TITLE PAGE

Information Type: Study Protocol

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

Compound Number: GR109714

Development Phase IV

Effective Date: 17-Dec-2018

Subject: Safety of 3TC in patients with renal impairment

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1. LIST OF ABBREVIATIONS

1.1. HIV Medications (generic name) and corresponding abbreviations

Abbreviation	Medication Name
3TC	lamivudine
DTG	dolutegravir
FTC	emtricitabine
RTV or /r	ritonavir
TAF	tenofovir alafenamide fumarate
TDF	tenofovir disoproxil fumarate
ZDV	zidovudine

1.2. Other abbreviations

Abbreviation	Description
AUC	area under the concentration-time curves
ADME	absorption-distribution-metabolism-excretion
ADAP	AIDS drug assistance program
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
BAA	business associates agreement
BID	twice daily
CMS	Centers for Medicare & Medicaid Services
CLcr	Creatinine Clearance
CCR5	chemokine receptor type 5
CDC	Centers for Disease Control & Prevention
DAIDS	Division of AIDS
DHHS	Department of Health and Human Services
eGFR	estimated glomerular filtration rate
EMR	electronic medical record
ELISA	enzyme-linked immunosorbent assay
HBV	hepatitis B virus
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HITECH	Health Information Technology for Economic and Clinical Health Act
HIV	human immunodeficiency virus
HIVAN	HIV-associated nephropathy
ICD-9	International Classification of Diseases v9
IQR	Inter-Quartile Range
µL	microliter
mL	milliliter

OPERA	Observational Pharmaco-Epidemiology Research & Analysis
PHI	protected health information
QA	quality assurance
QD	once daily
RNA	ribonucleic acid
SD	Standard Deviation
t1/2	half-life

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2. RESPONSIBLE PARTIES: SPONSOR INFORMATION PAGE

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Date

INVESTIGATOR PROTOCOL AGREEMENT PAGE

- I confirm agreement to conduct the study in compliance with the protocol.
- I acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.
- I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

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25 JUN 2018

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Investigator Name: Laurence Brunet

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In

Date

3. ABSTRACT

Introduction:

Lamivudine (3TC) is a cytosine dideoxynucleoside analogue with potent in vitro activity against human immunodeficiency virus (HIV) demonstrated through the inhibition of reverse transcriptase. 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 approximately 70% being renally excreted unchanged. Given the absorption-distribution-metabolism-excretion (ADME) profile of 3TC, current guidance is that dose adjustment should be considered in patients with renal insufficiency. Renal insufficiency is a common co-morbidity 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.

Objectives:

Primary:

1) Analytic (hypothesis testing): 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, among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

2) Descriptive: 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 with total daily 3TC dose prescribed of:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

Secondary:

1) To estimate and compare the frequency and rate, as well as estimate the association between the 3TC dose prescribed and the rate of a composite outcome consisting of gastrointestinal symptoms diagnoses, specific diagnoses of interest and moderate-severe laboratory abnormalities, among

patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2:

a. 300 mg (3TC 150 mg BID or 300 mg QD)
b. 150 mg (3TC 150 mg QD)

2) To describe changes in eGFR over follow-up among patients censored due to an improvement or worsening of eGFR with a total daily 3TC dose prescribed:
   a. 300 mg (3TC 150 mg BID or 300 mg QD)
   b. 150 mg (3TC 150 mg QD)

Study Design:

An observational clinical cohort analysis utilizing prospectively collected electronic medical record (EMR) data obtained from the OPERA® Observational Database will be used to address the study objectives. Subjects will be included from 17NOVEMBER1995 through 31MARCH2018 and observed through 30JUNE2018, thus allowing a potential minimum of 3 months of follow-up in all patients. Renally impaired, ART naïve or experienced HIV+ patients will be included. Exposure to 3TC 300 mg daily and 150 mg daily will be assessed among new users of 3TC with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2. Person-time will be censored upon discontinuation of 3TC, change in 3TC dose, improvement of eGFR to ≥50 ml/min/1.73m^2 or worsening of eGFR to <30 ml/min/1.73m^2, cessation of continuous clinical activity, death or study end.

