeks (p=0.41)

DISCUSSION

The primary results of the OPTIONS trial demonstrated that~~,~~ in ~~people with HIV-1~~PWH who have virologic failure on ART and who start a regimen with a cumulative activity of >2 active antiretroviral medications~~,~~ omitting NRTIs did not result in inferior rates of regimen (mostly virologic) failure compared to adding NRTIs by 48 weeks. Now, we report the 96-week results of the trial, which confirm that HIV-1 salvage therapy can safely omit NRTIs without compromising regimen efficacy or durable virologic response as long as the new regimen contains a sufficient number of active drugs. The observation that virologic failure was uncommon after week 48 (>85% of virologic

failures occurred before this time point) indicates that, even in highly treatment-experienced persons who have~~with~~ drug-resistant HIV-1, once virologic suppression is achieved, it is typically sustained.

The number of deaths between treatment initiation and 96 weeks was lower in the Omit NRTIs group than in the Add NRTIs group but the 95% confidence intervals on the cumulative probability of death for this timeframe overlapped. The causes of death were heterogeneous and there was no pattern to suggest a common mechanism or specific etiology for the imbalance. Additional investigations of mitochondrial function and inflammation in the two groups are underway and will be the topic of a separate report.

Several characteristics were associated with virologic failure in the randomized groups in OPTIONS. Compared to older participants, younger participants were more likely to experience virologic failure. Previous studies have shown that younger people have greater difficulties with adherence[7, 8], suggesting enhanced adherence support is needed to improve outcomes in this high-risk group. As in previous studies of second-line therapy (EARNEST, SECOND-LINE, ACTG A5273), in OPTIONS having virus with less NRTI resistance at time of regimen selection was associated with higher odds of virologic failure, perhaps related to poorer adherence[9-11]. Finally, starting fewer new antiretroviral medications was associated with a higher likelihood of virologic failure,

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emphasizing the importance of using new classes of active medications as part of salvage regimens whenever possible.

The importance of active agents in achieving virologic suppression was further demonstrated ~~by the results~~ in the Highly Resistant group who were directly assigned to receive active and partially-active medications. As expected, this group had lower rates of virologic suppression than the randomized groups, where the cumulative activity of the regimen was higher. Nevertheless, even in the Highly Resistant group, over half of participants achieved HIV-1 RNA <200 copies/mL at week 96, indicating that virologic suppression is possible in this difficult-to treat population. Current regimens may yield even more favorable results. In OPTIONS, the only integrase inhibitor available was raltegravir. Based on results of the SAILING trial[12], which showed that dolutegravir was superior to raltegravir in participants with previous virologic failure, one would anticipate that regimens with dolutegravir would be associated with even better virologic outcomes than those seen in OPTIONS.

The OPTIONS trial also confirmed that the frequency of treatment-emergent resistance varies by antiretroviral class. In participants who received the PI, darunavir, only 3.4% of those with virologic failure developed treatment-emergent darunavir resistance, a remarkably low proportion and consistent with the high barrier to resistance of this class even in highly treatment-experienced patients. ~~**On the opposite end of the spectrum,**~~ By contrast, 16% ~~18-24% of those who received an NNRTI, most frequently~~

~~etravirine, and experienced virologic failure had treatment-emergent~~ ~~an increase in resistance to etravirine compared to baseline~~of those with virologic failure developed treatment-emergent etravirine resistance**. The rate of treatment-emergent primary major INSTI resistance on raltegravir ~~fell~~ was similar ~~in between~~ (11%). These results comport to the higher barrier to resistance of boosted PIs as compared to NNRTI ~~and the intermediate barrier of~~or first-generation INSTIs, like raltegravir.**

~~In addition to assessing virologic outcomes, we~~We also evaluated quality-of-life scores, which significantly improved after starting a new regimen, demonstrating a strong link between effective treatment and better QoL. Participants in the Add NRTIs group who had lower QoL at baseline had higher likelihood of virologic failure; this association was not observed in the Omit NRTIs group. One potential explanation is that participants with lower quality of life were less able to tolerate NRTIs leading to higher rates of virologic failure.

Finally, we found expected changes in metabolic and renal parameters. Total cholesterol, non-HDL cholesterol and LDL cholesterol levels rose in the Omit NRTIs group compared to the Add NRTIs group, most likely because 95% of those in the latter group received TDF, which lowers lipids[5, 6]. There was a small decline in creatinine clearance (-2.7%) in the Add NRTI group, possibly from TDF, which ~~is known to affect~~affects renal function[13, 14].

The OPTIONS trial is unique in several aspects: participants received 2-3 active agents in the randomized arms and did not receive NRTIs in one arm. In contrast, recycling of NRTIs was a component of most previous treatment-experienced trials: In the DUET, RESIST, POWER, MOTIVATE and BENCHMRK trials[15-19], treatment-experienced participants received an optimized background regimen with or without a single new agent; response rates varied from 34%-72% at 48 weeks and 58-62% at 96 weeks. OPTIONS demonstrated sustained virologic responses in the majority of participants even without recycling NRTIs – a finding which ~~has~~ changed treatment guidelines[1].

A limitation of th~~e~~is analysis ~~presented here~~ is that most participants (82%) in the Add NRTIs group received TDF/FTC; the lipid and renal effects we observed would likely not be seen with tenofovir alafenamide or abacavir. Strengths of the study include the large sample size and the long duration of follow-up.

In conclusion, the 96-week results ~~presented here~~ confirm and extend the original findings of the OPTIONS trial: HIV-1 salvage therapy can safely omit NRTIs without compromising regimen efficacy or durable virologic response as long as the new regimen contains a sufficient number of active drugs. We have identified specific sub-groups at a higher risk of virologic failure; based on these findings, more careful attention to younger people and those receiving fewer new antiretroviral medications is warranted. Ultimately, including newer agents in salvage regimens, like second-generation integrase inhibitors or drugs against novel targets, are likely to improve

417 virologic outcomes even further, leading to sustained virologic suppression in the vast

418 majority of treatment-experienced people ~~living~~ with HIV-1.

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Figure legends

Figure 1: Participant disposition.

Figure 2: Cumulative probability of virologic failure over time by treatment group.

Figure 3: Mean quality of life score and change in quality of life score over time by treatment group. Quality of life was assessed using the general health score, which uses a visual analog scale that ranges from 0 (worst possible health) to 100 (perfect health).

Long-term Outcomes in a Large Randomized Trial of HIV-1 Salvage Therapy: 96-week Results of AIDS Clinical Trials Group A5241 (OPTIONS)

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Running title: Long-term Results of HIV Salvage Therapy

Word counts: Abstract: 200 words. Text: 3497 words.

