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The following guiding principles have been applied to the disclosure:

- *Information will be excluded in order to protect the privacy of patients and all named persons associated with the study*
- *Patient data listings will be completely removed* to protect patient privacy. Anonymized data from each patient may be made available subject to an approved research proposal. For further information please see the Patient Level Data section of the* ***GSK Clinical Study Register****.*
- *Aggregate data will be included; with any direct reference to individual patients excluded*

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An Evaluation of the Safety of Lamivudine in HIV-positive Patients with Renal Impairment:

Final Report

Effective Date: September 18, 2019

OPERA Data Through 04/10/2019

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PPD

18-Sep-2019

Vani Vannappagari
Global Head, Epidemiology and Real World Evidence

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17 Sep 2019

Nassrin Payvandi
VP & Head, Safety and Pharmacovigilance

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Jens-Ulrich Stegmann
ViiV QPPV

Date

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Nassrin Payvandi
VP & Head, Safety and Pharmacovigilance

Date

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Jens-Ulrich Stegmann
ViiV QPPV

Date

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1. Executive summary

Background

Renal insufficiency is a common co-morbidity in HIV infected patients. The pharmacokinetics of a single 300 mg dose of 3TC has been evaluated in subjects with normal, moderately impaired and severely impaired renal function; higher peak serum concentrations, longer half-lives ($t_{1/2}$) and larger areas under the concentration-time curves (AUC) have been reported in renally impaired patients. Renal clearance of 3TC has been shown to be linearly correlated with creatinine clearance (CLcr), suggesting the need for dose adjustments in the renally-impaired patient. However, as seen in patients with normal renal function, this single 300 mg dose was well tolerated by the renally-impaired patients. A two-compartment model has been described in which 3TC freely penetrates tissue beyond the circulatory system and distributes through peripheral compartments. Dose adjustments in patients with mild renal impairment have been recommended in several studies.

Given the absorption-distribution-metabolism-excretion (ADME) profile of 3TC, current guidelines are to consider dose adjustment in patients with renal insufficiency. With the availability of 3TC as a single agent in multiple dosing formulations and in multiple fixed-dose combination (FDC) formulations, a population-level assessment of 3TC's safety profile when prescribed in the renally-impaired was warranted.

The primary objectives of this study were to:

1) Estimate the association between 3TC dose and the rate of a composite outcome consisting of specific diagnoses of interest and severe laboratory abnormalities among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

2) Estimate the frequency and rate of specific diagnoses of interest and severe laboratory abnormalities among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

Methods

A total of four distinct study populations were identified from the OPERA Observational Database for analysis. All study populations were a subset of the Main Population, consisting of HIV-1 patients at least 13 years of age initiating 3TC for the first time between 17NOV1995 and 31DEC2018 with an eGFR ≤ 49 ml/min/1.73m^2 and ≥ 30 ml/min/1.73m^2 at 3TC initiation.

Exposure of interest was the 3TC dose; 300 mg vs. 150 mg. Outcomes of interest were defined as Composite 1 (diagnoses and/or severe lab abnormalities), Composite 2 (diagnoses and/or moderate/severe lab abnormalities and/or gastrointestinal symptoms), or Composite 3 (severe lab abnormalities only).

Demographic and clinical characteristics, as well as the prevalence of pre-existing Composite 1 and 2 were compared between patients in the Main Population on a full dose (300 mg) versus an adjusted dose (150 mg). Pairwise comparisons between 3TC dose groups were evaluated by p-values calculated from Pearson Chi-Square test or Fisher's exact test for categorical variables and Wilcoxon Rank Sum test for continuous variables.

For all outcomes of interest, a population restricted to patients without pre-existing events at baseline was defined to estimate incidence proportions and incidence rates of each outcome during follow-up. Multivariable Poisson regression was employed to estimate the incidence rate ratio comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Exposure time consisted of time from baseline until the occurrence of the first event or until a censoring event (3TC discontinuation, 3TC total daily dose change, out-of-range eGFR, loss to follow-up, death, or study end). Only the first event defined for each composite outcome was counted.

Analyses were conducted in the Main Population for Composite 1, 2, and 3. Analyses were also repeated for Composite 1 in a population including patients with pre-existing events, in a population uncensored at out-of-range eGFR, and in a population with in-patient records available.

Finally, changes in eGFR over follow-up and eGFR testing characteristics among patients censored due to an out-of-range eGFR were described.

Results

The Main Population consisted of 539 patients initiating 3TC with an eGFR between >30 and ≤49, of whom 103 (19.1%) received an adjusted dose and 436 (80.9%) received a full dose of 3TC. Dose adjustment was more frequent among females, African Americans, diabetics, persons with drug or alcohol abuse, with higher viral loads, with higher VACS mortality index (an indicator of comorbidities and frailty) and with lower eGFRs.

Diagnoses and severe lab abnormalities included in the Composite 1 were common within 12 months prior to 3TC initiation among dose-adjusted (n=36, 35.0%) and full dose recipients (n=124, 28.4%). Low hemoglobin was significantly more frequent in patients with dose adjustment (16.5%) than with the full 3TC dose (6.4%). Out of 67 patients on 150 mg without pre-existing conditions from Composite 1, only 5 experienced a Composite 1 outcome during follow-up (incidence rate [IR]: 12.55 per 100 person-year, 95% CI: 5.22, 30.15). Out of 312 patients on 300 mg dose without pre-existing Composite 1, 29 experienced conditions from Composite 1 outcomes during follow-up (incidence rate [IR]: 19.57 per 100 person-year, 95% CI: 13.60, 28.17). After adjusting for drug/alcohol abuse and baseline hemoglobin, there was no statistically significant difference in the rate of Composite 1 with the full dose compared to the adjusted dose, with an incidence rate ratio (IRR) of 1.51 (95% CI: 0.59, 3.92).

Diagnoses, moderate/severe lab abnormalities, and gastrointestinal symptoms were very common within 12 months prior to 3TC initiation in patients on 150 mg (n=79, 76.7%) and on 300 mg (n=351, 80.5%). Low hemoglobin was significantly more frequent in patients with dose adjustment (25.2%) than with the full 3TC dose (12.4%). Out of 24 patients on 150 mg without pre-existing conditions from Composite 2, 6 experienced a Composite 2 outcome during follow-up (IR: 38.12 per 100 person-year, 95% CI: 17.13, 84.85). Out of 85 patients on 300 mg without pre-existing conditions from Composite 2, 26 experienced a Composite 2 outcome during follow-up (IR: 80.17 per 100 person-year, 95% CI: 54.59, 117.75). After adjusting for drug/alcohol abuse and baseline hemoglobin, patients on a full 3TC dose had a statistically significantly increased risk of experiencing an event defined in Composite 2 (diagnosis, moderate/severe lab abnormality or gastrointestinal symptom) compared to those on an adjusted dose, with an IRR of 3.07 (1.12, 8.40).

Out of 79 patients on 150 mg without pre-existing severe lab abnormalities defined in Composite 3, only 4 experienced an incident event in Composite 3 during follow-up (IR: 8.55 per 100 person-year, 95% CI: 3.21, 22.77). Out of 379 patients on 300 mg without pre-existing Composite 3, 16 experienced incident severe lab abnormalities defined in Composite 3 during follow-up (IR: 8.70 per 100 person-year, 95% CI: 5.33, 14.21). After adjusting for drug/alcohol abuse and baseline hemoglobin, there was no statistically significant difference in the rate of Composite 3 with the full dose compared to the adjusted dose, with an IRR of 0.88 (0.29, 2.66).

A sensitivity analysis was conducted by including all patients, regardless of the presence of pre-existing conditions at baseline, to identify any event in Composite 1 occurring during follow-up. Out of 103 patients on 150 mg, 25 experienced an event in Composite 1 during follow-up (IR: 51.28 per 100 person-year, 95% CI: 34.65, 75.88). Out of 436 patients on 300 mg, 62 experienced an event defined in Composite 1 during follow-up (IR: 33.68 per 100 person-year, 95% CI: 26.26, 43.20). After adjusting for drug/alcohol abuse and baseline hemoglobin, there was no statistically significant difference in the rate of Composite 1 with the full dose compared to the adjusted dose, with an IRR of 0.63 (0.39, 1.01).

Another sensitivity analysis was conducted without censoring person-time at the first out-of-range eGFR. Out of 67 patients on 150 mg without pre-existing Composite 1, 10 experienced an incident event defined in Composite 1 during follow-up (IR: 13.87 per 100 person-year, 95% CI: 7.46, 25.78). Out of 312 patients on 300 mg without pre-existing Composite 1, 72 experienced an event defined in Composite 1 during follow-up (IR: 16.17 per 100 person-year, 95% CI: 12.84, 20.37). After adjusting for drug/alcohol abuse and baseline hemoglobin, there was no statistically significant difference in the rate of Composite 1 with the full dose compared to the adjusted dose, with an IRR of 1.14 (0.59, 2.21).

A last sensitivity analysis was conducted among 97 patients for whom in-patient records were available in OPERA, using only in-patient diagnoses and severe laboratories as the composite outcome. In this in-patient population, 15/26 (57.7%) of patients on 150 mg and 28/71 (39.4%) of those on 300 mg had pre-existing events defined in the composite within 12 months before baseline. Out of 11 patients on 150 mg without pre-existing in-patient Composite 1 events, only 1 experienced an incident event during follow-up (IR: 30.51 per 100 person-year, 95% CI: 4.30, 216.62). Out 43 patients on 300 mg without pre-existing in-patient Composite 1 events, only 2 experienced an incident event during follow-up (IR: 19.62 per 100 person-year, 95% CI: 4.91, 78.43). The number of patients and events in each group was too small to conduct modeling analyses in the sensitivity analysis.

Over follow-up, 404 patients were censored due to an out-of-range eGFR. Of the 79 patients with a follow-up eGFR < 30 ml/min/1.73m^2, most had a confirmation of worsening with a second eGFR < 30 ml/min/1.73m^2, including 10/18 (55.6%) patients on 150 mg and 33/47 (73.3%) patients on 300 mg. Patients on an adjusted 3TC dose had a greater number of eGFR measurements after their first eGFR < 30 ml/min/1.73m^2, compared to those on the full dose. However, there was no difference between the two groups in term of the magnitude of changes in eGFR observed. In both groups, the most frequent reason for censoring patients with a confirmed eGFR < 30 ml/min/1.73m^2 was discontinuation of 3TC. There was no statistical difference in the likelihood of changing 3TC dose after a confirmed worsening of eGFR.

Of the 325 patients with a follow-up eGFR > 49 ml/min/1.73m^2, those on the full dose were more likely to have a confirmation of improvement with a second eGFR > 49 ml/min/1.73m^2 (217/255, 85.1%), compared to those on an adjusted dose (38/55, 69.1%). Those on a full dose were followed for a longer

time after their out-of-range eGFR and had a greater number of eGFR measurements than those on an adjusted dose. However, those on 150 mg experienced a larger median increase in eGFR from baseline, compared to those on 300 mg. In both groups, the most frequent reason for censoring patients with a confirmed eGFR < 49 ml/min/1.73m^2 was discontinuation of 3TC. Patients initiating 3TC with a daily dose of 150 mg were statistically more likely to change total daily dose after confirmation of eGFR improvement > 49ml/min/1.73m^2 (39%), compared to those initiating with 300 mg (10.1%, p<0.001).

Conclusions

Compared to dose adjusted 3TC (150 mg), there was no statistically significant difference in the risk of severe diagnoses or laboratory abnormalities with the full dose (300 mg) among patients with renal impairment, defined as an eGFR ≥ 30 and ≤ 49 ml/min/1.73m^2. However, we observed statistically significantly increased risk of GI symptoms and/or moderate lab abnormalities with the full dose in this population. GI symptoms and moderate laboratory abnormalities were common in these patients prior to 3TC administration, resulting in a smaller sample size. Further, GI symptoms were not attributed to 3TC use by the caregiver in the medical record during follow-up, resulting in uncertainty as to their association with the drug. No difference was observed between doses in any other comparison.

To the best of our knowledge, this is the first observational study assessing the impact of 3TC dose adjustment or lack thereof among patients with renal impairment. The OPERA cohort utilized routine clinical data from electronic medical records and therefore, reflects clinical practices in the U.S. The small number of patients with renal impairment and the fact that many events of interest were commonly experienced in the months preceding 3TC initiation led to a reduced sample size. Statistical models therefore had to remain parsimonious and residual confounding is possible. Based on these results and because 3TC is a well-tolerated drug with a wide therapeutic index, dose adjustment may be unnecessary until the eGFR becomes very low (<30 ml/min/1.73m^2). Clinical judgement will be key in weighing the risks versus the benefits in this fragile patient population.

2. Background and Rationale

2.1. Background

Lamivudine (3TC) is a cytosine dideoxynucleoside analogue with potent in vitro activity against human immunodeficiency virus (HIV) demonstrated through the inhibition of reverse transcriptase [1,2,3]. Early phase clinical trials of 3TC monotherapy have demonstrated potent antiretroviral activity as well as a positive safety profile [4,5]. Late phase clinical trials have shown 3TC to effectively decrease HIV-1 RNA and increase CD4+ lymphocyte counts when combined with zidovudine (ZDV) [6,7]. Treatment guidelines have placed 3TC on the recommended list of agents for both antiretroviral (ART) naïve and experienced patients in combination with other ART medications [8].

Dose ranging studies of 3TC have evaluated doses between 0.25 and 20 mg/kg in asymptomatic, HIV patients with normal renal function and demonstrated that following oral administration, 3TC was rapidly absorbed with a mean absolute bioavailability of 82% with approximately 70% being renally excreted unchanged [9]. Given the absorption-distribution-metabolism-excretion (ADME) profile of 3TC, current guidelines are to consider dose adjustment in patients with renal insufficiency. Renal insufficiency is a common comorbidity in HIV infected patients [10].

The pharmacokinetics of a single 300 mg dose of 3TC was evaluated in subjects with normal, moderately impaired and severely impaired renal function [12]. This study demonstrated higher peak serum concentrations, longer half-lives ($t_{1/2}$) and larger areas under the concentration-time curves (AUC) in renally impaired patients. Renal clearance of 3TC was shown to be linearly correlated with creatinine clearance (CLcr), suggesting the need for dose adjustments in the renally-impaired patient. Similar to patients with normal renal function, this single 300 mg dose was well tolerated by the renally-impaired patients. The $t_{1/2}$ in patients with normal renal function in this study [12] ranged from 8 to 17.6 hours, whereas other studies demonstrated $t_{1/2}$ estimates ranging from 2 to 7 hours [4,9,13,14]. A retrospective analysis of 244 patients described a two-compartment model in which 3TC freely penetrates tissue beyond the circulatory system and distributes through peripheral compartments [15] and suggested dose adjustments in the patient with mild renal impairment; recommendations which are in agreement with other studies [12,16].

With the availability of 3TC as a single agent in multiple dosing formulations and in multiple fixed-dose combination (FDC) formulations, and with the prevalence of renal insufficiency in the HIV-positive population, a population-level assessment of 3TC's safety profile when prescribed in the renally-impaired was warranted.

2.2. Rationale

Given the ADME profile of 3TC, current guidelines state that dose adjustment should be considered in patients with renal insufficiency [17]. Renal insufficiency is a common comorbidity in HIV infected patients and with the availability of 3TC as a single agent in multiple dosing formulations and in multiple fixed-dose combination (FDC) formulations, a population-level assessment of 3TC's safety profile when

prescribed in the renally-impaired will provide insight into the clinical management of renally-impaired patients.