Endpoints:

The clinical endpoint will be a composite outcome consisting of specific diagnoses of interest and severe laboratory values, as defined by the DAIDS grading system.

4. AMENDMENTS AND UPDATES

Amendment or update no	Date	Section of study protocol	Amendment or update	Reason
<1>	<Date>	<Text>	<Text>	<Text>
<2>	<Date>	<Text>	<Text>	<Text>
<n>	<Date>	<Text>	<Text>	<Text>

5. MILESTONES

Milestone	**Planned date**
Start of data analysis	January 2019
End of data analysis	May 2019
Preliminary tables	June 2019
Draft report of study results	July 2019
Final Report	August 2019

6. BACKGROUND AND RATIONALE

6.1. Background

Lamivudine (3TC), (-)2',3'-dideoxynucleoside 3'-thiacytidine, 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] and is the (-)enantiomer of a dideoxy analogue of cytidine. 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 anti-retroviral (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 co-morbidity in HIV infected patients [10]. Prior to modern ART when 3TC guidelines were created, HIV-associated nephropathy (HIVAN) was a common diagnosis

[11].

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].

Table 1. Schedule of Recommended Dose Adjustments

CLcr (ml/min)	**Recommended 3TC Dose**
≥50	150 mg twice-daily or 300 mg once-daily
30-49	150 mg once-daily
15-29	150 mg first dose, then 100 mg once-daily
5-14	150 mg first dose, then 50 mg once-daily
<5	50 mg first dose, then 25 mg once-daily

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 is warranted.

6.2. 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 co-morbidity 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.

7. RESEARCH QUESTION AND OBJECTIVE(S)

Research Hypothesis:

There is no excess risk in the rate of the composite primary endpoint consisting of specific diagnoses of interest or severe laboratory abnormalities between patients prescribed 3TC 150 mg per day versus patients prescribed 3TC 300 mg per day.

Primary objectives:

1) Analytic (hypothesis testing): 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 among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

2) Descriptive: 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 with total daily 3TC dose prescribed of:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

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 among patients with a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

2) To describe changes in eGFR over follow-up among patients censored due to an improvement or worsening of eGFR with a total daily 3TC dose prescribed:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

Sensitivity analysis objective:

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, 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, with total daily 3TC dose prescribed:
    a. 300 mg (3TC 150 mg BID or 300 mg QD)
    b. 150 mg (3TC 150 mg QD)

    This assessment will be done without censoring the patients whose eGFR falls out of the range of interest during follow-up.

2) To estimate the association between 3TC dose prescribed and the rate of a composite outcome restricted to severe laboratory abnormalities 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 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 first event from 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

8. RESEARCH METHODS

8.1. Study Design

This is an observational clinical cohort study analysing prospectively collected electronic medical record (EMR) data obtained from the OPERA® Observational Database.

Study Period: Patients will be included if they initiate 3TC between 17NOVEMBER1995 and 31MARCH2018 and will be followed up until 30JUNE2018, allowing at least 3 months of follow-up for testing and laboratory results.

Index date will be defined as the date of first 3TC initiation.

Baseline will be defined as the last value on or up to 12 months before index date.

8.2. Study Population and Setting

The study sample will be identified from the OPERA Observational Database for analysis per the inclusion/exclusion criteria defined below.