Summary: HIV-1 salvage therapy can safely omit nucleoside reverse transcriptase inhibitors without compromising efficacy or durability of response as long as the new regimen has a cumulative activity of 2 or more active drugs.

Footnotes

Conflicts of Interest: RTG's institution has received educational grants from Gilead, Viiv, Janssen, Theratechnologies and Merck; RTG has served on scientific advisory boards for Merck, Gilead and Theratechnologies. KTT's institution receives research grants from ViiV, Janssen, Merck and Gilead, and she has served as an advisor for Gilead Sciences. JJE is an ad hoc consultant to Merck, Gilead Sciences, Janssen and ViiV Healthcare and receives contract support from Gilead Sciences, Janssen and ViiV Healthcare for work unrelated to this study. CJF receives grants to his institution for research and education from Gilead, ViiV, Janssen, Merck, Amgen, Cytodyn and is on the speakers bureau for Clinical Care Options. The views expressed are those of the authors and do not necessarily represent the views of the NIH or Department of Health and Human Services.

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ABSTRACT

Background: Short-term (48-week) results of the OPTIONS trial showed that nucleoside reverse transcriptase inhibitors (NRTIs) can be safely omitted from salvage therapy as long as the regimen has a cumulative activity of >2 active antiretroviral (ARV) medications. The long-term durability of this approach and outcomes in persons **who have** more-extensive **HIV-1** drug resistance are uncertain.

Methods: Participants with virologic failure and anticipated ARV susceptibility received an optimized regimen and were randomized to Omit or Add NRTIs. A separate group with more resistance (cumulative activity ≤2 active agents) received an optimized regimen including NRTIs.

Results: At week 96, among 360 participants randomized to Omit or Add NRTIs, 70% and 65% had HIV-1 RNA <200 copies/mL, respectively. Virologic failure was uncommon after week 48. Younger age and starting fewer new antiretroviral medications were associated with higher odds of virologic failure. In the Highly Resistant group, 53% had HIV-1 RNA <200 copies/mL at week 96.

Conclusions: HIV-1 salvage therapy can safely omit NRTIs without compromising efficacy or durability of response as long as the new regimen has a cumulative activity of >2 active drugs. Younger people and those receiving fewer new ARVs require careful monitoring. Even among individuals with more-extensive resistance, most achieve virologic suppression.

Keywords: HIV-1, antiretroviral therapy, treatment-experienced participants, randomized controlled trial, salvage therapy, drug resistance.

INTRODUCTION

In people with HIV-1 infection (PWH) who have virologic failure on antiretroviral therapy (ART), guidelines recommend starting at least two, and preferably three, new active antiretroviral medications[1]. The question of whether nucleoside reverse transcriptase inhibitors (NRTIs) should be included in a new regimen when other active agents are available was addressed in the OPTIONS trial (AIDS Clinical Trials Group (ACTG) A5241)[2]. In this study, participants who were failing protease inhibitor (PI)-based therapy but whose virus was sensitive to a new regimen with a cumulative activity of >2 active agents were randomized to add or omit NRTIs from their new regimen. At week 48, the Omit NRTIs group was not inferior to the Add NRTIs group for the primary outcome of regimen failure[2].

The initial report of the OPTIONS trial findings focused on week 48 results (primary outcome) leaving important questions unanswered, such as the long-term durability of the two strategies. In addition, the impact of NRTIs on metabolic outcomes and quality-of-life were not described. Now, we report on the virologic responses through 96 weeks (end of study follow-up) and factors associated with virologic failure. In participants who experienced virologic failure, we describe the frequency and type of treatment-emergent drug resistance. Because of the importance of safety and tolerability with long-term ART, we present the metabolic, renal and self-reported quality-of-life outcomes.

In addition to the two groups that were randomized to omit or add NRTIs, OPTIONS included a third, non-randomized, group with more drug resistance (sensitive only to a regimen with a cumulative phenotypic susceptibility score of ≤2 active agents as opposed to >2 in the randomized groups). Based on treatment history and resistance testing, the participants in this group were treated with a combination of active and partially-active agents that included NRTIs. Here, for the first time we report the outcomes following treatment in these individuals with highly drug-resistant HIV-1.

METHODS

The OPTIONS design, eligibility criteria and procedures were previously described[2]. OPTIONS (NCT00537394) was an open-label, Phase III, partially randomized strategy trial in treatment-experienced PWH (failing PI-based regimen with triple-class experience or drug resistance [non-nucleoside reverse transcriptase inhibitors (NNRTIs), NRTIs and PIs]) that used a continuous phenotype susceptibility score (cPSS) to select an optimized antiretroviral (ARV) regimen. The cPSS is the sum of the predicted activity of ARVs (excluding NRTIs) in each study regimen[3]. An optimized regimen was the combination of ARVs with the highest cPSS that was acceptable to the participant and local study investigators. Optimized regimens and NRTIs were recommended based upon treatment history, viral resistance and co-receptor tropism test results (PhenoSense GT® and Trofile®, respectively; Monogram Biosciences). Participants who had previously received enfuvirtide or an integrase strand transfer inhibitor (INSTI) were presumed to be resistant to these agents. Participants with cPSS >2 were randomly assigned to

receive their optimized regimen only (Omit NRTIs group) or to add NRTIs (Add NRTIs group) to their optimized regimen, stratified by INSTI experience and choice of maraviroc-containing study regimen. A separate group of participants with cPSS ≤2 (Highly Resistant group) were directly assigned to receive an optimized regimen and add NRTIs. Optimized regimens, consisting of medications available at the time of the trial, were composed of 3 or 4 of the following: ritonavir-boosted darunavir or tipranavir, raltegravir, etravirine, maraviroc or enfuvirtide. All participants were in the U.S. and provided informed consent in compliance with U.S. Department of Health and Human Services guidelines.

Procedures and outcomes

Study evaluations occurred before entry, at entry, weeks 1, 4, 8, 12, 16, and 24, and every 12 weeks thereafter through week 96. The primary efficacy outcome was regimen failure, which was a composite outcome of first confirmed virologic failure or discontinuation of NRTI assignment. Virologic failure was defined as any one of the following (with confirmation on repeat measurement): <1 $\log_{10}$ copies/mL HIV-1 RNA decrease from baseline to week 12; virologic rebound to >200 copies/mL after suppression to <200 copies/mL; lack of suppression to <200 copies/mL by week 24; or HIV -1 RNA ≥200 copies/mL at or after week 48. Following intention-to-treat principles, participants who experienced virologic failure or who discontinued their assigned NRTI strategy (primary study endpoint) continued to be followed through 96 weeks to be evaluated for secondary outcomes. Secondary outcomes included change in CD4 cell

count from baseline; occurrence of newly acquired HIV-1 drug resistance or tropism shift between baseline and confirmed virologic failure; change in lipids from baseline; change in cardiovascular risk score from baseline; and change in quality-of-life (QoL) scores from baseline. Fasting lipids were collected at entry, weeks 24, 48 and 96. Quality of life was assessed at baseline, weeks 8, 24 and every 24 weeks thereafter using the general health score, which uses a visual analog scale that ranges from 0 (worst possible health) to 100 (perfect health). Cardiovascular risk was assessed using the Framingham risk score as this study was completed in 2011 prior to the introduction of newer guidelines for assessing risk.