3. Objectives

3.1. Primary Objectives

1) To estimate the association between 3TC dose prescribed and the rate of a composite outcome consisting of specific diagnoses of interest and severe laboratory abnormalities (Composite 1) among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

2) To estimate the frequency and rate of specific diagnoses of interest and severe laboratory abnormalities among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

3.2. Secondary Objectives

1) To estimate and compare the frequency and estimate the association between 3TC dose prescribed and a composite outcome consisting of gastrointestinal symptoms diagnoses, specific diagnoses of interest and moderate-severe laboratory abnormalities (Composite 2) among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

2) To describe changes in eGFR over follow-up among patients censored due to an improvement or worsening of eGFR

3.3. Sensitivity Objectives

1) To estimate the association between 3TC dose prescribed and the rate of a composite outcome consisting of specific diagnoses of interest and severe laboratory abnormalities (Composite 1), regardless of changes in eGFR outside of the target range over follow-up, among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

2) To estimate the association between 3TC dose prescribed and the rate of a composite outcome restricted to severe laboratory abnormalities (Composite 3) among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

3) To estimate the association between 3TC dose prescribed and the rate of incident and prevalent composite outcome (Composite 1) consisting of specific diagnoses of interest and severe laboratory abnormalities among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2

4) To estimate the association between 3TC dose prescribed and the rate of a composite outcome consisting of specific diagnoses of interest and severe laboratory abnormalities (Composite 1) among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2 with in-patient records available

4. Methodology

4.1. Study design

Study population

A total of four distinct study populations were identified from the OPERA Observational Database for analysis per the inclusion criteria defined below. All study populations were a subset of the Main Population (Table 1). The OPERA cohort utilizes routine clinical data from electronic medical records from 85 clinics in 19 states and 1 territory in the U.S., accounting for about 7% of the population living with HIV in the U.S.

Eligibility period: 17NOV1995 to 31DEC2018

Baseline (index) date: Date of 3TC initiation

Baseline period: The 12-month baseline period preceding the index date were used to assess patient demographic and clinical characteristics

Table 1. Inclusion criteria for the four study populations

	Main Population	Secondary Population	Sensitivity Population	In-patient Population
Diagnosis of HIV, a positive HIV Western Blot, or a positive HIV enzyme-linked immunosorbent assay (ELISA); and a detectable HIV viral load test	X	X	X	X
At least 13 years of age at the index date	X	X	X	X
Prescribed 3TC (150 mg or 300 mg daily dose) for the first time between 11/17/1995 and 12/31/2018	X	X	X	X
eGFR ≤ 49 ml/min/1.73m^2 and ≥ 30 ml/min/1.73m^2 at 3TC initiation	X	X	X	X
Follow-up eGFR either <30 ml/min/1.73m^2 or >49 ml/min/1.73m^2		X		
In care at a health system with access to in-patient records				X

Observation period: Patients were observed from their index date until the first of the censoring events described for each population (Table 2).

Table 2. Censoring events for the three study populations

	Main Population	Secondary Population	Sensitivity Population	In-patient Population
Discontinuation of 3TC due to any cause, defined as a gap of 45 days or more	X	X	X	X
Change in total daily dose of 3TC	X	X	X	X
Cessation of continuous clinical activity, defined as at least one clinical contact, visit or telephone contact. Patients failing to meet the continuous clinical activity requirement will be censored 12 months after their last contact	X	X	X	X
Death	X	X	X	X
Study end (March 31, 2019)	X	X	X	X
First follow-up eGFR > 49 ml/min/1.73m^2 or < 30 ml/min/1.73m^2	X			X

Continuous Clinical Activity

Patients failing to meet the continuous clinical activity requirement (defined as any 12-month period in which no clinical contact is made) were censored 12 months after their last contact. Regimen gaps in therapy of 45 days or less were collapsed for continuity of treatment analysis.

4.2. Exposure definition

First exposure to 3TC with a total daily dose of either 150 mg (150 mg QD) or 300 mg (150 mg BID or 300 mg QD) after inclusion in the OPERA database were the exposures of interest. Exposure time was defined as the time from baseline until the occurrence of the first event defined for the composite outcomes, or a censoring event, whichever came first.

4.3. Outcomes definition

Composite 1

- Specific diagnoses of interest, with additional diagnosis terms to be used in parentheses:
    - Lactic Acidosis (hyperlactatemia, increased lactic acid)
    - Paraesthesia (tingling, numbness)
    - Peripheral Neuropathy (tingling, numbness)
    - Pancreatitis

- Rhabdomyolysis (rhabdomyolysis, increased CPK)
- Anemia (low/decrease Hemoglobin (Hb, Hgb), low/decreased hematocrit (Hct))
- Neutropenia (low neutrophils)
- Thrombocytopenia (low platelets)
- Nausea (Nausea or N in a combo of N/V/D for nausea, vomiting, diarrhea)

- Severe laboratory abnormalities (DAIDS grade 3 or greater):
  - Neutrophils < 600 cells/µL
  - Haemoglobin
    - < 8.5 g/dL in females
    - < 9 g/µL in males
  - Platelets < 50,000 cells/mm^3
  - ALT ≥ 5 x ULN
  - AST ≥ 5 x ULN
  - Total bilirubin > 2.6 x ULN
  - Lactate > 2.0 x ULN + pH < 7.3
  - Creatinine kinase > 10 x ULN

<u>Composite 2</u>

- Diagnoses of gastrointestinal symptoms:
  - Hyperlactataemia (lactic acidosis, increased lactic acid)
  - Nausea (Nausea or N in a combo of N/V/D)
  - Vomiting (Vomiting, emesis, hyperemesis)
  - Abdominal Pain (RLQ, RUQ, LUQ, LLQ for Right, left, upper, lower, quadrant; epigastric pain)
- Specific diagnoses of interest:
  - Lactic Acidosis (hyperlactatemia, increased lactic acid)
  - Paraesthesia (tingling, numbness)
  - Peripheral Neuropathy (tingling, numbness)
  - Pancreatitis
  - Rhabdomyolysis (rhabdomyolysis, increased CPK)
  - Anemia (low/decrease Hemoglobin (Hb, Hgb), low/decreased hematocrit (Hct))
  - Neutropenia (low neutrophils)
  - Thrombocytopenia (low platelets)
- Moderate or severe laboratory abnormalities (DAIDS grade 2 or greater):
  - Neutrophils < 800 cells/µL
  - Haemoglobin < 9.5 g/dL in females or < 10 g/dL in men
  - Platelet Count < 100,000 cells/mm^3
  - ALT ≥ 2.5 x ULN
  - AST ≥ 2.5 x ULN
  - Total bilirubin > 1.6 x ULN
  - Lactate > 2.0 x ULN + pH ≥ 7.3
  - Creatinine kinase > 6 x ULN

- Red blood count (RBC, not graded by DAIDS)
  - < 4.52 x10^{12}/L in adult male
  - < 4.10 x10^{12}/L in adult female
- Mean corpuscular volume (MCV) > 96 (Not graded by DAIDS)

Composite 3

- Severe laboratory abnormalities (DAIDS grade 3 or greater):
  - Neutrophils < 600 cells/µL
  - Haemoglobin
    - < 8.5 g/dL in females
    - < 9 g/µL in males
  - Platelets < 50,000 cells/mm^3
  - ALT ≥ 5 x ULN
  - AST ≥ 5 x ULN
  - Total bilirubin > 2.6 x ULN
  - Lactate > 2.0 x ULN + pH < 7.3
  - Creatinine kinase > 10 x ULN

Follow-up eGFR endpoints and testing characteristics

- Last eGFR value (median (IQR); above vs. within vs. below the target range)
- 3TC duration
- Change in eGFR between baseline and last eGFR (median (IQR); improved vs. maintained vs. worsened)
- Number of eGFR tests during follow-up

4.4. Analyses

Power calculations performed during protocol development are included in the appendix.

4.4.1. Baseline descriptive analyses (Main Population)

Descriptive analyses of demographic and clinical characteristics were conducted in patients in the Main Population. The prevalence of each component of Composite 1 and 2 was also described. Medians and interquartile ranges for continuous variables and frequencies (counts and percentages) for categorical variables were provided. Pairwise comparisons between 3TC dose groups were evaluated by p-values calculated from Pearson Chi-Square test for categorical variables. Fisher's exact test was used to

compare frequencies with few events. Wilcoxon Rank Sum test was used to calculate p-values for continuous variables.

4.4.2. Primary objective 1: Association between 3TC dose and Composite 1 (Main Population)

Among patients without pre-existing Composite 1 events at baseline, multivariable Poisson regression was employed to estimate the incidence rate ratio for Composite 1 comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Poisson regression is the preferred method to analyze count data, as it accounts for the fact that the variance tends to increase with the mean. Exposure time consisted of time from baseline until the occurrence of the first event defined for Composite 1 or until a censoring event (3TC discontinuation, 3TC total daily dose change, eGFR outside of specified range, loss to follow-up, death, or study end). Only the first event defined for Composite 1 occurring for a given patient was counted because independence of events could not be assumed, making the use of all events inappropriate.

4.4.3. Primary objective 2: Frequency and incidence rate of Composite 1 (Main Population)

Among patients without pre-existing Composite 1 events at baseline, frequency of incident Composite 1, as well as each of its components was detailed. Incidence rates were also estimated to avoid the loss of any exposed time and allow the use of uneven follow-up time across patients. Incidence rates were obtained from an unadjusted Poisson regression and corresponded to the number of events during follow-up divided by the total person-years at risk. Person-years at risk were defined as the sum of the total number of years contributed by each patient from baseline until the first of the following events: (a) first abnormal lab or diagnosis included in the composite outcome, (b) censoring event, or (c) end of study period. The incidence rate of Composite 1 and each of its components was compared across both total daily dosing groups (i.e. 300 mg vs. 150 mg) using univariate Poisson regression.

4.4.4. Secondary objective 1a: Frequency and incidence rate of Composite 2 (Main Population)

Among patients without pre-existing Composite 2 events at baseline, frequency of incident Composite 2, as well as each of its components was detailed. Incidence rates were also compared across both total daily dosing groups (i.e. 300 mg vs. 150 mg) using univariate Poisson regression. Incidence rates were calculated as the number of events during follow-up divided by the total person-years at risk. Person-years at risk were defined as the sum of the total number of years contributed by each patient from baseline until the first of the following events: (a) first abnormal lab or diagnosis included in the composite outcome, (b) censoring event, or (c) end of study period.

4.4.5. Secondary objective 1b: Association between 3TC dose and Composite 2 (Main Population)

Among patients without pre-existing Composite 2 at baseline, multivariable Poisson regression was employed to estimate the incidence rate ratio for Composite 2 comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Poisson regression is the preferred method to analyze count data, as it accounts for the fact that the variance tends to increase with the mean. Exposure time consisted of time from baseline until the occurrence of the first event defined for Composite 2 or until a censoring event (3TC discontinuation, 3TC total daily dose change, eGFR outside of specified range, loss to follow-up, death, or study end). Only the first event defined for Composite 2 occurring for a given patient was counted.

4.4.6. Secondary objective 2: Changes in eGFR over follow-up among patients censored due to an improvement or worsening of eGFR (Secondary Population)

Changes in eGFR over follow-up and eGFR testing characteristics among patients censored due to an improvement or worsening of eGFR were compared between 3TC daily dose groups using Wilcoxon Rank Sum test for continuous variables and Pearson Chi-Square test for categorical variables.

4.4.7. Sensitivity objective 1: Association between 3TC dose and Composite 1 without censoring when eGFR is out of range (Sensitivity Population)

This analysis was conducted among patients without pre-existing Composite 1 at baseline. Person-time was not censored when eGFR increased to > 49 ml/min/1.73m^2 or < 30 ml/min/1.73m^2. Multivariable Poisson regression was employed to estimate the incidence rate ratio for Composite 1 comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Poisson regression is the preferred method to analyze count data, as it accounts for the fact that the variance tends to increase with the mean. Exposure time consisted of time from baseline until the occurrence of the first event defined for Composite 1 or until a censoring event (3TC discontinuation, 3TC total daily dose change, loss to follow-up, death, or study end). Only the first event defined for Composite 1 occurring for a given patient was counted.

4.4.8. Sensitivity objective 2: Association between 3TC dose and Composite 3 (Main Population)

Among patients without pre-existing Composite 3 at baseline, multivariable Poisson regression was employed to estimate the incidence rate ratio for Composite 3 comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Poisson regression is the preferred method to analyze count data, as it accounts for the fact that the variance tends to increase with the mean. Exposure time consisted of time from baseline until the occurrence of the first event defined for Composite 3 or until a censoring event (3TC discontinuation, 3TC total daily dose change, eGFR outside

of specified range, loss to follow-up, death, or study end). Only the first event defined for Composite 3 occurring for a given patient was counted.

4.4.9. Sensitivity objective 3: Association between 3TC dose and Composite 1, regardless of pre-existing conditions (Main Population)

Among all patients (regardless of pre-existing Composite 1 at baseline), multivariable Poisson regression was employed to estimate the incidence rate ratio for Composite 1 comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Poisson regression is the preferred method to analyze count data, as it accounts for the fact that the variance tends to increase with the mean. Exposure time consisted of time from baseline until the occurrence of the first event defined for Composite 1 or until a censoring event (3TC discontinuation, 3TC total daily dose change, eGFR outside of specified range, loss to follow-up, death, or study end). Only the first event defined for Composite 1 occurring for a given patient was counted.

4.4.1. Sensitivity objective 4: Association between 3TC dose and Composite 1 (In-patient Population)

To explore the accuracy of the diagnoses and diagnostic codes in the database, a sensitivity analysis was conducted in a population restricted to patients in care at a healthcare system with access to in-patient records. The outcome for this sensitivity analysis consisted of Composite 1, with diagnoses identified in the in-patient setting only.

5. Results

5.1. Identification and description of the main study population

A total of 539 patients met all eligibility criteria and were included in the main study population, representing 0.5% of all HIV+ individuals in OPERA (Table 3). Of those, 19% initiated 3TC with a total daily dose of 150 mg and 81% initiated 3TC with a total daily dose of 300 mg (Table 4).

Table 3. Identification of the Main Population

		Patients Included	%	Patients Excluded	%
1	Patients who are HIV+	103,369	100.0	0	0.0
2	Patients with HIV-1 infection only (excluding HIV-2 infection)	102,445	99.1	924	0.9
3	HIV+ patients prescribed ART	86,928	84.9	15,517	15.1
4	Patients prescribed 3TC	28,236	32.5	58,692	67.5
5	Patients prescribed 3TC for the first time between 11/17/1995 and 12/31/2018	23,512	83.3	4,724	16.7
6	Patients who were 13 years of age or older at first ART regimen of interest	23,460	99.8	52	0.2
7	Patients with a baseline eGFR of >= 30 ml/min/1.73m^2 and <= 49 ml/min/1.73m^2	552	2.4	22,908	97.6
8	Patients initiating 3TC with a total daily dose of 150 mg or 300 mg	539	97.6	13	2.4

HIV+ = individuals living with HIV infection; ART = antiretroviral therapy; 3TC = lamivudine

Table 4. Main Population by 3TC dose

		N (%)
Patients initiating 3TC with 150 mg		103 (19.1%)
Patients initiating 3TC with 300 mg	Overall	436 (80.9%)
	150 mg BID	44 (10.1%)
	300 mg QD	389 (89.2%)
	Unknown dose frequency	3 (0.7%)
Total study population		**539 (100.0%)**

Compared to individuals on a total daily 3TC dose of 300 mg, those on 150 mg were more likely to be female, and African American (Table 5). They were also generally sicker, with higher viral loads (Table 6), higher VACS mortality index and lower eGFRs, as well as a higher likelihood of comorbid conditions such as diabetes and substance abuse (Table 9).