Inclusions:

1) A diagnosis of HIV, a positive HIV Western Blot, or a positive HIV enzyme-linked immunosorbent assay (ELISA); and a detectable HIV viral load test
2) At least 13 years of age at the time of 3TC initiation
3) eGFR ≤ 49 ml/min/1.73m^2 and ≥ 30 ml/min/1.73m^2 at baseline
4) Initiating 3TC for the first time while in the target eGFR range

Exclusions:

1) HIV negative
2) eGFR <30 ml/min/1.73m^2 at time of 3TC initiation
3) eGFR >49 ml/min/1.73m^2 at time of 3TC initiation

8.3. Variables

8.3.1. eGFR equation

eGFR calculations: The CKD-EPI equation will be used to calculate the eGFR. This equation is currently recommended in multiple clinical guidelines (2016 European AIDS Clinical Society (EACS) Guidelines, Kidney Disease, Improving Global Outcomes (KDIGO)). In an exploratory analysis, the number of patients initiating 3TC while in the target eGFR range of 30 to 49 ml/min/1.73m^2 did not vary substantially when using the CKD-EPI, MDRD or Cockcroft-Gault equations.

8.3.2. Exposure definitions

Exposures of interest, captured through prescription data:

1) Prescription of 3TC with a total daily dose of 300 mg (150 mg BID or 300 mg QD)
2) Prescription of 3TC with a total daily dose of 150 mg (150 mg QD)

Censoring events:

1) Discontinuation of 3TC due to any cause, defined as a gap of 45 days or more

   a. Patients discontinuing 3TC due to an event will still be captured in the primary analysis as if the event occurred on treatment.
2) Change in total daily dose of lamivudine
3) Improvement of eGFR to ≥50 ml/min/1.73m^2 or worsening of eGFR to <30 ml/min/1.73m^2. For the primary analysis, events occurring only during eGFR of ≥ 30 ml/min/1.73m^2 - ≤ 49 ml/min/1.73m^2 will be counted.
4) 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
5) Death
6) Study end (to 30 JUNE2018)

8.3.3. Outcomes of Interest

There will be 2 composite outcomes of interest. The composite outcome for the primary analysis will consist of specific diagnoses of interest and severe laboratory abnormalities. The composite outcome for the secondary analysis will consist of gastrointestinal symptoms, specific diagnoses of interest and moderate-severe laboratory abnormalities.

To identify outcomes of interest, diagnosis codes will be used in conjunction with text searches of the diagnosis field of the electronic medical records using terms consistent with each diagnosis of interest. The same outcomes will be used for the sensitivity analysis.

<u>Primary analysis:</u>

A composite outcome consisting of specific diagnoses of interest and laboratory abnormalities of grade 3-4 observed in 3TC clinical trials (4-7, 9) and as defined by the DAIDS grading system (18). The outcomes have been selected for inclusion based on the severity, clinical significance and inclusion in the label as adverse drug reactions. The composite variable for primary analysis excludes GI symptoms and moderate lab abnormalities both of which are not specific enough. This composite outcome will be considered present if a patient experiences at least one incident (not present at baseline) specific diagnosis of interest or laboratory abnormality of grade 3-4 during follow-up, defined as:

Specific diagnoses of interest, with additional diagnosis terms to be used in parenthesis:

- 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 (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 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

Diagnostic Codes

Primary Outcomes	**ICD-9 codes**	**ICD-10 codes**
Parathesia	782.0	R20.2
Lactic acidosis	276.2	E87.2
Peripheral neuropathy	337.9 excluding 357.2	G90.09 excluding E11.40
Acute pancreatitis	577.0	K85.90
Rhabdomyolysis	728.88	M62.82
Anemia	285.9/284.9	D64.9
Neutropenia	288.00	D70.9
Thrombocytopenia	287.5	D69.6
Nausea	787.03	R11.0
Secondary Outcomes		
Hyperlactatemia	276.2	E87.2
Nausea	787.03	R11.0
Vomiting	787	R11.10
Abdominal pain	789.00	R10.9