Statistical Analysis

Calculating percentages of participants with HIV-1 RNA below limits used two methods: observed analysis included only participants with an observed RNA result; imputed analysis included all participants, and missing values were imputed as greater than limit.

Cumulative probability of regimen or virologic failure by 96 weeks was estimated using a stratified Kaplan–Meier estimator. Stratum-specific estimates by group used inverse variance weights. Confidence Intervals (CIs) used log(–log)-transformed Greenwood-estimated variance. Participants without regimen failure were censored at last visit. Non-inferiority was concluded if the upper 95% confidence bound of the treatment difference was <15%.

Secondary outcomes used marginal modeling with generalized estimating equations incorporating equicorrelation structure for continuous outcomes and independence correlation and logit link for dichotomous outcomes. Non-linear time trends were included as suggested by goodness of fit using Quasi-AIC.

Association of baseline characteristics with **observed** virologic failure in the randomized groups used logistic regression, a stepwise covariate selection process, **reparameterization** of covariates exhibiting non-linearity in the logit, and testing for all 2-way statistical interactions in the main-effects model.

RESULTS

Study Participants

A total of 413 participants enrolled. Three hundred-sixty participants with cPSS of >2 were randomized to receive an optimized regimen without NRTIs (Omit NRTIs group, n=179) or an optimized regimen that added NRTIs (Add NRTIs group, n=181). An additional 53 participants who had highly resistant virus received an optimized regimen with a cumulative activity of 2 or fewer active agents (cPSS ≤2) and added NRTIs (Highly Resistant group). Table 1 summarizes baseline characteristics. Figure 1 shows participant disposition: 159 in the Omit NRTIs group (89%), 158 in the Add NRTIs group (87%), and 44 in the Highly Resistant group (83%) completed the study with a week 96 visit.

There were fewer deaths following treatment initiation in the Omit NRTIs group than

in the Add NRTIs group (Figure 1): 1 and 10, respectively; cumulative probability of death through 96 weeks (95% CI): 0.6% (0.1%, 4%) and 5.7% (3.1%, 10.3%), respectively. Because of the small number of events, the 95% confidence intervals on the cumulative probabilities of death overlap. The cumulative probability of death through 96 weeks (2 deaths) in the Highly Resistant group (also receiving NRTIs) was 4% (1%, 15.1%). The causes and timing of death were heterogeneous and there was no pattern suggesting a common mechanism or specific etiology.

Regimen and Virologic Failure

At week 96, 70% of all 179 participants in the Omit NRTIs group and 65% of all 181 participants in the Add NRTIs group had HIV-1 RNA <200 copies/mL; while 61% and 59% had HIV-1 RNA <50 copies/mL, respectively (Supplementary Table 1). Among the 158 participants in each randomized group who had a week 96 HIV-1 RNA value (observed analysis), 79% in the Omit NRTIs group and 75% in the Add NRTIs group had HIV-1 RNA <200 copies/mL; while 69% and 68% had HIV-1 RNA <50 copies/mL, respectively.

The cumulative probability for regimen failure (virologic failure or discontinuation of NRTI assignment) at week 96 was 33.6% in the Omit NRTIs group and 31.3% in the Add NRTIs group. The upper bound of the 95% CI on the difference in regimen failure between randomized groups (Omit – Add) was 11.5% and, thus, non-inferiority of Omit versus Add NRTIs (compared to the pre-specified bound of 15%) was achieved. Most

regimen failures were due to virologic failure (46 of 60 in the Omit NRTIs group, 50 of 57 in the Add NRTIs group). By week 96, the estimated cumulative probability for virologic failure was 28.2% in the Omit NRTIs group and 30.2% in the Add NRTIs group (Figure 2); the upper 95% confidence bound on the difference between groups was 7.4% and, thus, the Omit NRTIs group can be concluded to be non-inferior to the Add NRTIs group at a lower non-inferiority threshold of 10%. Most virologic failures occurred in the first 48 weeks: only 15 of 104 (14%) virologic failures occurred in the randomized groups after week 48.

In the Highly Resistant group, at week 96, 53% (of 53 participants) had HIV-1 RNA <200 copies/mL and 47% had HIV-1 RNA <50 copies/mL. Among 43 participants who had a week 96 HIV-1 RNA value (observed analysis), 65% had HIV-1 RNA <200 copies/mL and 58% had HIV-1 RNA <50 copies/mL.

Change in CD4 Cell Count

At week 96, the mean (95% CI) CD4 cell count was 391 (357-425)/mm^3 for the Omit NRTIs group and 428 (383-473)/mm^3 for the Add NRTIs group. Mean increases in CD4 cell count from baseline to week 96 were 143 (115-170) and 174 (145-203)/mm^3, respectively. In the Highly Resistant group, the mean CD4 cell count at week 96 was 307/mm^3 and the mean increase from baseline to week 96 was 133/mm^3.

Baseline Factors Associated with Virologic Failure in the Randomized Groups

The following factors were significantly and independently associated with virologic

failure in the randomized groups: age, number of active NRTIs chosen prior to randomization (regardless of treatment arm), total number of new antiretrovirals started following randomization, and quality-of-life (QoL) score (Supplementary Table 2). Younger participants (age 16-46 years) had significantly higher odds of virologic failure compared to older participants (age 47-69 years) (adjusted odds ratio (AOR) 4.4, 95%CI (2.4, 8.2). The number of active NRTI in the regimen chosen prior to randomization (reflecting the extent of resistance to this class) was associated with virologic failure; in general, having 1 active NRTI was associated with the lowest odds of virologic failure (Supplementary Table 2). Participants who started fewer new antiretrovirals had higher odds of virologic failure (1-2 new medications vs. 4-6, AOR 6.9, (2.0, 24.0); 3 vs. 4-6, AOR 3.0, (1.4, 6.5)). QoL score was not significantly associated with virologic failure in the Omit NRTIs group; however, in the Add NRTIs group, the AOR of virologic failure was higher in participants with low baseline QoL scores (quartile 1: 0-60 points) versus high QoL scores (quartiles 3 and 4: 76-100 points): AOR 5.1 (2.0, 13.2); or medium (quartile 2: 61-75 points) versus high scores: AOR 3.4 (1.2, 9.3).