Table 5. Baseline Demographic Characteristics (Main Population)

		3TC Daily Dose: 150 mg N= 103	3TC Daily Dose: 300 mg N= 436	p-value
Age	Median (IQR)	53.9 (48.2, 61.0)	54.3 (46.7, 60.5)	0.9280
	13-25	1 (1.0%)	5 (1.1%)	0.9267
	26-49	36 (35.0%)	145 (33.3%)	
	50+	66 (64.1%)	286 (65.6%)	
Sex	Male	63 (61.2%)	317 (72.7%)	**0.0209**
	Female	40 (38.8%)	119 (27.3%)	
Race	African American	67 (65.0%)	202 (46.3%)	**0.0006**
	Other	36 (35.0%)	234 (53.7%)	
Ethnicity	Hispanic	11 (10.7%)	55 (12.6%)	0.5996
	Non-Hispanic	84 (81.6%)	336 (77.1%)	
	Missing	8 (7.8%)	45 (10.3%)	
Region	Northeast	8 (7.8%)	26 (6.0%)	0.0688
	South	71 (68.9%)	281 (64.4%)	
	Midwest	6 (5.8%)	14 (3.2%)	
	West	18 (17.5%)	115 (26.4%)	
Payer	Medicaid	35 (34.0%)	146 (33.5%)	0.9239
	Medicare	35 (34.0%)	128 (29.4%)	0.3583
	Commercial Insurance	33 (32.0%)	154 (35.3%)	0.5291
	Cash	17 (16.5%)	57 (13.1%)	0.3628
	ADAP/Ryan White	17 (16.5%)	93 (21.3%)	0.2745
	Other	4 (3.9%)	10 (2.3%)	0.3192
	No Payer info	12 (11.7%)	64 (14.7%)	0.4270

Table 6. Baseline Virologic and Immunologic Characteristics (Main Population)

		3TC Daily Dose: 150 mg N= 103	3TC Daily Dose: 300 mg N= 436	p-value
HIV viral load (copies/ml)	Median (IQR)	135.0 (19.0, 30150.0)	47.0 (19.0, 631.0)	**0.0097**
	< 1000	60 (58.3%)	303 (69.5%)	**0.0330**
	>= 1,000 to < 10,000	8 (7.8%)	22 (5.0%)	
	>= 10,000 to < 100,000	17 (16.5%)	36 (8.3%)	
	>= 100,000	12 (11.7%)	36 (8.3%)	
	Missing	6 (5.8%)	39 (8.9%)	
Log10 HIV viral load	Median (IQR)	2.1 (1.3, 4.5)	1.7 (1.3, 2.9)	**0.0103**
CD4 cell count (cell/µl)	Median (IQR)	401.5 (180.0, 633.0)	458.0 (216.0, 702.0)	0.0988
	> 500	40 (38.8%)	194 (44.5%)	0.8577
	> 350 to <= 500	13 (12.6%)	57 (13.1%)	
	> 200 to <= 350	16 (15.5%)	65 (14.9%)	
	> 50 to <= 200	20 (19.4%)	68 (15.6%)	
	<= 50	9 (8.7%)	29 (6.7%)	
	Missing	5 (4.9%)	23 (5.3%)	

Table 7. Baseline HIV Infection Characteristics (Main Population)

		3TC Daily Dose: 150 mg N= 103	3TC Daily Dose: 300 mg N= 436	p-value
Calendar year of HIV diagnosis	Median (IQR)	2004 (1996, 2009)	2004 (1996, 2010)	0.6384
	< 1995	19 (18.4%)	88 (20.2%)	0.3895
	1995 to 1999	16 (15.5%)	58 (13.3%)	
	2000 to 2004	18 (17.5%)	76 (17.4%)	
	2005 to 2009	25 (24.3%)	95 (21.8%)	
	2010 to 2014	22 (21.4%)	79 (18.1%)	
	2015 to 2018	3 (2.9%)	40 (9.2%)	
Calendar year at baseline (3TC initiation)	Median (IQR)	2013 (2011, 2015)	2014 (2011, 2015)	0.3723
	2000 to 2004	2 (1.9%)	5 (1.1%)	0.6217
	2005 to 2009	14 (13.6%)	69 (15.8%)	
	2010 to 2014	51 (49.5%)	190 (43.6%)	
	2015 to 2018	36 (35.0%)	172 (39.4%)	
Years from HIV diagnosis to baseline	Median (IQR)	8.4 (1.9, 15.4)	8.2 (1.8, 16.9)	0.7150
	0	1 (1.0%)	18 (4.1%)	0.1795
	> 0 to <= 1	15 (14.6%)	72 (16.5%)	
	> 1 to <= 2	10 (9.7%)	17 (3.9%)	
	> 2 to <= 5	10 (9.7%)	55 (12.6%)	
	> 5 to <= 10	21 (20.4%)	68 (15.6%)	
	> 10 to <= 15	18 (17.5%)	68 (15.6%)	
	> 15 to <= 20	12 (11.7%)	52 (11.9%)	
	> 20 to <= 25	7 (6.8%)	45 (10.3%)	
	> 25	9 (8.7%)	35 (8.0%)	
	Unknown HIV diagnosis date	0	6 (1.4%)	
Months from first OPERA visit to baseline	Median (IQR)	13.1 (0.8, 52.9)	10.3 (0.5, 43.3)	0.3592
	0	12 (11.7%)	70 (16.1%)	0.8076
	> 0 to 6	28 (27.2%)	119 (27.3%)	
	> 6 to 12	11 (10.7%)	37 (8.5%)	
	> 12 to 24	12 (11.7%)	48 (11.0%)	
	> 24	40 (38.8%)	162 (37.2%)	
History of AIDS diagnosis	Yes	48 (46.6%)	185 (42.4%)	0.4422

Table 8. Baseline HIV Treatment Characteristics (Main Population)

		3TC Daily Dose: 150 mg N= 103	3TC Daily Dose: 300 mg N= 436	p-value
Previous ART experience	ART-naïve	12 (11.7%)	49 (11.2%)	0.3896
	ART-experienced	88 (85.4%)	358 (82.1%)	
	No known experience, no HIV VL	3 (2.9%)	29 (6.7%)	
Co-prescription of ARV requiring dose adjustment	Any	41 (39.8%)	154 (35.3%)	0.3943
	tenofovir disproxil fumarate (TDF)	6 (5.8%)	34 (7.8%)	0.4921
	tenofovir alafenamide (TAF)	0	4 (0.9%)	1.0000
	emtricitabine (FTC)	6 (5.8%)	27 (6.2%)	0.8887
	atazanavir (ATV)	21 (20.4%)	71 (16.3%)	0.3194
	lopinavir/r (LPV/r)	7 (6.8%)	37 (8.5%)	0.5731
	stavudine (d4T)	0	1 (0.2%)	1.0000
	didanosine (ddI)	3 (2.9%)	2 (0.5%)	0.0505
	zalcitabine (ddC)	0	0	
	zidovudine (AZT)	9 (8.7%)	33 (7.6%)	0.6906
	maraviroc	0	4 (0.9%)	1.0000
Co-prescription of ARV known to inhibit tubular secretion of creatinine	Any	74 (71.8%)	332 (76.1%)	0.3624
	dolutegravir (DTG)	24 (23.3%)	170 (39.0%)	**0.0028**
	cobicistat	3 (2.9%)	22 (5.0%)	0.4445
	ritonavir	53 (51.5%)	164 (37.6%)	**0.0100**
	rilpivirine (RPV)	5 (4.9%)	13 (3.0%)	0.3594
	darunavir (DRV)	25 (24.3%)	80 (18.3%)	0.1722

Table 9. Baseline Clinical Characteristics (Main Population)

		3TC Daily Dose: 150 mg N= 103	3TC Daily Dose: 300 mg N= 436	p-value
eGFR (ml/min/1.73m^2)	Median (IQR)	39.9 (36.4, 45.5)	43.3 (38.4, 46.5)	**0.0036**
	> 40 to <= 49	51 (49.5%)	288 (66.1%)	**0.0018**
	>= 30 to <= 40	52 (50.5%)	148 (33.9%)	
VACS mortality index	Median (IQR)	47.0 (32.0, 70.0)	36.0 (24.0, 54.0)	**0.0010**
	0 to < 15	5 (4.9%)	28 (6.4%)	**0.0317**
	>= 15 to < 30	13 (12.6%)	108 (24.8%)	
	>= 30 to < 45	24 (23.3%)	108 (24.8%)	
	>= 45	47 (45.6%)	142 (32.6%)	
	Missing	14 (13.6%)	50 (11.5%)	
BMI	Median (IQR)	25.3 (21.5, 28.5)	25.9 (22.8, 30.0)	0.1497
	Underweight	8 (7.8%)	25 (5.7%)	0.2750
	Normal weight	37 (35.9%)	143 (32.8%)	
	Overweight	38 (36.9%)	138 (31.7%)	
	Obese	17 (16.5%)	102 (23.4%)	
	Missing	3 (2.9%)	28 (6.4%)	
Comorbid conditions	Diabetes (diagnosis, prescription or labs)	33 (32.0%)	98 (22.5%)	**0.0419**
	Hypertension	68 (66.0%)	246 (56.4%)	0.0757
	Cardiovascular Disease	30 (29.1%)	92 (21.1%)	0.0800
	Hepatitis B	11 (10.7%)	27 (6.2%)	0.1096
	Hepatitis C	20 (19.4%)	74 (17.0%)	0.5564
	Liver diseases (other than viral hepatitis)	9 (8.7%)	44 (10.1%)	0.6781
	Substance abuse (drug and alcohol)	28 (27.2%)	79 (18.1%)	**0.0380**

VACS Mortality Index characterizes the 5-year risk of all-cause mortality; BMI = body mass index (kg/m^2)

5.2. Composite 1 in the main study population

Composite 1 consisted of specific diagnoses of interest (lactic acidosis, paraesthesia, peripheral neuropathy, pancreatitis, rhabdomyolysis, anemia, neutropenia, thrombocytopenia, nausea) and severe laboratory abnormalities (DAIDS grade 3 or greater: neutrophils < 600 cells/µL, hemoglobin < 8.5 g/dL in females or < 9 g/µL in males, platelets < 50,000 cells/mm^3, ALT ≥ 5 x ULN, AST ≥ 5 x ULN, total bilirubin > 2.6 x ULN, lactate > 2.0 x ULN + pH < 7.3, creatinine kinase > 10 x ULN).

5.2.1. Prevalence of pre-existing Composite 1 in the main study population

At baseline there was no difference in the prevalence of pre-existing Composite 1 overall within 12 months before initiating 3TC between individuals receiving 150 mg vs. 300 mg. However, individuals initiating 3TC with an adjusted dose were more likely to have pre-existing severe lab abnormalities. This was largely driven by a higher prevalence of low hemoglobin in those taking a total daily dose of 150 mg compared to those taking a total daily dose of 300 mg (Table 10).

Table 10. Prevalence of Pre-existing Composite 1 within 12 months before or at baseline[a] (Main Population)

		3TC Daily Dose: 150 mg N= 103	3TC Daily Dose: 300 mg N= 436	p-value
Composite 1	Any	36 (35.0%)	124 (28.4%)	0.1933
Diagnoses of interest [b]	Any	27 (26.2%)	92 (21.1%)	0.2605
	Lactic Acidosis	3 (2.9%)	7 (1.6%)	0.4123
	Paraesthesia	0	4 (0.9%)	1.0000
	Peripheral Neuropathy	6 (5.8%)	28 (6.4%)	0.8227
	Pancreatitis	3 (2.9%)	3 (0.7%)	0.0871
	Rhabdomyolysis	0	0	.
	Anemia	20 (19.4%)	56 (12.8%)	0.0847
	Neutropenia	1 (1.0%)	2 (0.5%)	0.4714
	Thrombocytopenia	5 (4.9%)	15 (3.4%)	0.5599
	Nausea (within 3 months)	2 (1.9%)	12 (2.8%)	1.0000
Severe laboratory abnormalities (DAIDS grade 3+)[b]	Any	24 (23.3%)	57 (13.1%)	**0.0090**
	Neutrophils < 600 cells/µL	1 (1.0%)	5 (1.1%)	1.0000
	Haemoglobin < 8.5 g/dL in female or < 9 g/µL in male	17 (16.5%)	28 (6.4%)	**0.0009**
	Platelets <50,000 cells/mm3	4 (3.9%)	7 (1.6%)	0.2345
	ALT >= 5 x ULN	3 (2.9%)	14 (3.2%)	1.0000
	AST >= 5 x ULN	4 (3.9%)	6 (1.4%)	0.1037
	Total bilirubin > 2.6 x ULN	2 (1.9%)	23 (5.3%)	0.1953
	Creatinine kinase > 10 x ULN	0	1 (0.2%)	1.0000

[a] The baseline period used to assess the presence of prevalent pre-existing events was defined as the 12-month period preceding the index date, inclusive of the index date

[b] Categories are not mutually exclusive

5.2.2. Frequency and incidence rate of Composite 1 in the main study population after excluding patients with pre-existing Composite 1 (Primary objective 2)

After excluding all individuals with pre-existing Composite 1 at baseline, 67/103 (65.0%) individuals remained in the 150 mg group and 312/436 (71.6%) remained in the 300 mg group. No statistically significant difference could be detected in the incidence proportion of new Composite 1 during follow-up between the two groups (Table 11). Incidence rates (IR) of Composite 1 and their confidence intervals completely overlapped between 150 mg and 300 mg total daily doses, with an IR (95% CI) of 12.55 per 100 person-years (5.22, 30.15) with 150 mg and of 19.57 per 100 person-year (13.60, 28.17) with 300 mg (Table 12, Figure 1).

Table 11. Incidence proportions of Composite 1 during follow-up (Main Population without pre-existing Composite 1)

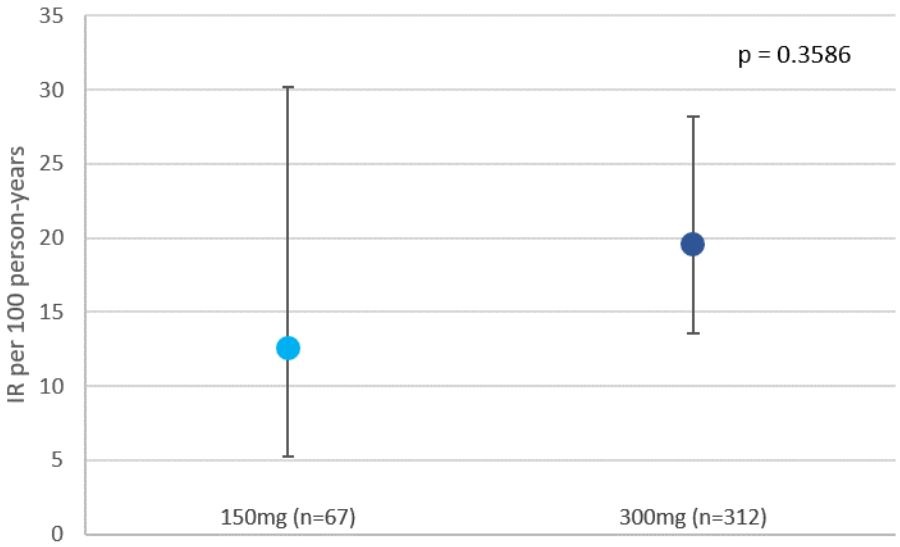
		3TC Daily Dose: 150 mg n=67	3TC Daily Dose: 300 mg n=312	p-value
Composite 1	Any	5 (7.5%)	29 (9.3%)	0.8146
Diagnoses of interest [a]	Any	4 (6.0%)	20 (6.4%)	1.0000
	Lactic Acidosis	0	0	.
	Paraesthesia	0	2 (0.6%)	1.0000
	Peripheral Neuropathy	0	4 (1.3%)	1.0000
	Pancreatitis	0	1 (0.3%)	1.0000
	Rhabdomyolysis	0	0	.
	Anemia	4 (6.0%)	6 (1.9%)	0.0809
	Neutropenia	0	1 (0.3%)	1.0000
	Thrombocytopenia	0	0	.
	Nausea	1 (1.5%)	9 (2.9%)	1.0000
Severe laboratory abnormalities (DAIDS grade 3+) [a]	Any	2 (3.0%)	10 (3.2%)	1.0000
	Neutrophils < 600 cells/µL	0	0	.
	Haemoglobin < 8.5 g/dL in female or < 9 g/µL in male	0	7 (2.2%)	0.2159
	Platelets <50,000 cells/mm3	0	0	.
	ALT >= 5 x ULN	0	3 (1.0%)	1.0000
	AST >= 5 x ULN	0	1 (0.3%)	1.0000
	Total bilirubin > 2.6 x ULN	2 (3.0%)	0	**0.0309**
	Creatinine kinase > 10 x ULN	0	0	.