Secondary analyses

1) Composite outcome consisting of gastrointestinal symptoms, select diagnoses and moderate-severe laboratory abnormalities of grade 2-4 observed in 3TC

clinical trials (4-7, 9) and as defined by the DAIDS grading system (18). The composite variable for secondary analysis includes GI symptoms and moderate lab abnormalities both of which are not specific enough for inclusion in the primary objectives, but could be clinically relevant for patients. This composite outcome will be considered present if a patient experiences at least one incident (not present at baseline) gastrointestinal symptom or moderate-severe laboratory abnormality during follow-up, defined as:

Diagnoses of gastrointestinal symptoms:

- Hyperlactataemia (lactic acidosis, increased lactic acid)
- Nausea (Nausea)
- Vomiting (Vomiting, emesis, hyperemesis)
- Abdominal Pain (RLQ, RUQ, LUQ, LLQ for Right, left, upper, lower, quadrant; epigastric pain)

Specific diagnoses of interest:

- Lactic Acidosis
- Paraesthesia
- Peripheral Neuropathy
- Pancreatitis
- Rhabdomyolysis
- Anemia
- Neutropenia
- Thrombocytopenia

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)

2) Follow-up eGFR endpoints and testing characteristics, defined as:
   - Last eGFR value
     i. Continuous: Median (IQR)

ii. Categorical: Above the target range, within the target range, below the target range
- 3TC duration
- Change in eGFR between baseline and last eGFR
  i. Continuous: Median (IQR)
  ii. Categorical: Improved, remained the same, worsened
- Number of eGFR tests during follow-up

8.3.4. Study Covariates/Potential Confounders

The following patient demographic and clinical characteristics will be described when first eligible for the study. Selection of covariates for multivariable models will be from this list based on expert knowledge and observed differences between 3TC dose groups and observed association of each covariate on the endpoints used in the modelling.

Demographic variables: These variables will be described and only relevant variables will be assessed for confounding

- Age
  - Continuous (years)
  - Categorical:
    i. 13-25 years old
    ii. 26-49 years old
    iii. 50+ years old
- Gender
- Race (African American or not)
- Ethnicity (Hispanic or not)
- Geographic region (i.e., Northeast, Midwest, South, West)
- Payer type (i.e. Medicaid, Medicare, commercial insurance, AIDS drug assistance programs (ADAP)/Ryan White, cash)

Virologic and immunologic variables: These variables will be described and only relevant variables will be assessed for confounding

- HIV viral load at baseline
  - Continuous (copies/mL and $\log_{10}$ copies/mL)
  - Categorical:
    - Low (<10,000 copies/mL)
    - Moderate (≥10,000 to <100,000 copies/mL)
    - High (≥100,000 copies/mL)
    - Missing
- CD4 cell count at baseline

  - Continuous (cells/µL)
  - Categorical:
    - High (CD4 > 500 cells/µL)
    - Moderately High (CD4 count >350 to ≤500 cells/µL)
    - Moderate (CD4 count >200 to ≤350 cells/µL)
    - Moderately Low (CD4 count >50 to ≤200 cells/µL)
    - Low (CD4 count ≤50 cells/µL)
    - Missing

Clinical variables: These variables will be described and only relevant variables will be assessed for confounding

- eGFR at baseline
  - Continuous (ml/min/1.73m^2)
  - Categorical:
    i. eGFR ≤ 49 ml/min/1.73m^2 and >40 ml/min/1.73m^2
    ii. eGFR ≤ 40 ml/min/1.73m^2 and ≥ 30 ml/min/1.73m^2
- Year of HIV diagnosis
- Time from HIV diagnosis date to baseline (years)
- ART experienced vs. naive
- Year of 3TC ART initiation
- Time from first active date in the database to baseline (months)
- AIDS defining illness at baseline
- Mortality Risk (VACS Mortality Index)
- Comorbid Conditions
  - Diabetes
  - Hypertension
  - Hyperlipidemia
  - CVD
  - Viral hepatitis B, C, and other liver diseases
- BMI
- Illicit drug use
- Co-prescribed ART requiring dose adjustment
  - tenofovir disproxil fumarate (TDF)
  - tenofovir alafenamide (TAF)
  - emtricitabine (FTC)
  - atazanavir
  - lopinavir/r
  - stavudine
  - didanosine
  - zalcitabine
  - zidovudine
  - maraviroc
- Co-prescribed ART known to inhibit secretion of tubular creatinine