Tropism Changes at Virologic Failure

A total of 177 randomized participants had R5 virus at screening; most received a regimen containing maraviroc (71% in the Add NRTIs group; 69% in the Omit NRTIs group). Within the R5 subgroup, 27 of 89 participants in the Add NRTIs group (30%) and 22 of 88 participants in the Omit NRTIs (25%) experienced virologic failure. At virologic failure, only 5 of 45 (11%) who had a tropism result had non-R5 virus.

Treatment-emergent Resistance among Participants with Virologic Failure

Among the 131 participants across all three groups who experienced virologic failure, 9 did not have resistance test results. For the 122 participants with results, we assessed changes in HIV-1 sensitivity to NRTIs, NNRTIs and PIs using phenotypic testing (Monogram PhenoSense GT®) for the randomized groups and changes in INSTI resistance using genotyping (for participants who received raltegravir) for all groups. For phenotypic testing, a drug was considered susceptible if the individual's net assessment from the report was either "partially sensitive" or "sensitive". The findings are summarized by antiretroviral class.

NRTI: Treatment-emergent phenotypic resistance to NRTIs at virologic failure was uncommon. For example, for tenofovir, in the Add NRTIs group, 6 participants (11%) with virologic failure had an increase in fold-change resistance and 2 (4%) had reversion to less resistance; in the Omit NRTIs group, 2 participants (4%) had an increase in resistance and 5 (10%) had reversion to less resistance.

NNRTI: Eighty-two percent of randomized participants received an ARV regimen containing etravirine. **Of the 82 etravirine-exposed participants who experienced virologic failure and had resistance data, 13 (16%) developed treatment-emergent etravirine resistance.**

PI: Eighty-six percent of participants in the randomized groups with virologic failure received ritonavir-boosted darunavir. Treatment-emergent darunavir resistance was

rare: Of the 89 darunavir-exposed participants with virologic failure, only 3 (3.4%) developed treatment-emergent darunavir resistance.

INSTI: Among the 131 participants with virologic failure, 116 received raltegravir; of these, 104 had integrase genotyping completed at baseline and 104 had testing completed at time of virologic failure. At baseline, 4 participants (all in the Highly Resistant group) had ≥1 major primary integrase resistance mutation (Supplementary Table 3)[4]; 15 participants had ≥1 major accessory integrase resistance mutation; and 88 participants had no mutations. At time of virologic failure, 24 participants had ≥1 major primary or major accessory mutation; 11 participants had both major primary and major accessory mutations (8 of these were in the Highly Resistant group), 4 participants had 1 major primary mutation (1 in Highly Resistant group), and 9 participants had ≥1 major accessory mutations (none from the Highly Resistant group). The rate of treatment-emergent major primary integrase resistance among participants who did not have such a mutation at baseline was 11% (11/100).

Effect of NRTIs on Metabolic and Renal Outcomes

We examined the effect of NRTIs on lipids by comparing the randomized groups. There was a greater increase in total cholesterol from baseline in the Omit NRTIs group compared to the Add NRTIs group (Omit NRTIs group estimated changes 17 mg/dL higher than Add NRTIs group; p=0.0007), non-HDL cholesterol from fasting samples (Omit NRTIs estimated changes 17 mg/dL higher than Add NRTIs group p= 0.0013), and LDL cholesterol (Omit NRTIs estimated changes 13 mg/dL higher than Add NRTIs group,

p=0.0026). Ninety-five percent of participants in the Add NRTIs group received tenofovir disoproxil fumarate (TDF), which decreases lipids[5, 6].

We also assessed the Framingham Risk Score (FRS) in the randomized groups (FRS was the most widely-used cardiovascular risk prediction tool at the time of the study). The Omit NRTIs group had increasingly higher proportions (39% at week 24, 43% at week 48, 46% at week 96) of participants with moderate-to-high (>10%) risk scores compared to the Add NRTIs group (38% at week 24, 40% at week 48, 43% at week 96) (p=0.04 for treatment-by-time interaction), perhaps related to differences in lipids between the groups.

Commented [TD1]: CF

We examined changes in estimated creatinine clearance among participants in the randomized groups. There was greater decline in creatinine clearance from baseline in the Add NRTIs group than in the Omit NRTIs group at week 96: mean -2.7% vs. +1.7% (p=0.037).

Quality-of-Life Scores

In all three groups, the mean QoL score significantly increased from baseline to week 96 (Figure 3). There were no significant differences between randomized treatment groups in change in QoL from baseline over 96 weeks (p=0.41)

DISCUSSION

The primary results of the OPTIONS trial demonstrated that in PWH who have virologic failure on ART and who start a regimen with a cumulative activity of >2 active antiretroviral medications omitting NRTIs did not result in inferior rates of regimen (mostly virologic) failure compared to adding NRTIs by 48 weeks. Now, we report the 96-week results of the trial, which confirm that HIV-1 salvage therapy can safely omit NRTIs without compromising regimen efficacy or durable virologic response as long as the new regimen contains a sufficient number of active drugs. The observation that virologic failure was uncommon after week 48 (>85% of virologic failures occurred before this time point) indicates that, even in highly treatment-experienced persons who have drug-resistant HIV-1, once virologic suppression is achieved, it is typically sustained.

The number of deaths between treatment initiation and 96 weeks was lower in the Omit NRTIs group than in the Add NRTIs group but the 95% confidence intervals on the cumulative probability of death for this timeframe overlapped. The causes of death were heterogeneous and there was no pattern to suggest a common mechanism or specific etiology for the imbalance. Additional investigations of mitochondrial function and inflammation in the two groups are underway and will be the topic of a separate report.

Several characteristics were associated with virologic failure in the randomized groups in OPTIONS. Compared to older participants, younger participants were more likely to

experience virologic failure. Previous studies have shown that younger people have greater difficulties with adherence[7, 8], suggesting enhanced adherence support is needed to improve outcomes in this high-risk group. As in **previous** studies of second-line therapy (EARNEST, SECOND-LINE, ACTG A5273), **in OPTIONS** having virus with less NRTI resistance at **time of regimen selection** was associated with **higher odds** of virologic failure, perhaps related to poorer adherence[9-11]. Finally, starting fewer new antiretroviral medications was associated with a higher likelihood of virologic failure, emphasizing the importance of using new classes of active medications as part of salvage regimens whenever possible.