[a] Categories are not mutually exclusive

Table 12. Incidence rates of Composite 1 during follow-up (Main Population without pre-existing Composite 1)

dose	N	# Composite 1	person-years	IR per 100 person-years (95% CI)
150 mg	67	5	39.844	12.55 (5.22, 30.15)
300 mg	312	29	148.156	19.57 (13.60, 28.17)

Figure 1. Incidence rates per 100 person-years of Composite 1 during follow-up (Main Population without pre-existing Composite 1)



The specific events leading to censoring of person-time were assessed. If two events were recorded on the same day, they were reported together as a single event. In both groups, the most frequent reasons for censoring were a follow-up eGFR >49, discontinuation of 3TC and a follow-up eGFR <30. There was no detected difference in the frequency of total daily dose changes among individuals initiating 3TC with 150 mg compared to 300 mg (Table 13).

Table 13. Censoring during follow-up (Main Population without pre-existing Composite 1)

Censoring events[a]	3TC Daily Dose: 150 mg n=67	3TC Daily Dose: 300 mg n=312	p-value
Discontinuation of 3TC, defined as a gap ≥ 45 days	13 (19.4%)	55 (17.6%)	0.7312
Change in 3TC total daily dose	5 (7.5%)	23 (7.4%)	1.0000
Change in 3TC total daily dose and eGFR < 30 or > 49	2 (3.0%)	1 (0.3%)	0.0820
First follow-up eGFR > 49	31 (46.3%)	176 (56.4%)	0.1303
Discontinuation of 3TC and eGFR > 49	2 (3.0%)	2 (0.6%)	0.1453
First follow-up eGFR < 30	9 (13.4%)	35 (11.2%)	0.6076
Discontinuation of 3TC and eGFR < 30	1 (1.5%)	0	0.1768
Cessation of continuous clinical activity (censored 12 months after their last contact)	2 (3.0%)	8 (2.6%)	0.6920
Death	1 (1.5%)	2 (0.6%)	0.4431
Study end (March 31, 2019)	1 (1.5%)	10 (3.2%)	0.6970

[a] Censoring events are mutually exclusive

Incidence rates for each individual component of Composite 1 are listed in Table 14. These diagnoses and severe lab abnormalities were rare in both groups with no events recorded for many of them.

Table 14. Incidence rates of Composite 1 during follow-up (Main Population without pre-existing Composite 1)

		3TC Daily Dose: 150 mg n=67			3TC Daily Dose: 300 mg n=312		
		Incident events n (%)	person-years	IR per 100 person-years (95% CI)	Incident events n (%)	person-years	IR per 100 person-years (95% CI)
Composite 1	Any	5 (7.5%)	39.84	12.55 (5.22, 30.15)	29 (9.3%)	148.16	19.57 (13.60, 28.17)
Diagnoses of interest[a]	Any	4 (6.0%)	40.17	9.96 (3.74, 26.53)	20 (6.4%)	150.58	13.28 (8.57, 20.59)
	Lactic Acidosis	0	40.71	0	0	159.72	0
	Paraesthesia	0	40.71	0	2 (0.6%)	158.60	1.26 (0.32, 5.04)
	Peripheral Neuropathy	0	40.71	0	4 (1.3%)	157.41	2.54 (0.95, 6.77)
	Pancreatitis	0	40.71	0	1 (0.3%)	159.22	0.63 (0.09, 4.46)
	Rhabdomyolysis	0	40.71	0	0	159.72	0
	Anemia	4 (6.0%)	40.17	9.96 (3.74, 26.53.)	6 (1.9%)	157.14	3.82 (1.72, 8.50)
	Neutropenia	0	40.71	0	1 (0.3%)	159.70	0.63 (0.09, 4.45)
	Thrombocytopenia	0	40.71	0	0	159.72	0
	Nausea	1 (1.5%)	40.70	2.46 (0.35, 17.44)	9 (2.9%)	155.03	5.81 (3.02, 11.16)
Severe laboratory abnormalities (DAIDS grade 3+)[a]	Any	2 (3.0%)	40.38	4.95 (1.24, 19.80)	10 (3.2%)	156.88	6.37 (3.43, 11.85)
	Neutrophils < 600 cells/µL	0	40.71	0	0	159.72	0
	Haemoglobin < 8.5 g/dL in female or < 9 g/µL in male	0	40.71	0	7 (2.2%)	157.29	4.45 (2.12, 9.34)
	Platelets <50,000 cells/mm3	0	40.71	0	0	159.72	0

		3TC Daily Dose: 150 mg n=67			3TC Daily Dose: 300 mg n=312		
		Incident events n (%)	person-years	IR per 100 person-years (95% CI)	Incident events n (%)	person-years	IR per 100 person-years (95% CI)
	ALT >= 5 x ULN	0	40.71	0	3 (1.0%)	159.31	1.88 (0.61, 5.84)
	AST >= 5 x ULN	0	40.71	0	1 (0.3%)	159.31	0.63 (0.09, 4.46)
	Total bilirubin > 2.6 x ULN	2 (3.0%)	40.38	4.95 (1.24, 19.80)	0	159.72	0
	Creatinine kinase > 10 x ULN	0	40.71	0	0	159.72	0

[a] Categories are not mutually exclusive

5.2.3. Association between 3TC dose and Composite 1 in the main study population after excluding patients with pre-existing Composite 1 (Primary objective 1)

Recommended Poisson regression models were selected based on the trade-off between completeness/appropriateness of the adjustment set and the number of patients and incident events excluded due to missing data. The number of patients and events excluded are noted in the table's footnotes and the recommended model is marked in blue. Statistically significant associations are bolded.

After adjusting for baseline substance abuse and hemoglobin, there was no statistically significant association between 3TC total daily dose and incidence of Composite 1, with an IRR (95% CI) of 1.51 (0.59, 3.92). Substance abuse was associated with a statistically significantly increased rate of Composite 1, while higher hemoglobin levels were statistically significantly associated with lower rates of Composite 1 (Table 15).

Table 15. Incidence rate ratios from a Poisson regression for the association between 3TC dose and Composite 1

	Unadjusted IRR (95% CI)	**Adjusted model A[a] IRR (95% CI)**	**Adjusted model B1[b] IRR (95% CI)**	**Adjusted model B2[b] IRR (95% CI)**	**Adjusted model C[c] IRR (95% CI)**	**Adjusted model D[d] IRR (95% CI)**
3TC 300 mg vs. 150 mg	1.56 (0.60, 4.03)	1.48 (0.57, 3.82)	1.51 (0.59, 3.92)	1.51 (0.59, 3.92)	1.93 (0.68, 5.52)	1.54 (0.59, 4.00)
Drug or alcohol abuse	-	**3.97 (1.89, 8.34)**	**4.00 (1.91, 8.38)**	**4.00 (1.91, 8.38)**	**3.64 (1.67, 7.94)**	**4.00 (1.89, 8.46)**
Baseline eGFR	-	0.98 (0.92, 1.04)	1.00 (0.93, 1.07)	-	-	-
Baseline hemoglobin (mg/dL)	-	-	**0.81 (0.69, 0.96)**	**0.81 (0.69, 0.96)**	0.89 (0.73, 1.09)	0.85 (0.71, 1.02)
VACS score	-	-	-	-	1.01 (0.99, 1.03)	-
$\log_{10}$ HIV viral load	-	-	-	-	-	1.17 (0.92, 1.49)

[a] All patients included (no missing values)

[b] Excluding 5 patients with missing hemoglobin (no events excluded) *** Recommended model ***

[c] Excluding 40 patients with missing hemoglobin and/or VACS (2 events excluded: 1 on 150 mg and 1 on 300 mg)

[d] Excluding 34 patients with missing hemoglobin and/or HIV viral load (1 event excluded: on 300 mg)

5.3. Composite 2 in the main study population

Composite 2 consisted of gastrointestinal symptoms (hyperlactataemia, nausea, vomiting, abdominal pain), diagnoses of interest (lactic acidosis, paraesthesia, peripheral neuropathy, pancreatitis, rhabdomyolysis, anemia, neutropenia, thrombocytopenia) and moderate to severe laboratory abnormalities (DAIDS grade 2 or greater: neutrophils < 800 cells/µL, hemoglobin < 9.5 g/dL in females or < 10 g/µL in males, platelets < 100,000 cells/mm^3, ALT ≥ 2.5 x ULN, AST ≥ 2.5 x ULN, total bilirubin > 1.6 x ULN, lactate > 2.0 x ULN + pH < 7.3, creatinine kinase > 6 x ULN, red blood count < 4.52 $x10^{12}$/L in male or < 4.10 $x10^{12}$/L in female, mean corpuscular volume > 96)

5.3.1. Prevalence of pre-existing Composite 2 in the main study population

As for Composite 1, there was no difference in the prevalence of pre-existing Composite 2 overall within 12 months before initiating 3TC between individuals receiving 150 mg vs. 300 mg. However, those taking a total daily dose of 150 mg were more likely to have low hemoglobin prior to 3TC initiation compared to those taking a total daily dose of 300 mg. Pre-existing gastrointestinal symptoms were also more frequent in individuals initiating 3TC with 150 mg than with 300 mg (Table 16).

Table 16. Prevalence of Pre-existing Composite 2 within 12 months before/at baseline [a] (Main Population)

		3TC Daily Dose: 150 mg N= 103	**3TC Daily Dose: 300 mg N= 436**	**p-value**
Composite 2	Any	79 (76.7%)	351 (80.5%)	0.3871
Diagnoses of interest [b]	Any	27 (26.2%)	90 (20.6%)	0.2174
	Lactic Acidosis	3 (2.9%)	7 (1.6%)	0.4123
	Paraesthesia	0	4 (0.9%)	1.0000
	Peripheral Neuropathy	6 (5.8%)	28 (6.4%)	0.8227
	Pancreatitis	3 (2.9%)	3 (0.7%)	0.0871
	Rhabdomyolysis	0	0	.
	Anemia	20 (19.4%)	56 (12.8%)	0.0847
	Neutropenia	1 (1.0%)	2 (0.5%)	0.4714
	Thrombocytopenia	5 (4.9%)	15 (3.4%)	0.5599
Moderate/Severe laboratory abnormalities (DAIDS grade 2+) [b]	Any	77 (74.8%)	333 (76.4%)	0.7291
	Neutrophils < 800 cells/µL	3 (2.9%)	12 (2.8%)	1.0000
	Haemoglobin < 9.5 g/dL in female or < 10 g/µL in male	26 (25.2%)	54 (12.4%)	**0.0010**
	Platelets <100,000 cells/mm^3	11 (10.7%)	27 (6.2%)	0.1096
	ALT >= 2.5 x ULN	12 (11.7%)	32 (7.3%)	0.1507

	AST >= 2.5 x ULN	7 (6.8%)	16 (3.7%)	0.1580
	Total bilirubin > 1.6 x ULN	10 (9.7%)	48 (11.0%)	0.7017
	Creatinine kinase > 6 x ULN	1 (1.0%)	1 (0.2%)	0.3460
	Red blood count <4.52 x1012/L in male or <4.10 x1012/L in female	67 (65.0%)	278 (63.8%)	0.8066
	Mean corpuscular volume (MCV) >96	36 (35.0%)	174 (39.9%)	0.3535
Diagnoses of gastrointestinal symptoms [b]	Any	12 (11.7%)	22 (5.0%)	**0.0131**
	Hyperlactataemia	3 (2.9%)	7 (1.6%)	0.4123
	Nausea (within 3 months)	2 (1.9%)	12 (2.8%)	1.0000
	Vomiting (within 3 months)	3 (2.9%)	11 (2.5%)	0.7372
	Abdominal Pain (within 3 months)	8 (7.8%)	16 (3.7%)	0.0698

[a] The baseline period used to assess the presence of prevalent pre-existing events was defined as the 12-month period preceding the index date, inclusive of the index date

[b] Categories are not mutually exclusive

5.3.2. Frequency and incidence rate of Composite 2 in the main study population after excluding patients with pre-existing Composite 2 (Secondary objective 1)

After excluding all individuals with pre-existing Composite 2 at baseline, 24/103 (23.3%) individuals remained in the 150 mg group and 85/436 (19.5%) remained in the 300 mg group. No statistically significant difference could be detected in the incidence proportion of new Composite 2 during follow-up between the two groups (Table 17). Incidence rates (IR) of Composite 2 overlapped greatly between 150 mg and 300 mg total daily doses, with an IR (95% CI) of 38.12 per 100 person-years (17.13, 84.85) with 150 mg and of 80.17 per 100 person-year (54.59, 117.75) with 300 mg (Table 18, Figure 2).

Table 17. Incidence proportions of Composite 2 during follow-up (Main Population without pre-existing Composite 2)

		3TC Daily Dose: 150 mg n=24	**3TC Daily Dose: 300 mg n=85**	**p-value**
Composite 2	Any	6 (25.0%)	26 (30.6%)	0.5955
Diagnoses of interest [a]	Any	1 (4.2%)	3 (3.5%)	1.0000
	Lactic Acidosis	0	0	.
	Paraesthesia	0	1 (1.2%)	1.0000
	Peripheral Neuropathy	0	1 (1.2%)	1.0000
	Pancreatitis	0	0	.