- dolutegravir
- cobicistat
- ritonavir
- rilpivirine
- darunavir

8.4. Data sources

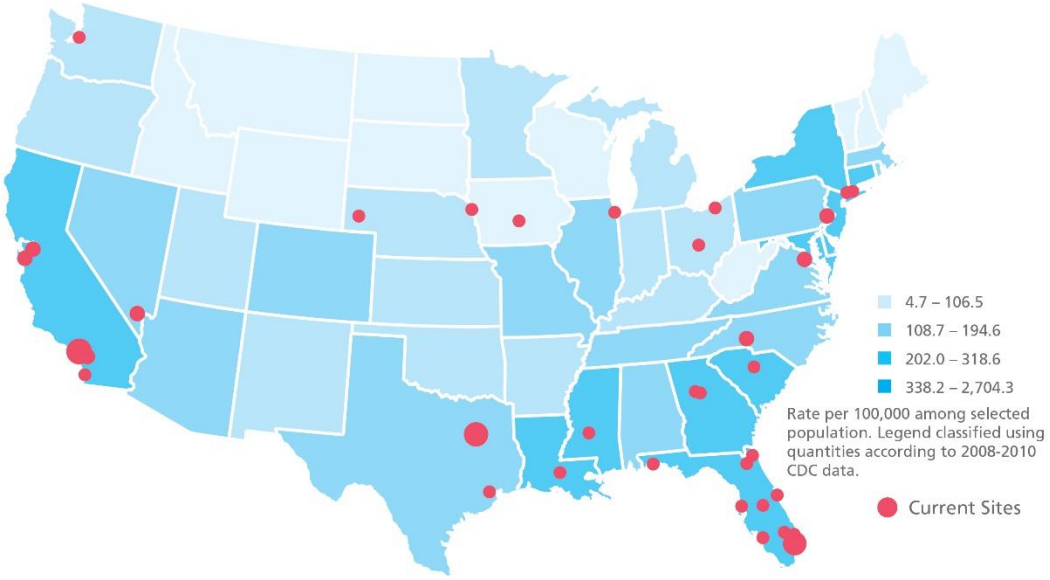
The OPERA® (Observational Pharmaco-Epidemiology Research & Analysis) database and research network is a multi-site observational database built from the complete patient health records managed in Electronic Health Record (EHR) systems from more than 400 participating caregivers 84 separate locations throughout the U.S. (see coverage map below).

Through their membership in OPERA, medical practices meet the Centers for Medicare & Medicaid Services (CMS) Electronic Health Record (EHR) Incentive Program for Integration with a Specialized Registry. OPERA-participating physicians and ancillary healthcare providers have documented the care of over 868,000 patients in their EHRs, including 86,000 HIV+ patients of which 17% are women, representing 7 percent of all the HIV+ patients linked to care in the U.S. The OPERA database is refreshed from these EHR databases at each clinic on a daily basis, making the OPERA database the largest continuously operating cohort of HIV+ patients in the U.S.

As of April 10, 2018, there are more than 2.6 million documented prospective visits in the EHR systems for these HIV+ patients and 2.8 million prescriptions written for ART medications. The average years of follow-up (years of documenting patient visits prospectively in the EHR) for patients in OPERA is 3.7 years with 7,959 HIV+ patients who have ten years or more of follow-up.

In addition to HIV treatment, OPERA captures the diagnosis and treatment of co-morbid conditions; such as mental health conditions (31.6%), syphilis (30.6%), hypertension (28.2%), hepatitis C (10.7%), and hepatitis B (7.1%).