The importance of active agents in achieving virologic suppression was further demonstrated in the Highly Resistant group who were directly assigned to receive active and partially-active medications. As expected, this group had lower rates of virologic suppression than the randomized groups, where the cumulative activity of the regimen was higher. Nevertheless, even in the Highly Resistant group, over half of participants achieved HIV-1 RNA <200 copies/mL at week 96, indicating that virologic suppression is possible in this difficult-to treat population. Current regimens may yield even more favorable results. In OPTIONS, the only integrase inhibitor available was raltegravir. Based on results of the SAILING trial[12], which showed that dolutegravir was superior to raltegravir in participants with previous virologic failure, one would anticipate that regimens with dolutegravir would be associated with even better virologic outcomes than those seen in OPTIONS.

The OPTIONS trial also confirmed that the frequency of treatment-emergent resistance varies by antiretroviral class. In participants who received the PI, darunavir, only 3.4% of those with virologic failure developed treatment-emergent darunavir resistance, a remarkably low proportion and consistent with the high barrier to resistance of this class even in highly treatment-experienced patients. **By contrast, 16% of those with virologic failure developed treatment-emergent etravirine resistance. The rate of treatment-emergent primary major INSTI resistance on raltegravir was similar (11%). These results comport to the higher barrier to resistance of boosted PIs as compared to NNRTI or first-generation INSTIs, like raltegravir.**

We also evaluated quality-of-life scores, which significantly improved after starting a new regimen, demonstrating a strong link between effective treatment and better QoL. Participants in the Add NRTIs group who had lower QoL at baseline had higher likelihood of virologic failure; this association was not observed in the Omit NRTIs group. One potential explanation is that participants with lower quality of life were less able to tolerate NRTIs leading to higher rates of virologic failure.

Finally, we found expected changes in metabolic and renal parameters. Total cholesterol, non-HDL cholesterol and LDL cholesterol levels rose in the Omit NRTIs group compared to the Add NRTIs group, most likely because 95% of those in the latter group received TDF, which lowers lipids[5, 6]. There was a small decline in creatinine

clearance (-2.7%) in the Add NRTI group, possibly from TDF, which affects renal function[13, 14].

The OPTIONS trial is unique in several aspects: participants received 2-3 active agents in the randomized arms and did not receive NRTIs in one arm. In contrast, recycling of NRTIs was a component of most previous treatment-experienced trials: In the DUET, RESIST, POWER, MOTIVATE and BENCHMRK trials[15-19], treatment-experienced participants received an optimized background regimen with or without a single new agent; response rates varied from 34%-72% at 48 weeks and 58-62% at 96 weeks. OPTIONS demonstrated sustained virologic responses in the majority of participants even without recycling NRTIs – a finding which changed treatment guidelines[1].

A limitation of this analysis is that most participants (82%) in the Add NRTIs group received TDF/FTC; the lipid and renal effects we observed would likely not be seen with tenofovir alafenamide or abacavir. Strengths of the study include the large sample size and the long duration of follow-up.

In conclusion, the 96-week results confirm and extend the original findings of the OPTIONS trial: HIV-1 salvage therapy can safely omit NRTIs without compromising regimen efficacy or durable virologic response as long as the new regimen contains a sufficient number of active drugs. We have identified specific sub-groups at a higher risk of virologic failure; based on these findings, more careful attention to younger people

391 and those receiving fewer new antiretroviral medications is warranted. Ultimately,
392 including newer agents in salvage regimens, like second-generation integrase inhibitors
393 or drugs against novel targets, are likely to improve virologic outcomes even further,
394 leading to sustained virologic suppression in the vast majority of treatment-experienced
395 people with HIV-1.

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Figure legends

Figure 1: Participant disposition.

Figure 2: Cumulative probability of virologic failure over time by treatment group.

Figure 3: Mean quality of life score and change in quality of life score over time by treatment group. Quality of life was assessed using the general health score, which uses a visual analog scale that ranges from 0 (worst possible health) to 100 (perfect health).

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic		Omit NRTIs Group (n=179)	Add NRTIs Group (n=181)	Randomized Groups Total (n = 360)	Highly Resistant Group (n=53)	P-value†
Age (years)						
Median (Q1, Q3)		46 (40, 51)	46 (41, 52)	46 (40, 52)	43 (40, 50)	0.233*
Female sex		47 (26%)	46 (25%)	93 (26%)	6 (11%)	0.021**
Race/Ethnicity	White Non-Hispanic	55 (31%)	59 (33%)	114 (32%)	18 (35%)	0.885**
	Black Non-Hispanic	69 (39%)	79 (44%)	148 (41%)	19 (37%)	
	Hispanic	46 (26%)	37 (21%)	83 (23%)	14 (27%)	
	Other	8 (4%)	4 (2%)	12 (3%)	1 (2%)	
Baseline CD4+ (cells/mm^3)						
Median (Q1, Q3)		212 (105, 348)	193 (104, 376)	207 (104, 363)	85 (25, 232)	<0.001*
Baseline HIV-1 RNA (log10 copies/mL)						
Median (Q1, Q3)		4.2 (3.6, 4.6)	4.2 (3.6, 4.7)	4.2 (3.6, 4.6)	4.4 (4.1, 4.8)	0.023*
Years of previous ARV exposure						
Median (Q1, Q3)		12 (9, 16)	10.7 (7.5, 14.0)	11.4 (0.5, 25.0)	13.1 (10.7, 16.5)	0.016*
Previous enfuvirtide or integrase inhibitor exposure		32 (18%)	34 (19%)	66 (18%)	40 (75%)	<0.001**
Screening HIV-1 Tropism	CCR5 (R5)	88 (49%)	89 (49%)	177 (49%)	10 (19%)	<0.001**
	Dual/Mixed (DM)	72 (40%)	71 (39%)	143 (40%)	31 (58%)	
	CXCR4 (R4)	8 (4%)	10 (6%)	18 (5%)	8 (15%)	
	Non-reportable (NR)	11 (6%)	11 (6%)	22 (6%)	4 (8%)	
Number of active NRTIs chosen prior to randomization‡	0	18 (10%)	21 (12%)	39 (11%)	6 (11%)	0.476**
	1	100 (56%)	103 (57%)	203 (56%)	34 (64%)	
	2 or 3	61 (34%)	57 (31%)	118 (33%)	13 (25%)	
Total number of new ARVs (including NRTIs) started following randomization	0	0 (0%)	0 (0%)	0 (0%)	9 (17%)	<0.001**
	1 – 2	17 (9%)	9 (5%)	26 (7%)	15 (28%)	
	3	138 (77%)	92 (51%)	230 (64%)	17 (32%)	
	4 – 6	24 (13%)	80 (44%)	104 (29%)	12 (23%)	
Total cholesterol from all samples (mg/dL)						
Median (Q1, Q3)		164 (140, 187)	164 (137, 192)	164 (139, 191)	178 (133, 207)	0.128*
Number missing		16	19	35	5	
Non-HDL cholesterol from fasting samples (mg/dL)						
Median (Q1, Q3)		124 (101, 149)	131 (102, 156)	126 (101, 152)	140 (109, 171)	0.053*
Number missing		27	28	55	8	
LDL cholesterol from all samples (mg/dL)						
Median (Q1, Q3)		90 (69, 115)	97 (69, 120)	93 (69, 117)	88 (62, 126)	0.963*
Number missing		30	30	60	10	
Framingham risk score (%)						
Median (Q1, Q3)		7.4 (3.4, 13.2)	8.5 (3.7, 13.3)	8.1 (3.6, 13.3)	8.6 (5.5, 14.5)	0.200*
Number missing		12	18	30	4	
Calculated Creatinine clearance (mL/min)						
Median (Q1, Q3)		108.4 (86.5, 134.4)	107.0 (88.4, 127.3)	107.3 (87.1, 130.7)	105.3 (97.1, 132.2)	0.419*
Number missing		1	0	1	0	
Quality of life score (points)						
Categories††	0 – 60 (quartile 1)	47 (26%)	51 (28%)	98 (27%)	10 (19%)	0.123**
	61 –75 (quartile 2)	42 (23%)	38 (21%)	80 (22%)	9 (17%)	
	76-100 (quartiles 3 & 4)	83 (46%)	89 (49%)	172 (48%)	34 (64%)	
	Missing	7 (4%)	3 (2%)	10 (3%)	0 (0%)	