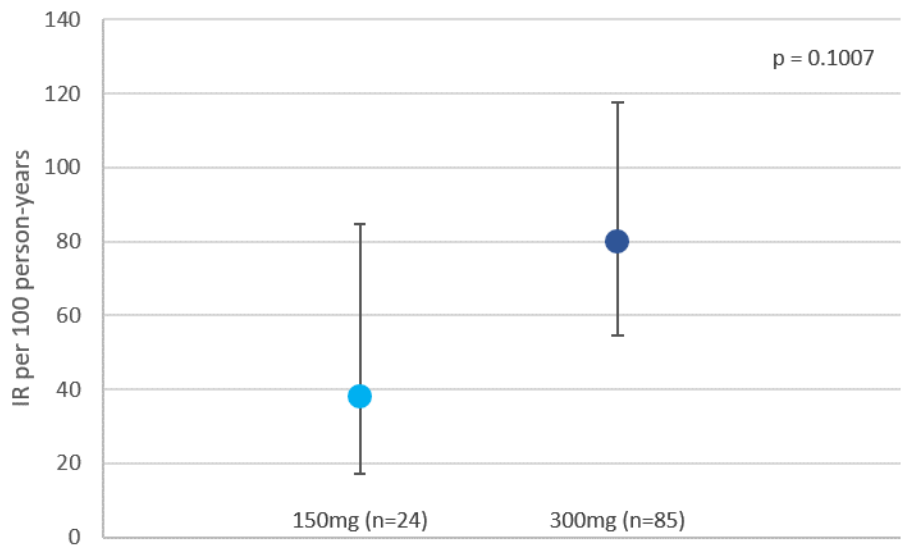
	Rhabdomyolysis	0	0	.
	Anemia	1 (4.2%)	1 (1.2%)	0.3935
	Neutropenia	0	0	.
	Thrombocytopenia	0	0	.
Moderate/Severe laboratory abnormalities (DAIDS grade 2+) [a]	Any	5 (20.8%)	24 (28.2%)	0.6040
	Neutrophils < 800 cells/µL	0	0	.
	Haemoglobin < 9.5 g/dL in female or < 10 g/µL in male	0	2 (2.4%)	1.0000
	Platelets <100,000 cells/mm^3	0	0	.
	ALT >= 2.5 x ULN	0	0	.
	AST >= 2.5 x ULN	0	1 (1.2%)	1.0000
	Total bilirubin > 1.6 x ULN	1 (4.2%)	2 (2.4%)	0.5295
	Creatinine kinase > 6 x ULN	0	0	.
	Red blood count < 4.52 x1012/L in male or < 4.10 x1012/L in female	3 (12.5%)	20 (23.5%)	0.3950
	Mean corpuscular volume (MCV) > 96	1 (4.2%)	10 (11.8%)	0.4500
Diagnoses of gastrointestinal symptoms [a]	Any	1 (4.2%)	2 (2.4%)	0.5295
	Hyperlactataemia	0	0	.
	Nausea	1 (4.2%)	2 (2.4%)	0.5295
	Vomiting	1 (4.2%)	2 (2.4%)	0.5295
	Abdominal Pain	0	0	.

[a] Categories are not mutually exclusive

Table 18. Incidence rates per 100 person-years of Composite 2 during follow-up (Main Population without pre-existing Composite 2)

dose	N	# Composite 2	person-years	IR per 100 person-years (95% CI)
150 mg	24	6	15.7399	38.12 (17.13, 84.85)
300 mg	85	26	32.4298	80.17 (54.59, 117.75)

Figure 2. Incidence rates per 100 person-years of Composite 2 during follow-up (Main Population without pre-existing Composite 2)



In this population restricted to individuals without pre-existing Composite 2, the most frequent reasons for censoring were a follow-up eGFR >49, discontinuation of 3TC and a follow-up eGFR <30. However, those initiating 3TC with an adjusted dose were more likely to be censored due to discontinuation, while those initiating 3TC with a full dose were more likely to be censored due to an eGFR >49 (Table 19).

Table 19. Censoring during follow-up (Main Study Population without pre-existing Composite 2)

Censoring events[a]	3TC Daily Dose: 150 mg n=24	3TC Daily Dose: 300 mg n=85	p-value
Discontinuation of 3TC, defined as a gap ≥ 45 days	10 (41.7%)	14 (16.5%)	**0.0085**
Change in 3TC total daily dose	2 (8.3%)	7 (8.2%)	1.0000
First follow-up eGFR > 49 ml/min/1.73m^2	7 (29.2%)	47 (55.3%)	**0.0238**
First follow-up eGFR < 30 ml/min/1.73m^2	2 (8.3%)	11 (12.9%)	0.7291
Discontinuation of 3TC and eGFR < 30	0	0	.
Discontinuation of 3TC and eGFR > 49	2 (8.3%)	1 (1.2%)	0.1214
Change in 3TC total daily dose and eGFR < 30 or > 49	0	0	.
Cessation of continuous clinical activity, defined as at least one clinical contact, visit or telephone contact (censored 12 months after their last contact)	0	0	.
Death	1 (4.2%)	0	0.2202
Study end (March 31, 2019)	0	5 (5.9%)	0.5840

[a] Censoring events are mutually exclusive

Incidence rates for each individual component of Composite 2 are listed in Table 20. These diagnoses, moderate to severe lab abnormalities and gastrointestinal symptoms were rare in both groups with no events recorded for many of them.

Table 20. Incidence rates per 100 person-years of Composite 2 during follow-up (Main Population without pre-existing Composite 2)

		3TC Daily Dose: 150 mg n=24			3TC Daily Dose: 300 mg n=85		
		Incident events n (%)	person-years	IR per 100 person-years (95% CI)	Incident events n (%)	person-years	IR per 100 person-years (95% CI)
Composite 2	Any	6 (25.0%)	15.74	38.12 (17.13, 84.85)	26 (30.6%)	32.43	80.17 (54.59, 117.75)
Diagnoses of interest [a]	Any	1 (4.2%)	16.52	6.05 (0.85, 42.98)	3 (3.5%)	36.80	8.15 (2.63, 25.28)
	Lactic Acidosis	0	16.52	0	0	38.37	0
	Paraesthesia	0	16.52	0	1 (1.2%)	38.02	2.63 (0.37, 18.67)
	Peripheral Neuropathy	0	16.52	0	1 (1.2%)	37.27	2.68 (0.38, 19.05)
	Pancreatitis	0	16.52	0	0	38.37	0
	Rhabdomyolysis	0	16.52	0	0	38.37	0
	Anemia	1 (4.2%)	16.52	6.05 (0.85, 42.98)	1 (1.2%)	38.24	2.62 (0.37, 18.57)
	Neutropenia	0	16.52	0	0	38.37	0
	Thrombocytopenia	0	16.52	0	0	38.37	0
Moderate/Severe laboratory abnormalities (DAIDS grade 2+) [a]	Any	5 (20.8%)	15.74	31.76 (13.22, 76.31)	24 (28.2%)	34.08	70.42 (47.20, 105.06)
	Neutrophils < 800 cells/µL	0	16.52	0	0	38.37	0
	Haemoglobin < 9.5 g/dL in female or < 10 g/µL in male	0	16.52	0	2 (2.4%)	38.37	5.21 (1.30, 20.84)
	Platelets <100,000 cells/mm^3	0	16.52	0	0	38.37	0
	ALT >= 2.5 x ULN	0	16.52	0	0	38.37	0

		3TC Daily Dose: 150 mg n=24			3TC Daily Dose: 300 mg n=85		
		Incident events n (%)	person-years	IR per 100 person-years (95% CI)	Incident events n (%)	person-years	IR per 100 person-years (95% CI)
	AST >= 2.5 x ULN	0	16.52	0	1 (1.2%)	38.37	2.61 (0.37, 18.50)
	Total bilirubin > 1.6 x ULN	1 (4.2%)	16.19	6.18 (0.87, 43.84)	2 (2.4%)	37.47	5.34 (1.34, 21.34)
	Creatinine kinase > 6 x ULN	0	16.52	0	0	38.37	0
	Red blood count < 4.52 x1012/L in male or < 4.10 x1012/L in female	3 (12.5%)	16.19	18.53 (5.98, 57.44)	20 (23.5%)	35.51	56.32 (36.33, 87.29)
	Mean corpuscular volume (MCV) > 96	1 (4.2%)	16.39	6.10 (0.86, 43.32)	10 (11.8%)	35.04	28.54 (15.36, 53.05)
Diagnoses of gastrointestinal symptoms [a]	Any	1 (4.2%)	16.51	6.06 (0.85, 42.99)	2 (2.4%)	36.26	5.52 (1.38, 22.05)
	Hyperlactataemia	0	16.52	0	0	38.37	0
	Nausea	1 (4.2%)	16.51	6.06 (0.85, 42.99)	2 (2.4%)	36.26	5.52 (1.38, 22.05)
	Vomiting	1 (4.2%)	16.51	6.06 (0.85, 42.99)	2 (2.4%)	36.26	5.52 (1.38, 22.05)
	Abdominal Pain	0	16.52	0	0	38.37	0

[a] Categories are not mutually exclusive

5.3.3. Association between 3TC dose and Composite 2 in the main study population after excluding patients with pre-existing Composite 2 (Secondary objective 1)

Recommended Poisson regression models were selected based on the trade-off between completeness/appropriateness of the adjustment set and the number of patients and incident events excluded due to missing data. The number of patients and events excluded are noted in the table's footnotes and the recommended model is marked in blue. Statistically significant associations are bolded. In this case, two models were deemed appropriate and similar inferences can be drawn from both.

After adjusting for baseline substance abuse and hemoglobin, the full 3TC dose was associated with a three times higher rate of Composite 2 compared to the adjusted dose, with an IRR (95% CI) of 3.07 (1.12, 8.40). In this model, substance abuse was not associated with a statistically significantly increased rate of Composite 2, while higher hemoglobin levels were statistically significantly associated with lower rates of Composite 2 (Table 21).

After adjusting for baseline substance abuse, eGFR and hemoglobin, the full 3TC dose was associated with a 3.23 times higher rate of Composite 2 compared to the adjusted dose, with an IRR (95% CI) of 3.23 (1.17, 8.91). In this model, substance abuse was associated with a statistically significantly increased rate of Composite 2, while higher eGFR levels were statistically significantly associated with lower rates of Composite 2, but hemoglobin levels were not statistically significantly associated with rates of Composite 2 (Table 21).

Table 21. Incidence rate ratio from a Poisson regression for the association between 3TC dose and Composite 2

	Unadjusted IRR (95% CI)	**Adjusted model A[a] IRR (95% CI)**	**Adjusted model B1[b] IRR (95% CI)**	**Adjusted model B2[b] IRR (95% CI)**	**Adjusted model C[c] IRR (95% CI)**	**Adjusted model D[d] IRR (95% CI)**
3TC 300 mg vs. 150 mg	2.10 (0.87, 5.11)	2.05 (0.84, 5.04)	**3.23 (1.17, 8.91)**	**3.07 (1.12, 8.40)**	**4.32 (1.42, 13.12)**	**2.82 (1.03, 7.70)**
Drug or alcohol abuse	-	**2.78 (1.21, 6.38)**	**2.41 (1.02, 5.73)**	2.13 (0.91, 5.02)	**2.98 (1.17, 7.59)**	2.39 (0.99, 5.77)
Baseline eGFR	-	**0.90 (0.85, 0.96)**	**0.92 (0.87, 0.99)**	-	**0.93 (0.87, 0.99)**	**0.93 (0.87, 0.99)**
Baseline hemoglobin (mg/dL)	-	-	0.80 (0.63, 1.00)	**0.76 (0.62, 0.95)**	0.81 (0.62, 1.04)	**0.75 (0.58, 0.96)**
VACS score	-	-	-	-	1.01 (0.98, 1.04)	-
$\log_{10}$ HIV viral load	-	-	-	-	-	0.87 (0.65, 1.17)

[a] All patients included (no missing values)

[b] Excluding 5 patients with missing hemoglobin (2 events excluded: 1 on 300 mg, 1 on 150 mg) *** Recommended models ***

[c] Excluding 16 patients with missing hemoglobin and/or VACS (4 events excluded: 2 on 300 mg, 2 on 150 mg)

[d] Excluding 12 patients with missing hemoglobin and/or HIV viral load (4 events excluded: 3 on 300 mg, 1 on 150 mg)

5.4. Composite 3 in the main study population (Sensitivity objective 2)

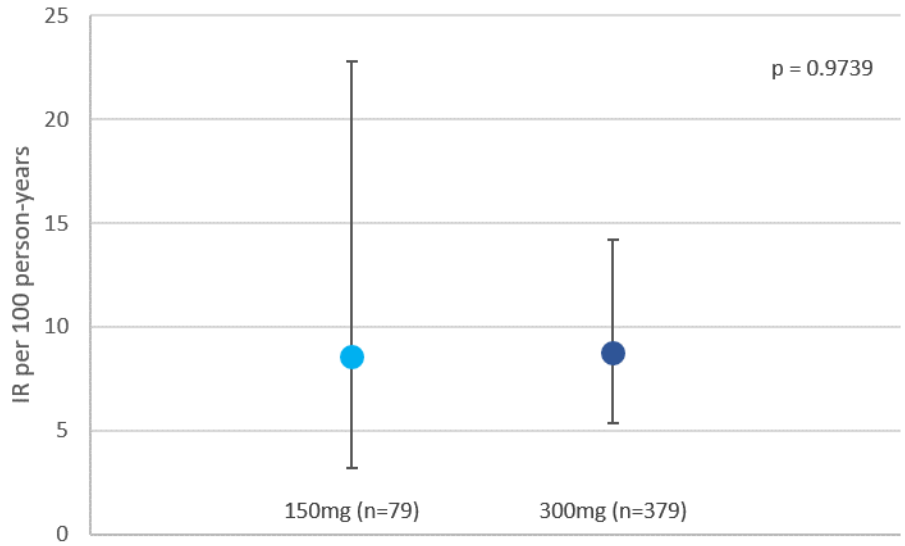
Composite 3 consisted of severe laboratory abnormalities (DAIDS grade 3 or greater: neutrophils < 600 cells/µL, hemoglobin < 8.5 g/dL in females or < 9 g/µL in males, platelets < 50,000 cells/mm^3, ALT ≥ 5 x ULN, AST ≥ 5 x ULN, total bilirubin > 2.6 x ULN, lactate > 2.0 x ULN + pH < 7.3, creatinine kinase > 10 x ULN).

After excluding all individuals with pre-existing Composite 3 at baseline, 79/103 (76.7%) individuals remained in the 150 mg group and 379/436 (86.9%) remained in the 300 mg group. Incidence rates (IR) of Composite 3 completely overlapped between 150 mg and 300 mg total daily doses, with an IR (95% CI) of 8.55 per 100 person-years (3.21, 22.77) with 150 mg and of 8.70 per 100 person-year (5.33, 14.21) with 300 mg (Table 22, Figure 3).

Table 22. Incidence rates per 100 person-years of Composite 3 during follow-up (Main Population without pre-existing Composite 3)

dose	N	# Composite 3	person-years	IR per 100 person-years (95% CI)
150 mg	79	4	46.801	8.55 (3.21, 22.77)
300 mg	379	16	183.811	8.70 (5.33, 14.21)

Figure 3. Incidence rates per 100 person-years of Composite 3 during follow-up (Main Population without pre-existing Composite 3)



Recommended Poisson regression models were selected based on the trade-off between completeness/appropriateness of the adjustment set and the number of patients and incident events excluded due to missing data. The number of patients and events excluded are noted in the table's footnotes and the recommended model is marked in blue. Statistically significant associations are bolded.

After adjusting for baseline substance abuse and hemoglobin, there was no statistically significant association between 3TC total daily dose and incidence of Composite 3, with an IRR (95% CI) of 0.88 (0.29, 2.66). Substance abuse was not statistically significantly associated with rate of Composite 3, while higher hemoglobin levels were statistically significantly associated with lower rates of Composite 3 (Table 15).

Table 23. Incidence rate ratio from a Poisson regression for the association between 3TC dose and Composite 3

	Unadjusted IRR (95% CI)	**Adjusted model A[a] IRR (95% CI)**	**Adjusted model B1[b] IRR (95% CI)**	**Adjusted model B2[b] IRR (95% CI)**	**Adjusted model C[c] IRR (95% CI)**	**Adjusted model D[d] IRR (95% CI)**
3TC 300 mg vs. 150 mg	1.02 (0.34, 3.05)	1.03 (0.34, 3.08)	0.91 (0.30, 2.76)	0.88 (0.29, 2.66)	0.78 (0.25, 2.40)	0.92 (0.30, 2.79)
Drug or alcohol abuse	-	1.59 (0.46, 5.47)	1.85 (0.53, 6.39)	1.88 (0.54, 6.53)	1.94 (0.55, 6.86)	1.73 (0.50, 6.00)
Baseline eGFR	-	0.96 (0.88, 1.04)	0.96 (0.88, 1.05)	-	-	-
Baseline hemoglobin (mg/dL)	-	-	**0.73 (0.58, 0.91)**	**0.72 (0.58, 0.90)**	0.95 (0.71, 1.28)	**0.71 (0.55, 0.93)**
VACS score	-	-	-	-	**1.03 (1.01, 1.05)**	-
$\log_{10}$ HIV viral load	-	-	-	-	-	0.95 (0.68, 1.33)

[a] All patients included (no missing values)

[b] Excluding 8 patients with missing hemoglobin (1 event excluded: 1 on 300 mg, 0 on 150 mg) *** Recommended model ***

[c] Excluding 55 patients with missing hemoglobin and/or VACS (2 events excluded: 2 on 300 mg, 0 on 150 mg)

[d] Excluding 46 patients with missing hemoglobin and/or HIV viral load (1 event excluded: 1 on 300 mg, 0 on 150 mg)

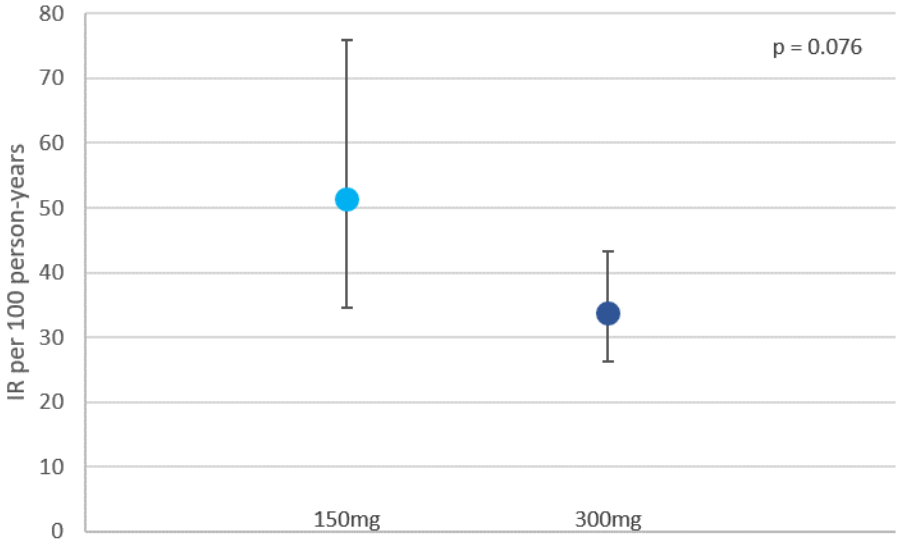
5.5. Association between 3TC dose and Composite 1 in the main study population including patients with or without pre-existing Composite 1 (Sensitivity objective 3)

When individuals with pre-existing Composite 1 were included in the analytical population, incidence rates (IR) of Composite 1 overlapped between 150 mg and 300 mg total daily doses, with an IR (95% CI) of 51.28 per 100 person-years (34.65, 75.88) with 150 mg and of 33.68 per 100 person-year (26.26, 43.20) with 300 mg (Table 24, Figure 4).

Table 24. Incidence rates per 100 person-years of Composite 1 during follow-up (Main Population, with or without pre-existing Composite 1)

dose	N	# Composite 1	person-years	IR per 100 person-years (95% CI)
150 mg	103	25	48.756	51.28 (34.65, 75.88)
300 mg	436	62	184.101	33.68 (26.26, 43.20)

Figure 4. Incidence rates per 100 person-years of Composite 1 during follow-up (Main Population, with or without pre-existing Composite 1)



Recommended Poisson regression models were selected based on the trade-off between completeness/appropriateness of the adjustment set and the number of patients and incident events excluded due to missing data. The number of patients and events excluded are noted in the table's footnotes and the recommended model is marked in blue. Statistically significant associations are bolded.

After adjusting for baseline substance abuse and hemoglobin, there was no statistically significant association between 3TC total daily dose and incidence of Composite 1, with an IRR (95% CI) of 0.63 (0.39, 1.01). Substance abuse was associated with a statistically significantly increased rate of Composite 1, while higher hemoglobin levels were statistically significantly associated with lower rates of Composite 1 (Table 25).

Table 25. Incidence rate ratios from a Poisson regression for the association between 3TC dose and Composite 1 (Main Population with or without pre-existing Composite 1)

	Unadjusted IRR (95% CI)	**Adjusted model A[a] IRR (95% CI)**	**Adjusted model B1[b] IRR (95% CI)**	**Adjusted model B2[b] IRR (95% CI)**	**Adjusted model C[c] IRR (95% CI)**	**Adjusted model D[d] IRR (95% CI)**
3TC 300 mg vs. 150 mg	0.66 (0.41, 1.04)	**0.61 (0.38, 0.98)**	0.62 (0.39, 1.01)	0.63 (0.39, 1.01)	0.68 (0.40, 1.14)	**0.60 (0.37, 0.99)**
Drug or alcohol abuse	-	**3.67 (2.32, 5.81)**	**3.43 (2.16, 5.45)**	**3.38 (2.13, 5.36)**	**2.77 (1.66, 4.63)**	**3.19 (1.96, 5.18)**
Baseline eGFR	-	0.96 (0.93, 1.00)	1.01 (0.97, 1.05)	-	-	-
Baseline hemoglobin (mg/dL)	-	-	**0.70 (0.63, 0.77)**	**0.70 (0.64, 0.77)**	0.82 (0.71, 0.94)	**0.73 (0.66, 0.82)**
VACS score	-	-	-	-	1.01 (1.00, 1.03)	-
$\log_{10}$ HIV viral load	-	-	-	-	-	1.04 (0.89, 1.22)

[a] All patients included (no missing values)

[b] Excluding 8 patients with missing hemoglobin (2 events excluded: 1 on 150 mg and 1 on 300 mg) *** Recommended model ***

[c] Excluding 65 patients with missing hemoglobin and/or VACS (13 events excluded: 5 on 150 mg and 8 on 300 mg)

[d] Excluding 57 patients with missing hemoglobin and/or HIV viral load (10 events excluded: 2 on 150 mg and 8 on 300 mg)

5.6. Composite 1 without censoring when follow-up eGFR is out of range (Sensitivity objective 1)

Without censoring at the first eGFR <30 or >49, the total years of follow-up and median follow-up time are greatly increased compared to the main analysis in which person-time was censored at the first out-of-range eGFR (Table 26).

Table 26. Comparison of follow-up time between the Main and Sensitivity Populations

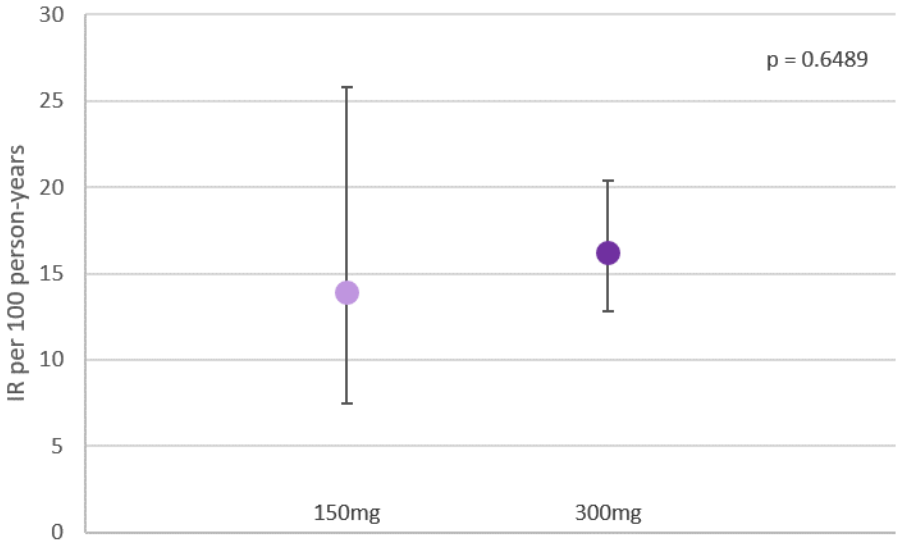
	Main Study Population (censored at first out-of-range eGFR)	Sensitivity Study Population (not censored at first out-of-range eGFR)
N	539	539
Total years of follow-up	259.31	919.53
Median (IQR) months of follow-up	2.6 (1.1, 7.5)	13.0 (3.7, 30.2)

After excluding all individuals with pre-existing Composite 1 at baseline, 67/103 (65.0%) individuals remained in the 150 mg group and 312/436 (71.6%) remained in the 300 mg group. Incidence rates (IR) of Composite 1 completely overlapped between 150 mg and 300 mg total daily doses, with an IR (95% CI) of 13.87 per 100 person-years (7.46, 25.78) with 150 mg and of 16.17 per 100 person-year (12.84, 20.37) with 300 mg (Table 27, Figure 5).

Table 27. Incidence rates per 100 person-years of Composite 1 during follow-up (Main Population without pre-existing Composite 1, without censoring when eGFR is out of range)

dose	N	# Composite 1	person-years	IR per 100 person-years (95% CI)
150 mg	67	10	72.104	13.87 (7.46, 25.78)
300 mg	312	72	445.207	16.17 (12.84, 20.37)

Figure 5. Incidence rates per 100 person-years of Composite 1 during follow-up (Main Population without pre-existing Composite 1, without censoring when eGFR is out of range)



Recommended Poisson regression models were selected based on the trade-off between completeness/appropriateness of the adjustment set and the number of patients and incident events excluded due to missing data. The number of patients and events excluded are noted in the table's footnotes and the recommended model is marked in blue. Statistically significant associations are bolded.

After adjusting for baseline substance abuse and hemoglobin, there was no statistically significant association between 3TC total daily dose and incidence of Composite 1, with an IRR (95% CI) of 1.14 (0.59, 2.21). Substance abuse was associated with a statistically significantly increased rate of Composite 1, while higher hemoglobin levels were statistically significantly associated with lower rates of Composite 1 (Table 28).

Table 28. Incidence rate ratios from a Poisson regression for the association between 3TC dose and Composite 1 (Main Population without pre-existing Composite 1, without censoring when eGFR is out of range)

	Unadjusted IRR (95% CI)	**Adjusted model A[a] IRR (95% CI)**	**Adjusted model B1[b] IRR (95% CI)**	**Adjusted model B2[b] IRR (95% CI)**	**Adjusted model C[c] IRR (95% CI)**	**Adjusted model D[d] IRR (95% CI)**
3TC 300 mg vs. 150 mg	1.17 (0.60, 2.26)	1.19 (0.61, 2.31)	1.14 (0.59, 2.21)	1.14 (0.59, 2.21)	1.27 (0.63, 2.56)	1.17 (0.60, 2.28)
Drug or alcohol abuse	-	**2.15 (1.29, 3.60)**	**2.22 (1.33, 3.71)**	**2.22 (1.33, 3.71)**	**2.10 (1.24, 3.57)**	**2.36 (1.40, 3.97)**
Baseline eGFR	-	0.99 (0.95, 1.03)	1.00 (0.96, 1.04)	-	-	-
Baseline hemoglobin (mg/dL)	-	-	**0.85 (0.76, 0.96)**	**0.85 (0.76, 0.95)**	0.89 (0.77, 1.02)	**0.88 (0.79, 0.99)**
VACS score	-	-	-	-	1.01 (0.99, 1.02)	-
$\log_{10}$ HIV viral load	-	-	-	-	-	1.14 (0.97, 1.34)

[a] All patients included (no missing values)

[b] Excluding 5 patients with missing hemoglobin (1 event excluded: 1 on 300 mg) *** Recommended model ***

[c] Excluding 40 patients with missing hemoglobin and/or VACS (6 events excluded: 1 on 150 mg and 5 on 300 mg)

[d] Excluding 34 patients with missing hemoglobin and/or HIV viral load (5 event excluded: 5 on 300 mg)

5.7. Graphical summary and comparison of estimates across composite outcome definitions and study populations

Figure 6. Incidence rates (unadjusted) per 100 person-years by 3TC dose and outcome definition

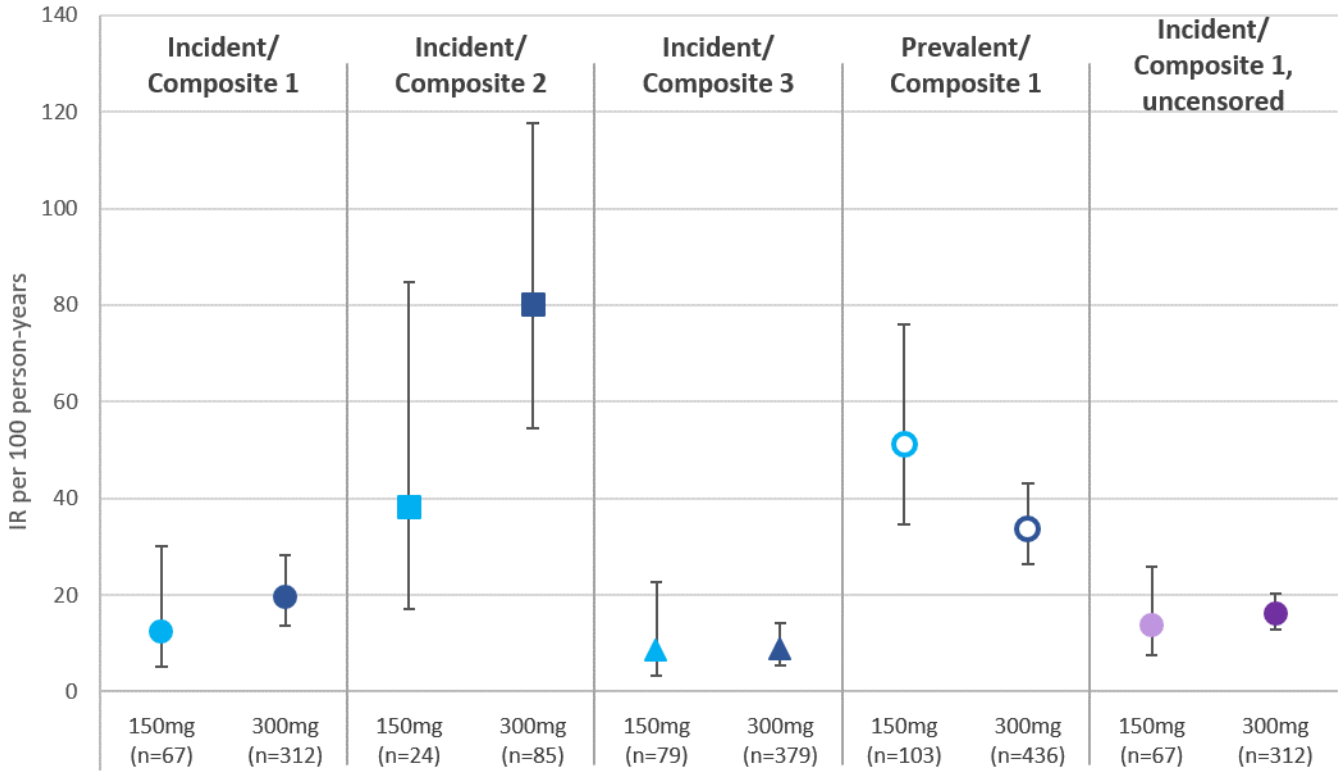
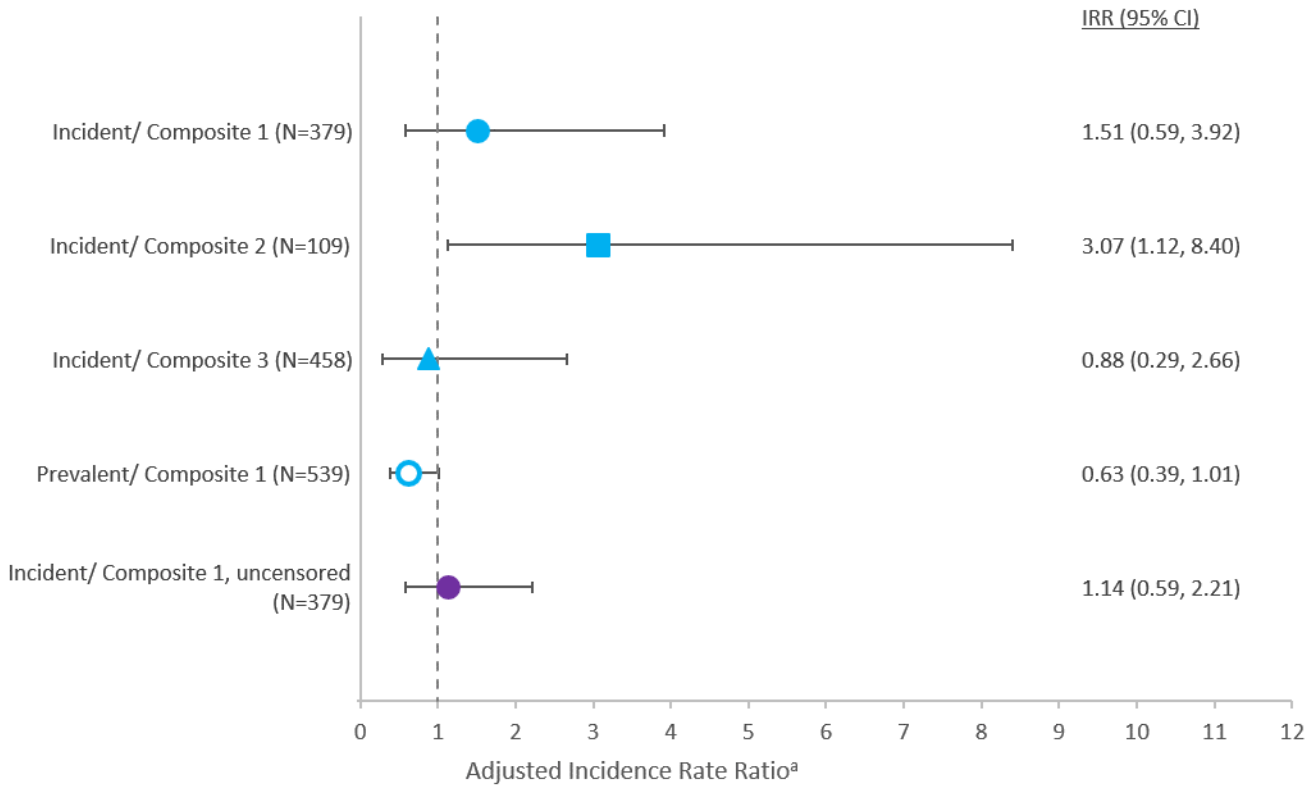