Baseline characteristics above include the entire study sample except in cases where missing values are noted.
†Statistical comparisons of baseline characteristics between combined randomized groups and highly resistant group.
ǂ An active NRTIs is defined to be either 'Partially sensitive' or 'Sensitive' from a net assessment by Monogram PhenoSense GT ® testing at screening.
†† Quality of life categories defined by grouped quartiles as informed by correlates of virologic failure analysis.
** Two sample Wilcoxon test with continuity correction.*
*** Chi-square test.*

Figure 1 revised

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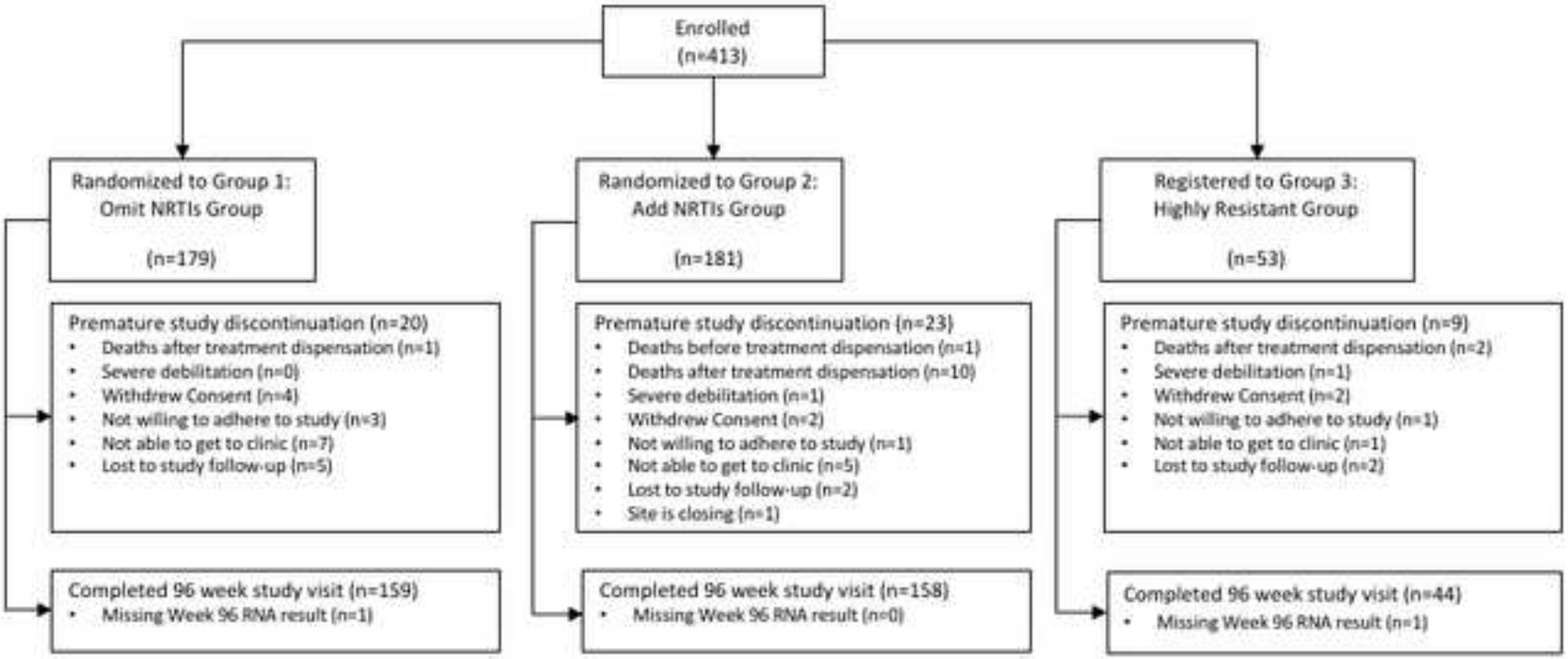

Figure 2

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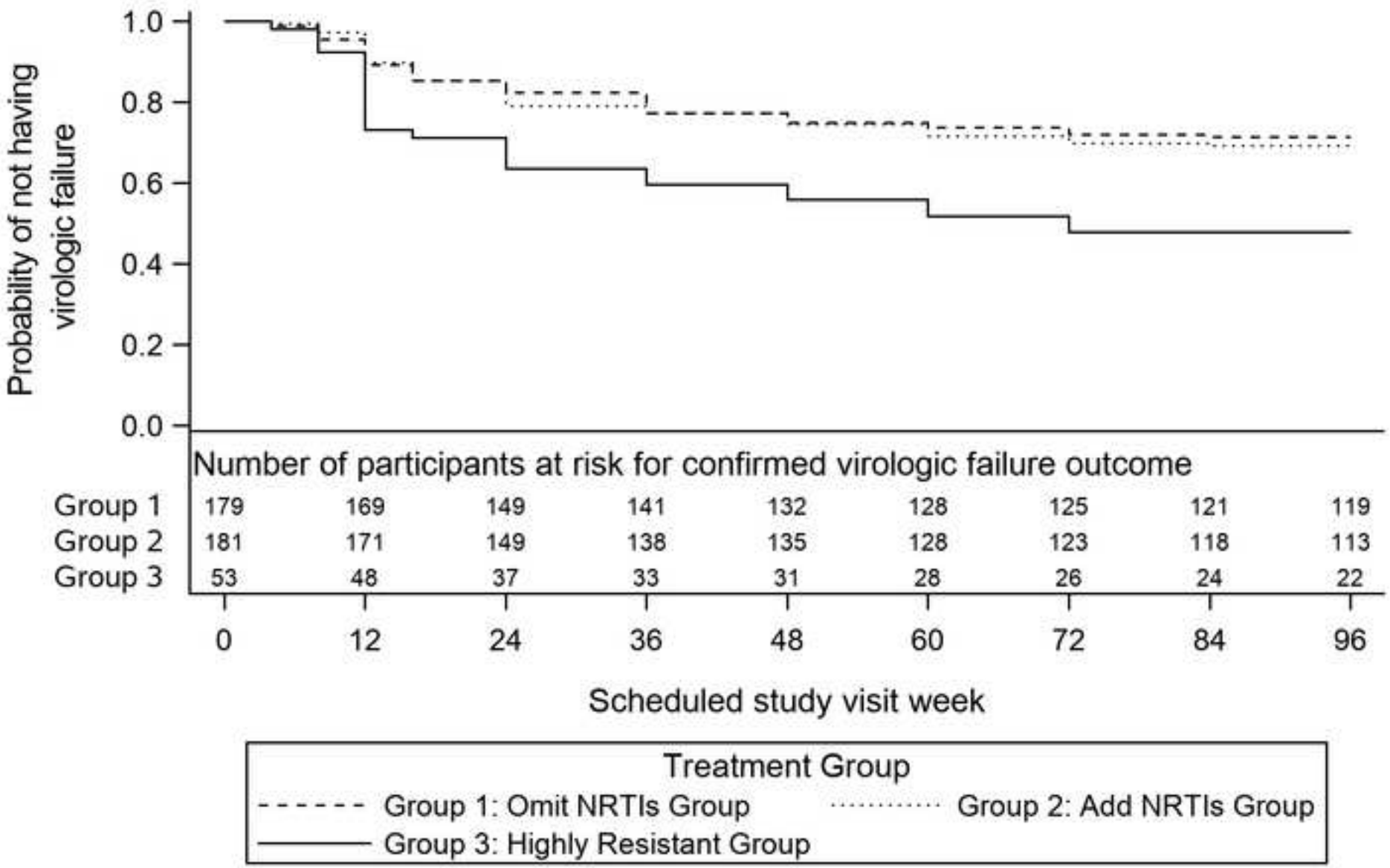

Figure 3

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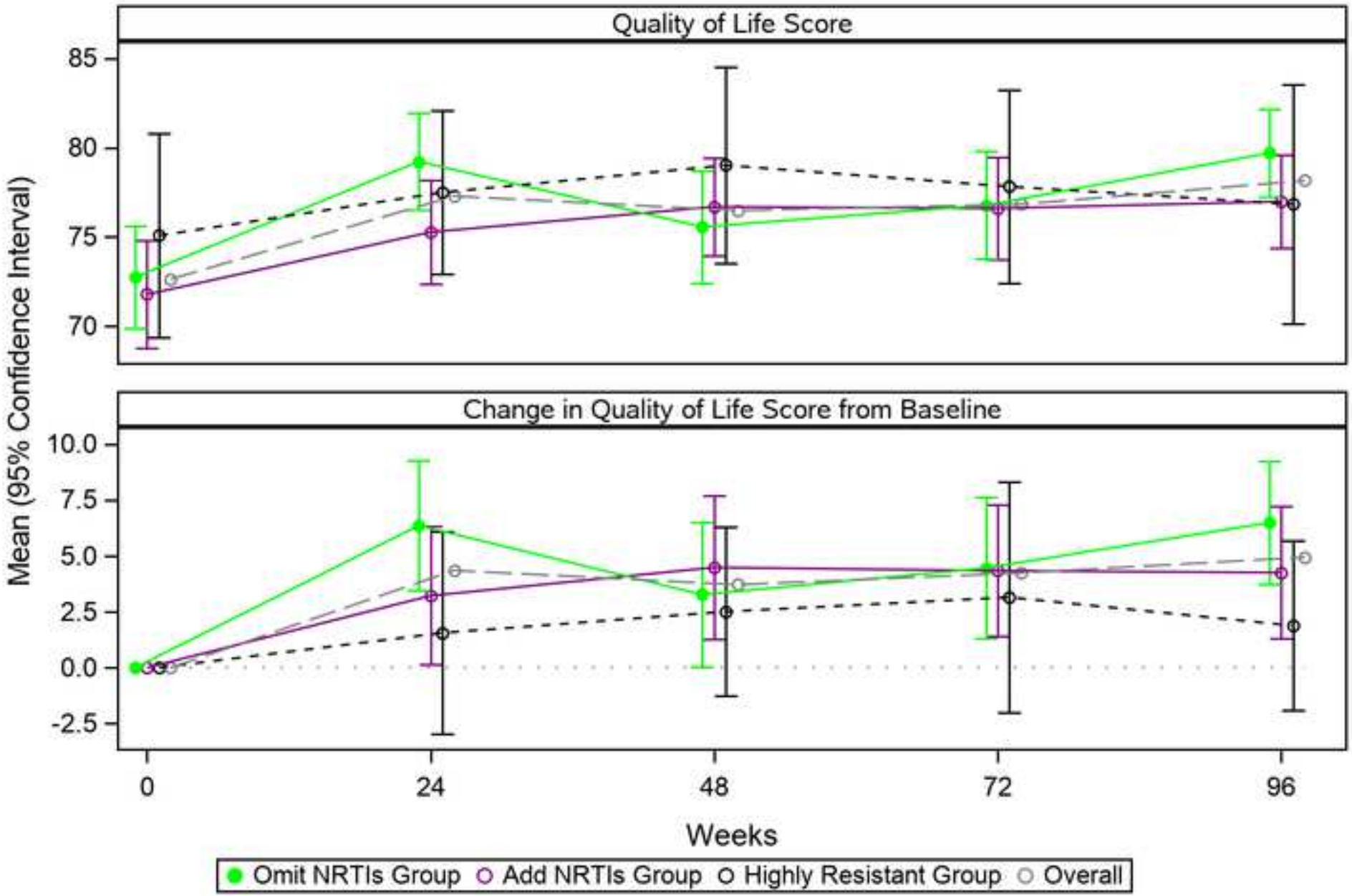

Supplementary Table 1: Percentage of participants below HIV RNA thresholds at week 96