Figure 7. Adjusted[a] incidence rate ratios from a Poisson regression comparing 300 mg vs. 150 mg of 3TC by outcome definition



[a] *Incidence rate ratios adjusted for drug/alcohol abuse and baseline hemoglobin*

5.8. In-patient diagnoses (sensitivity objective 1)

After adding the requirement that patients must receive care in a center for which in-patient records are available, only 97 individuals remained (Table 29). Of those, 27% initiated 3TC with an adjusted daily dose of 150 mg and 73% initiated 3TC with the full daily dose of 300 mg (Table 30).

Table 29. Identification of the Inpatient Study Population

		Patients Included	%	Patients Excluded	%
1	Main study population	539	.	0	.
2	Patients from center with in-patient records	97	18.0	442	82.0

Table 30. In-patient Study Population by 3TC dose

		N (%)
Patients initiating 3TC with 150 mg		26 (26.8%)
Patients initiating 3TC with 300 mg	Overall	71 (73.2%)
	150 mg BID	14 (19.7%)
	300 mg QD	55 (77.5%)
	Unknown dose frequency	2 (2.8%)
Total study population		**97 (100.0%)**

Among individuals with in-patient records available, there was no difference at baseline in the prevalence of pre-existing Composite 1 overall within 12 months before initiating 3TC between individuals receiving 150 mg vs. 300 mg. However, individuals initiating 3TC with an adjusted dose were more likely to have pre-existing severe lab abnormalities. This was largely driven by a higher prevalence of low hemoglobin in those taking a total daily dose of 150 mg compared to those taking a total daily dose of 300 mg consistent with the main study population (Table 31).

Table 31. *Prevalence of Pre-existing Composite 1 within 12 months before or at baseline[a] (In-patient Study Population)*

		3TC Daily Dose: 150 mg N= 26	3TC Daily Dose: 300 mg N= 71	p-value
Composite 1	Any	15 (57.7%)	28 (39.4%)	0.1089
Diagnoses of interest[b]	Any	10 (38.5%)	20 (28.2%)	0.3313
	Lactic Acidosis	1 (3.8%)	5 (7.0%)	1.0000
	Paraesthesia	0	1 (1.4%)	1.0000
	Peripheral Neuropathy	1 (3.8%)	1 (1.4%)	0.4663
	Pancreatitis	2 (7.7%)	2 (2.8%)	0.2907
	Rhabdomyolysis	0	0	.
	Anemia	9 (34.6%)	12 (16.9%)	0.0606
	Neutropenia	1 (3.8%)	1 (1.4%)	0.4663
	Thrombocytopenia	4 (15.4%)	8 (11.3%)	0.7284
	Nausea (within 3 months)	0	4 (5.6%)	0.5711
Severe laboratory abnormalities (DAIDS grade 3+)[b]	Any	13 (50.0%)	19 (26.8%)	**0.0311**
	Neutrophils < 600 cells/µL	1 (3.8%)	3 (4.2%)	1.0000
	Haemoglobin < 8.5 g/dL in female or < 9 g/µL in male	11 (42.3%)	12 (16.9%)	**0.0092**
	Platelets <50,000 cells/mm^3	3 (11.5%)	6 (8.5%)	0.6976
	AST >= 5 x ULN	0	6 (8.5%)	0.1259
	ALT >= 5 x ULN	0	3 (4.2%)	0.5618
	Total bilirubin > 2.6 x ULN	2 (7.7%)	9 (12.7%)	0.7219
	Lactate > 2.0 x ULN + pH <7.3	0	1 (1.4%)	1.0000
	Creatinine kinase > 10 x ULN	0	0	.

[a] The baseline period used to assess the presence of prevalent pre-existing events was defined as the 12-month period preceding the index date, inclusive of the index date

[b] Categories are not mutually exclusive

After excluding all individuals with pre-existing Composite 1 at baseline, 11/26 (42.3%) individuals remained in the 150 mg group, with only 1 incident Composite 1 event; 43/71 (60.6%) remained in the 300 mg group, with 2 Composite 1 events (Table 32). Incidence rates (IR) of

Composite 1 and their confidence intervals completely overlapped between 150 mg and 300 mg total daily doses, with an IR (95% CI) of 30.51 per 100 person-years (4.30, 216.62) with 150 mg dose and of 19.62 per 100 person-year (4.91, 78.43) with 300 mg dose (Table 33).

Table 32. Incidence proportions of Composite 1 during follow-up (In-patient Study Population without pre-existing Composite 1)

		3TC Daily Dose: 150 mg, n=11	3TC Daily Dose: 300 mg, n=43	p-value
Composite 1	Any	1 (9.1%)	2 (4.7%)	0.5025
Diagnoses of interest[a]	Any	1 (9.1%)	0	0.2037
	Lactic Acidosis	0	0	.
	Paraesthesia	0	0	.
	Peripheral Neuropathy	0	0	.
	Pancreatitis	0	0	.
	Rhabdomyolysis	0	0	.
	Anemia	1 (9.1%)	0	0.2037
	Neutropenia	0	0	.
	Thrombocytopenia	0	0	.
	Nausea	1 (9.1%)	0	0.2037
Severe laboratory abnormalities (DAIDS grade 3+)[a]	Any	0	2 (4.7%)	1.0000
	Neutrophils < 600 cells/µL	0	0	.
	Haemoglobin < 8.5 g/dL in female or < 9 g/µL in male	0	1 (2.3%)	1.0000
	Platelets <50,000 cells/mm3	0	0	.
	AST >= 5 x ULN	0	1 (2.3%)	1.0000
	ALT >= 5 x ULN	0	1 (2.3%)	1.0000
	Total bilirubin > 2.6 x ULN	0	0	.
	Lactate > 2.0 x ULN + pH <7.3	0	0	.
	Creatinine kinase > 10 x ULN	0	0	.

[a] Categories are not mutually exclusive

Table 33. Incidence rates of Composite 1 during follow-up (Inpatient Study Population without pre-existing Composite 1)

		3TC Daily Dose: 150 mg n=11			3TC Daily Dose: 300 mg n=43		
		Incident events n (%)	person-years	IR per 100 person-years (95% CI)	Incident events n (%)	person-years	IR per 100 person-years (95% CI)
Composite 1	Any	1 (9.1%)	3.28	30.51 (4.30, 216.62)	2 (4.7%)	10.20	19.62 (4.91, 78.43)
Diagnoses of interest[a]	Any	1 (9.1%)	3.28	30.51 (4.30, 216.62)	0	10.24	0
	Lactic Acidosis	0	3.28	0	0	10.24	0
	Paraesthesia	0	3.28	0	0	10.24	0
	Peripheral Neuropathy	0	3.28	0	0	10.24	0
	Pancreatitis	0	3.28	0	0	10.24	0
	Rhabdomyolysis	0	3.28	0	0	10.24	0
	Anemia	1 (9.1%)	3.28	30.49 (4.30, 216.44)	0	10.24	0
	Neutropenia	0	3.28	0	0	10.24	0
	Thrombocytopenia	0	3.28	0	0	10.24	0
	Nausea	1 (9.1%)	3.28	30.51 (4.30, 216.62)	0	10.24	0
Severe laboratory abnormalities (DAIDS grade 3+)[a]	Any	0	3.28	0	2 (4.7%)	10.20	19.62 (4.91, 78.43)
	Neutrophils < 600 cells/µL	0	3.28	0	0	10.24	0
	Haemoglobin < 8.5 g/dL in female or < 9 g/µL in male	0	3.28	0	1 (2.3%)	10.23	9.77 (1.38, 69.39)
	Platelets < 50,000 cells/mm3	0	3.28	0	0	10.24	0
	AST >= 5 x ULN	0	3.28	0	1 (2.3%)	10.20	9.80 (1.38, 69.59)
	ALT >= 5 x ULN	0	3.28	0	1 (2.3%)	10.20	9.80 (1.38, 69.59)

		3TC Daily Dose: 150 mg n=11			3TC Daily Dose: 300 mg n=43		
		Incident events n (%)	person-years	IR per 100 person-years (95% CI)	Incident events n (%)	person-years	IR per 100 person-years (95% CI)
	Total bilirubin > 2.6 x ULN	0	3.28	0	0	10.24	0
	Lactate > 2.0 x ULN + pH < 7.3	0	3.28	0	0	10.24	0
	Creatinine kinase > 10 x ULN	0	3.28	0	0	10.24	0

[a] Categories are not mutually exclusive

5.9. Out-of-range follow-up eGFRs

A total of 404 individuals had follow-up eGFR out of the range of interest and were thus censored in the main analysis (Table 34). Of those, 20% had a follow-up eGFR < 30 and 80% had a follow-up eGFR > 49 (Table 35).

Table 34. Identification of the Secondary Population (follow-up eGFR outside of range)

		Patients Included	%	Patients Excluded	%
1	Patients in Main Study Population	539	.	0	.
2	Patients with an out-of-range follow-up eGFR (<30 or >49 ml/min/1.73m^2)	404	75.0	135	25.0

Table 35. Secondary Population by follow-up eGFR (first out-of-range eGFR)

	N (%)
Patients with a follow-up eGFR < 30 ml/min/1.73m^2	79 (19.6%)
Patients with a follow-up eGFR > 49 ml/min/1.73m^2	325 (80.4%)
Total study population	**404 (100.0%)**

5.9.1. Changes in eGFR over follow-up in the secondary study population: patients censored due to an improvement or worsening of eGFR (Secondary objective 1)

Out of the individuals with a follow-up eGFR <30, a second confirmatory eGFR <30 was recorded in 10/18 (55.6%) individuals on 150 mg and in 33/45 (73.3%) individuals on 300 mg. Among those with confirmation of eGFR <30, those on an adjusted 3TC dose had a greater number of creatinine tests performed after their first low eGFR, compared to individuals on the full dose. No other difference in testing or eGFR changes could be detected (Table 36).

Table 36. Follow-up eGFR among patients with a follow-up eGFR below range (< 30 ml/min/1.73m^2), N=79

		3TC Daily Dose: 150 mg N= 18	3TC Daily Dose: 300 mg N= 45	p-value
Confirmation of eGFR < 30	Presence of a second eGFR < 30	10 (55.6%)	33 (73.3%)	0.1709
Out of those with confirmation of eGFR <30:				
Censoring events	Discontinuation of 3TC (any cause), defined as a gap ≥45 days	6 (60.0%)	11 (33.3%)	0.1578
	Change in 3TC total daily dose	2 (20.0%)	10 (30.3%)	0.6983
	Cessation of continuous clinical activity (censored 12 months after last contact)	0	0	.
	Death	1 (10.0%)	1 (3.0%)	0.4153
	Study end (March 31, 2019)	1 (10.0%)	11 (33.3%)	0.2366
Time from baseline to first eGFR < 30 (months)	Median (IQR)	2.1 (0.5, 6.8)	7.8 (2.2, 15.7)	0.0898
Time from baseline to last eGFR during follow-up (months)	Median (IQR)	18.1 (2.5, 35.8)	18.2 (10.2, 31.6)	0.8971
Time from first eGFR < 30 to last eGFR during follow-up (months)	Median (IQR)	14.5 (2.1, 19.9)	5.6 (3.0, 12.0)	0.3501
Number of eGFR tests during follow-up	Min	3.00	2.00	0.1121
	Q1	4.00	3.00	.
	Median	9.00	6.00	.
	Q3	18.00	8.00	.
	Max	24.00	17.00	.
Number of eGFR tests after the first eGFR < 30	Min	1.00	1.00	**0.0052**
	Q1	3.00	1.00	.
	Median	7.00	2.00	.
	Q3	14.00	3.00	.
	Max	20.00	13.00	.
Baseline eGFR value	Min	30.88	30.32	0.7037
	Q1	34.50	33.86	.
	Median	37.82	36.83	.

		3TC Daily Dose: 150 mg N= 18	3TC Daily Dose: 300 mg N= 45	p-value
	Q3	40.89	45.06	.
	Max	48.35	48.89	.
First eGFR < 30	Min	7.40	0.12	0.3613
	Q1	19.29	23.15	.
	Median	25.66	27.33	.
	Q3	28.00	28.31	.
	Max	29.83	29.77	.
Last eGFR value during follow-up	Min	15.43	5.36	0.3075
	Q1	26.40	23.46	.
	Median	31.64	28.18	.
	Q3	40.34	33.30	.
	Max	49.09	56.40	.
	< 30 ml/min/1.73m^2	3 (30.0%)	19 (57.6%)	0.2291
	>= 30 to <= 49 ml/min/1.73m^2	6 (60.0%)	13 (39.4%)	.
	> 49 ml/min/1.73m^2	1 (10.0%)	1 (3.0%)	.
Change in eGFR between baseline and last eGFR	Min	-20.80	-40.11	0.1466
	Q1	-8.95	-19.34	.
	Median	-2.30	-7.34	.
	Q3	2.14	-1.87	.
	Max	12.72	8.99	.
	Worsening (> 4 ml/min/1.73m^2 decrease)	5 (50.0%)	21 (63.6%)	0.6688
	Unchanged (± 4 ml/min/1.73m^2)	3 (30.0%)	8 (24.2%)	.
	Improvement (> 4 ml/min/1.73m^2 increase)	2 (20.0%)	4 (12.1%)	.
Change in eGFR between first eGFR < 30 and last eGFR	Min	-14.40	-17.54	0.5177
	Q1	-1.60	-3.49	.
	Median	4.20	3.75	.
	Q3	16.26	8.01	.
	Max	30.00	43.20	.
	Worsening (> 4 ml/min/1.73m^2 decrease)	2 (20.0%)	7 (21.2%)	1.0000
	Unchanged (± 4 ml/min/1.73m^2)	3 (30.0%)	10 (30.3%)	.
	Improvement (> 4 ml/min/1.73m^2 increase)	5 (50.0%)	16 (48.5%)	.

Out of the individuals with a follow-up eGFR >49, a second confirmatory eGFR >49 was recorded in 38/55 (69.1%) individuals on 150 mg and in 217/255 (85.1%) individuals on 300 mg. Among those with confirmation of eGFR > 49, those on an adjusted 3TC dose were more likely to change dose and therefore had shorter follow-up times and fewer creatinine tests performed after their first improved eGFR, compared to individuals on the full dose (Table 37).

Table 37. Follow-up eGFR among patients with a follow-up eGFR above range (> 49 ml/min/1.73m^2) (Secondary Population)

		3TC Daily Dose: 150 mg N= 55	3TC Daily Dose: 300 mg N= 255	p-value
Confirmation of eGFR > 49	Presence of a second eGFR > 49	38 (69.1%)	217 (85.1%)	**0.0048**
Out of those with confirmation of eGFR > 49:				
Censoring events	Discontinuation of 3TC (any cause), defined as a gap ≥ 45 days	17 (44.7%)	120 (55.3%)	0.2283
	Change in 3TC total daily dose	15 (39.5%)	22 (10.1%)	**<.0001**
	Cessation of continuous clinical activity (censored 12 months after last contact)	0	9 (4.1%)	0.2012
	Death	1 (2.6%)	5 (2.3%)	1.0000
	Study end (March 31, 2019)	5 (13.2%)	61 (28.1%)	0.0693
Time from baseline to first eGFR > 49 (months)	Median (IQR)	1.6 (0.2, 3.4)	2.2 (1.1, 4.5)	0.0862
Time from baseline to last eGFR during follow-up (months)	Median (IQR)	7.7 (2.5, 19.3)	24.4 (11.9, 40.2)	**<.0001**
Time from first eGFR > 49 to last eGFR during follow-up (months)	Median (IQR)	3.4 (2.3, 16.9)	19.3 (8.3, 35.9)	**<.0001**
Number of eGFR tests during follow-up	Min	2.00	2.00	**<.0001**
	Q1	3.00	4.00	.
	Median	4.00	7.00	.
	Q3	6.00	12.00	.
	Max	26.00	59.00	.

		3TC Daily Dose: 150 mg N= 55	3TC Daily Dose: 300 mg N= 255	p-value
Number of eGFR tests after the first eGFR > 49	Min	1.00	1.00	**<.0001**
	Q1	1.00	3.00	.
	Median	2.00	6.00	.
	Q3	5.00	11.00	.
	Max	18.00	58.00	.
Baseline eGFR value	Min	30.26	30.07	**0.0177**
	Q1	38.58	40.29	.
	Median	42.30	44.72	.
	Q3	45.97	46.96	.
	Max	48.83	48.85	.
First eGFR > 49	Min	49.04	49.01	0.3306
	Q1	51.77	52.44	.
	Median	53.85	56.48	.
	Q3	64.53	63.84	.
	Max	130.98	113.81	.
Last eGFR value during follow-up	Min	25.19	0.11	0.1153
	Q1	50.03	44.87	.
	Median	60.25	56.42	.
	Q3	72.61	66.54	.
	Max	98.17	127.15	.
	< 30 ml/min/1.73m^2	1 (2.6%)	12 (5.5%)	0.1815
	>= 30 to <= 49 ml/min/1.73m^2	6 (15.8%)	61 (28.1%)	.
	> 49 ml/min/1.73m^2	31 (81.6%)	144 (66.4%)	.
Change in eGFR between baseline and last eGFR	Min	-12.95	-45.74	**0.0432**
	Q1	5.13	2.02	.
	Median	18.51	11.32	.
	Q3	31.60	24.02	.
	Max	59.36	96.29	.
	Worsening (> 4 ml/min/1.73m^2 decrease)	3 (7.9%)	34 (15.7%)	0.5094
	Unchanged (± 4 ml/min/1.73m^2)	5 (13.2%)	32 (14.7%)	.
	Improvement (> 4 ml/min/1.73m^2 increase)	30 (78.9%)	151 (69.6%)	.
Change in eGFR between first eGFR > 49 and last eGFR	Min	-28.44	-83.62	**0.0352**
	Q1	-11.47	-11.53	.
	Median	2.77	-2.63	.

		3TC Daily Dose: 150 mg N= 55	3TC Daily Dose: 300 mg N= 255	p-value
	Q3	13.54	6.56	.
	Max	34.59	48.10	.
	Worsening (> 4 ml/min/1.73m^2 decrease)	12 (31.6%)	99 (45.6%)	0.1774
	Unchanged (± 4 ml/min/1.73m^2)	8 (21.1%)	47 (21.7%)	.
	Improvement (> 4 ml/min/1.73m^2 increase)	18 (47.4%)	71 (32.7%)	.

6. Discussion

This study aimed at assessing the risk of adverse diagnoses and laboratory abnormalities associated with the total daily dose of 3TC initiated by patients with renal impairment, defined as a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2.

The Composite 1 event rate was 12.55 per 100 person years (95% CI: 5.22, 30.15) with an adjusted dose of 150 mg and 19.57 per 100 person-years (95% CI: 13.60, 28.17) with a full dose of 300 mg. After adjusting for drug/alcohol abuse and baseline hemoglobin, the rate of Composite 1 events was increased by 51% with the full dose compared to the adjusted dose. However, since the 95% confidence interval included 1, the increase was not statistically significant (aIRR: 1.51 [95% CI: 0.59, 3.92]).

To assess the robustness of these main findings, several sensitivity analyses were conducted, using different composite outcome definitions or study populations. Results of the main analysis with Composite 1 were confirmed in three additional analyses: (1) changing the outcome to Composite 3 – severe lab abnormalities only (aIRR: 0.88 [95% CI:0.29, 2.66]); (2) including PLWH with pre-existing events at baseline in the analytical population (aIRR: 0.63 [95% CI: 0.39, 1.01]); and (3) not censoring person-time at an out-of-range eGFR (aIRR: 1.14 [95% CI: 0.59, 2.21]). While some point estimates were elevated and others pointed towards a lower rate of events with the full dose, all confidence intervals included the null. Restricting the study population to in-patients resulted in a sample size too small for analysis.

However, with Composite 2 – diagnoses, GI symptoms and moderate/severe lab abnormalities, a statistically significantly higher rate of events was observed with the full dose compared to the adjusted dose (aIRR: 3.07 [95% CI: 1.12, 8.40]). Of note, GI symptoms and moderate lab abnormalities were common in these patients prior to 3TC administration. This generated a small and highly selected population; therefore, results for Composite 2 should be interpreted with caution. Further, GI symptoms were not attributed to 3TC use by the caregiver in the medical record during follow up resulting in uncertainty as to their association with the drug.

Of note, only 19% of individuals initiating 3TC with an eGFR ≥ 30 and ≤ 49 received an adjusted dose of 150 mg daily. The full dose may be prescribed in some instances when fixed dose combinations are preferred, as the adjusted dose would result in a regimen with an increased number of tablets, potentially increasing the risk of suboptimal adherence. In addition, dose adjustment was more frequently prescribed to women, African Americans, and sicker patients (higher viral load, lower eGFR, higher VACS index, more diabetes and substance abuse, low hemoglobin). This suggests that physicians weighed the risks and benefit of prescribing a full dose, including the tradeoff between potential unintended events with the full dose vs. potentially lower adherence and effectiveness with the adjusted dose.

To the best of our knowledge, this is the first observational study assessing the impact of 3TC dose adjustment or lack thereof among patients with renal impairment. This study has several strengths. The OPERA cohort utilizes routine clinical data from electronic medical records from 85 clinics in 19 states and 1 territory, accounting for about 7% of the population living with HIV in the U.S. Thus, these data reflect routine clinical care in the U.S., where the 3TC dose-adjustment recommendation is not always followed in renal impaired patients.

A limitation of the study was the small number of patients with renal impairment and the fact that many events of interest were commonly experienced in the months preceding 3TC initiation which led to a reduced sample size. Statistical models therefore had to remain parsimonious and residual confounding is possible. Because symptoms were identified through diagnoses only, symptoms included in the Composite 2 definition were likely more severe, therefore warranting documenting them as a diagnosis in the electronic medical record rather than in a note in the review of systems. However, severity could not be graded from the information available. Another limitation of the Composite 2 definition is that symptoms such as headache, fatigue and malaise were not included because they were deemed too common and non-specific. However, such common symptoms are a major cause of dose adjustment and medication discontinuation. Given that many diagnoses, abnormal labs and symptoms included in the composite outcomes were non-specific, it is likely that some of them may have been unrelated to 3TC dosing but were rather associated with comorbid conditions. Reasons for discontinuation were not detailed sufficiently in the EMRs to assess if they differed by 3TC dose. However, discontinuation and dose modification frequency did not differ significantly between dosing groups in the main analysis. Nevertheless, discontinuation of 3TC was more common with the adjusted than the full dose in the population used to assess incidence of Composite 2. This could have led to an overestimation of the risk associated with the full dose if 3TC was discontinued before events of interest were recorded in the EMR in the adjusted dose group only. No adjustment was performed for multiple testing in the descriptive analyses, as these were performed for informational purposes only and do not represent the main objectives of the study. While it is possible that some events could be missed when censoring at the first out-of-range eGFR and that doing so would result in a population with more persistently diminished renal function than the target population, a sensitivity analysis in which person-time was not censored based on eGFR value yielded comparable results.

3TC is a well-tolerated drug with a wide therapeutic index. Therefore, dose adjustment may be unnecessary among renal impaired patients with an eGFR ≥ 30 to ≤ 49 ml/min/1.73m^2. Indeed, in this study, there was no statistically significant difference in the risk of severe events with the full compared to adjusted dose. However, we observed a statistically significant increased risk of GI symptoms and/or moderate lab abnormalities with a total daily dose of 300 mg in patients with an eGFR ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2. These findings suggest that dose adjustment may be considered for renally impaired patients who are experiencing such events. Clinical judgement will be key in weighing the risks versus the benefits in this fragile patient population

7. References

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8. Appendix

8.1. Study Size Considerations from the protocol

8.1.1. Feasibility numbers:

As of March 23, 2018, over 400 patients in the OPERA database have initiated 3TC in the renal impairment target range of interest (30-49 ml/min/1.73m^2). Preliminary results from feasibility assessment for the number of potential study participants are tabulated below.

Table 38. Identification of the study population as of 23MAR2018

		Patients Included	%	Patients Excluded	%
1	Patients who are HIV+	85,779	.	0	.
2	Patients with HIV-1 infection (excluding HIV-2 infection)	85,695	99.9	84	0.1
3	HIV+ patients prescribed ART	74,848	87.3	10,847	12.7
4	Patients prescribed 3TC	26,531	35.4	48,317	64.6
5	Patients prescribed 3TC for the first time between 11/17/1995 and 3/23/2018	20,514	77.3	6,017	22.7
6	Patients who were 13 years of age or older at first 3TC	20,489	99.9	25	0.1
7	Patients with a baseline eGFR of >=30 ml/min/1.73m2 and <=49 ml/min/1.73m2	471	2.3	20,018	97.7

Table 39. 3TC doses prescribed in the study population as of 23MAR2018

	N	%
Patients initiating 3TC with 150 mg	77	16.3
Patients initiating 3TC with 300 mg	377	80.0
Patients initiating 3TC with < 150 mg	9	1.9
Patients initiating 3TC with unspecified dose	8	1.7

Table 40. 3TC dose switches over follow-up in the study population as of 12FEB2018

	Patients initiating 3TC with 150 mg		Patients initiating 3TC with 300 mg	
	N	%	N	%
No change in dose	56	72.7	316	83.8
Changes dose once	16	20.8	39	10.3
Changes dose more than once	5	6.5	22	5.8

8.1.2. Power calculation

The power to detect a difference in rates of specific events of interest with 150 mg or 300 mg daily doses of 3TC has been computed under varying conditions, assuming a one-sided alternative hypothesis, and a sample size of 77 patients on 150 mg daily and 377 patients on 300 mg daily. A Wald test was used to test the mean difference between the estimated and null parameters [22-25]. All power calculations for composite endpoint, were performed for incidence rate ratios ranging from 0.50 to 0.98. A baseline rate of 0.03 adverse events per person-month on 3TC was derived from the 3TC registrational clinical trials and 3TC expanded access trials in which 600 patients had adverse events of significance out of 16,000 patients treated with 3TC for an average of 48 weeks. Additional curves were produced for baseline rates of 0.01 adverse events per person-month and 0.05 adverse events per person-month to account for potential differences in occurrence and capture of adverse events in a real-world setting. Finally, all calculations were performed with varying degrees of over-dispersion, ranging from a normal Poisson (SD = 1) to a more extreme scenario (SD=2).

Table 41. Power to detect a range of incidence rate ratio for a Poisson regression

Over-dispersion	3TC 150 mg N	3TC 300 mg N	3TC 150 mg Incidence rate (events/month)	3TC 300 mg Incidence rate (events/month)	Rate Ratio	Power
1.00	77	377	0.01	0.006	0.60	0.987
				0.007	0.70	0.875
				0.008	0.80	0.537
			0.03	0.018	0.60	1.000
				0.021	0.70	0.999
				0.024	0.80	0.932
			0.05	0.030	0.60	1.000
				0.035	0.70	1.000
				0.040	0.80	0.993
1.33	77	377	0.01	0.006	0.60	0.898
				0.007	0.70	0.668
				0.008	0.80	0.351
			0.03	0.018	0.60	1.000
				0.021	0.70	0.979
				0.024	0.80	0.749
			0.05	0.030	0.60	1.000
				0.035	0.70	0.999
				0.040	0.80	0.919
1.67	77	377	0.01	0.006	0.60	0.750
				0.007	0.70	0.496
				0.008	0.80	0.250
			0.03	0.018	0.60	0.991
				0.021	0.70	0.896
				0.024	0.80	0.565
			0.05	0.030	0.60	1.000

				0.035	0.70	0.984
				0.040	0.80	0.771
2.00	77	377	0.01	0.006	0.60	0.614
				0.007	0.70	0.384
				0.008	0.80	0.194
			0.03	0.018	0.60	0.957
				0.021	0.70	0.778
				0.024	0.80	0.435
			0.05	0.030	0.60	0.996
				0.035	0.70	0.932
				0.040	0.80	0.625

Figure 8. Power to detect a range of incidence rate ratio for a Poisson regression with 77 patients on 150 mg daily and 377 patients on 300 mg daily