	Imputed Analysis*		Observed Analysis*	
Groups	**HIV RNA Threshold**		**HIV RNA Threshold**	
	< 50 copies/mL	< 200 copies/mL	< 50 copies/mL	< 200 copies/mL
Omit NRTIs Group				
N	179	179	158	158
# of participants below threshold	109	125	109	125
Percentage (95% CI)	61% (53%, 68%)	70% (63%, 76%)	69% (61%, 76%)	79% (72%, 85%)
Add NRTIs Group				
N	181	181	158	158
# of participants below threshold	107	118	107	118
Percentage (95% CI)	59% (52%, 66%)	65% (58%, 72%)	68% (60%, 75%)	75% (67%, 81%)
Highly Resistant Group				
N	53	53	43	43
# of participants below threshold	25	28	25	28
Percentage (95% CI)	47% (33%, 61%)	53% (39%, 67%)	58% (42%, 73%)	65% (49%, 79%)

**For the imputed analysis, missing RNA value(s) of any reason at week 96 were assumed to be greater than either 50 or 200 copies/mL; therefore, all participants in each group were included in the denominator. The observed analysis at week 96 only included participants with non-missing RNA values. All 95% binomial confidence intervals were calculated using normal approximation.*

Supplementary Table 2: Mulitvariable model for the outcome of virologic failure within randomized arms

Modeling Component	Covariate	2nd Covariate†	Comparison group vs reference group	Odds Ratio (95% CI)	P-value
Main effects					
	Age				<.01*
			Younger (ages: 16-46 years) vs Older (ages: 47-69 years)	4.4 (2.4, 8.2)	
	Total number of new study ARVs started following randomization (including NRTIs)				<.01*
			1-2 vs 4-6	6.9 (2.0, 24.0)	
			3 vs 4-6	3.0 (1.4, 6.5)	
Statistical interactions					
	Quality of life score				0.03**
		Omit NRTIs Group	0 - 60 (quartile 1) vs 76 -100 (quartiles 3 & 4)	1.0 (0.4, 2.5)	
			61 - 75 (quartile 2) vs 76-100 (quartiles 3 & 4)	0.8 (0.3, 2.2)	
		Add NRTIs Group	0 -60 (quartile 1) vs 76 – 100 (quartiles 3 & 4)	5.1 (2.0, 13.2)	
			61 – 75 (quartile 2) vs 76 – 100 (quartile 3 & 4)	3.4 (1.2, 9.3)	
	Number of active NRTIs chosen prior to randomization				0.02**
		Omit NRTIs Group	0 vs 1	7.6 (2.1, 28.0)	
			2 or 3 vs 1	7.4 (3.1, 17.8)	
		Add NRTIs Group	0 vs 1	0.2 (0.0, 2.1)	
			2 or 3 vs 1	8.2 (3.5, 19.0)	

† *2nd covariate is only included in cases of statistical interactions.*
* *Wald Chi-Square tests for main effects in multivariable model.*
***Type 3 tests for a statistical interaction between two covariates in multivariable model.*

Supplementary Table 3: Listing of major integrase inhibitor resistance mutations

Classification	Mutation
Major Primary Resistance Mutations	
	T66A/I/K
	E92Q
	E138K/A/T
	G140S/A/C
	Y143C/R/H
	S147G
	Q148H/K/R/N
	N155H
Major Accessory Resistance Mutations	
	H51Y
	L74M/I
	T97A
	Q95K
	V151I/L/A
	S153Y/F
	E157Q
	G163R/K
	S230R
Rare Primary INSTI-Resistance Mutations	
	G118R
	F121Y
	P145S
	Q146P
	R263K
Miscellaneous INSTI-Associated Mutations	
	V54I
	L68V
	H114Y
	A128T

List of resistance mutation classifications based on Stanford HIV Drug Resistance Database Version 8.2.

Cover Letter

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January 12, 2019

Dear Editor:

We respectfully submit the manuscript entitled "**Long-term Outcomes in a Large Randomized Trial of HIV-1 Salvage Therapy: 96-week Results of AIDS Clinical Trials Group A5241 (OPTIONS)**" for consideration for publication as a Major Article in the *Journal of Infectious Diseases.*

This manuscript provides long-term 96-week final results of the OPTIONS trial (AIDS Clinical Trials Group A5241), a large phase 3 partially randomized strategy trial in treatment-experienced participants who were failing HIV protease inhibitor (PI)-based therapy. Short-term (48-week) results had shown that nucleoside reverse transcriptase inhibitors (NRTIs) can be safely omitted from salvage therapy as long as the regimen has a cumulative activity of >2 active antiretroviral (ARV) medications. However, the long-term durability and safety of this approach are uncertain. In addition, virologic outcomes in participants who have more-extensive resistance was not previously reported.

In this manuscript, we report the 96-week virologic and immunologic results among 360 participants with virologic failure and anticipated ARV susceptibility who received an optimized regimen and were randomized to Omit or Add NRTIs. Our main findings are that, at week 96, 70% of those who were randomized to Omit NRTIs and 67% of those randomized to Add NRTIs had HIV RNA <200 copies/mL. Based on this result, we conclude that HIV-1 salvage therapy can safely omit NRTIs with compromising efficacy or durability of virologic response as long as the new regimen has a cumulative activity of >2 active drugs. Notably, we found that younger participants and those receiving fewer new ARVs had higher odds of virologic failure; these groups warrant careful monitoring.

We also report, for the first time, the virologic and immunologic outcomes in a separate group of participants with more-extensive resistance (cumulative ARV activity of 2 or fewer active agents) who received an optimized regimen including NRTIs. In this highly resistant group, 53% had HIV RNA <200 copies/mL at week 96, indicating that even among individuals with more-extensive resistance, most achieve virologic suppression.

In participants who experienced virologic failure in this trial, we present, for the first time, the frequency and type of treatment-emergent drug resistance. In participants in the randomized groups who received the PI, darunavir, only 3.4% of those with virologic failure developed treatment-emergent darunavir resistance. The rate of treatment-emergent etravirine resistance in the randomized groups, by contrast, was closer to 20% and the rate of treatment-emergent raltegravir resistance in the overall population was about 10%. These results in this treatment-experienced population comport to the higher barrier to resistance of boosted PIs as compared to NNRTIs and the intermediate barrier to resistance of first-generation INSTIs.

Finally, given the importance of safety and tolerability with long-term ART, we present, for the first time, the metabolic, renal and quality of life outcomes of participants in this large trial of treatment-experienced participants.

This manuscript has not been accepted for publication nor is it under consideration at any other journal. All the authors have seen and approved the content and have contributed significantly to the work. The manuscript was entirely prepared by the authors with no outside writing assistance.

Based on their expertise in conducting and analyzing clinical trials of antiretroviral therapy, we suggest the following individuals as potential reviewers for this manuscript:

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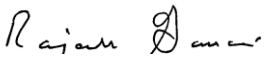
Sincerely,



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