Figure 1: U.S. Map of OPERA HIV+ Population & CDC (2010) State-by-State Estimates



8.5. Data analysis

8.5.1. Analysis of primary objectives

Descriptive analyses will be conducted for patients prescribed 3TC 150 mg and 300 mg per day between 17NOV1995 and 31MARCH2018 whether previously ART naïve or ART experienced. Patient characteristics at baseline will include demographics; such as age, sex, race, ethnicity and geographic region (section 8.3). Clinical characteristics at baseline will include treatment experience; such as year of ART initiation, number of ART classes experienced, baseline HIV-1 RNA viral load and CD4 cell counts (section 8.3). The history and prevalence of selected specific diagnoses of interest and/or laboratory tests at or before baseline will be described (section 8.3). Baseline descriptive statistics will be provided. The proportion of patients on another ARV requiring dose adjustment, as well as the proportion of patients prescribed an adjusted dose of these ARVs will be described. Medians and interquartile ranges for continuous variables will be compared between daily dose groups using Wilcoxon Rank Sum test. Frequencies (counts and percentages) for categorical variables will be compared using Pearson Chi-Square test.

The incidence rate of the composite outcome (specific diagnoses of interest and/or laboratory abnormalities of grade 3-4) will be estimated within each dose group and also compared across each of the total daily dosing groups (i.e. 300 mg vs. 150 mg) using univariate Poisson regression (including treatment term only). Multivariable Poisson regression adjusting for retained covariates will be employed to estimate the incidence rate ratio for the composite outcome comparing total 3TC daily doses of 300 mg vs. 150 mg, using time since 3TC initiation as the offset. Statistical hypothesis test will be based on the adjusted rate ratio from the final Poisson model.

Required assumptions of statistical models will be investigated and if determined to be untenable then alternative approaches will be employed. Model-fitting strategy will follow a sequential process of identifying factors in univariate models and parsing clinically relevant ones into multivariable model with consideration of both main effects and pairwise interaction terms of each covariate versus treatment group and each other. The final model will retain clinically relevant factors.

8.5.2. Analysis of secondary objectives

1) Gastrointestinal symptom diagnoses and moderate-severe lab abnormalities

   Analyses of this secondary objective will follow the plan described for the primary objective. The end-point will be defined as a composite outcome of specified gastrointestinal symptoms diagnoses of interest and/or moderate-severe laboratory abnormalities.

2) Changes in eGFR

   Changes in eGFR over follow-up and eGFR testing characteristics among patients censored due to an improvement or worsening of eGFR will be compared between 3TC daily dose groups using Wilcoxon Rank Sum test for continuous variables and Pearson Chi-Square test for categorical variables. Graphical representation of median change in eGFR over follow-up period will be generated for each of the dosing groups.

8.5.3. Sensitivity Analyses

1) No censoring based on eGFR

The proposed sensitivity analysis assesses the effect of allowing for fluctuations in eGFR during follow up period, on the outcomes. Patients will not be censored when their eGFR changes to a value below or above the target range (<30 ml/min/1.73m^2 and >49 ml/min/1.73m^2). Patients included in the analysis will all have a baseline eGFR between ≥ 30 ml/min/1.73m^2 and ≤ 49 ml/min/1.73m^2, but their eGFR will be allowed to fluctuate in and out of the target range during follow-up. The analyses conducted will be the same as analyses of the primary objective.

2) Composite outcome restricted to severe laboratory abnormalities

Analyses will be repeated using a composite outcome consisting of severe laboratory abnormalities only because laboratory values may have less misclassification than the diagnosis information.

3) Composite outcome consisting of both prevalent and incident specific diagnoses of interest and severe laboratory abnormalities

To test the impact of excluding prevalent diagnoses and laboratory abnormalities, analyses will be repeated including diagnoses and laboratory abnormalities of interest in the composite outcome, even if they are present at baseline.

4) Composite outcome consisting of the first specific diagnosis of interest or severe laboratory abnormality only

Analyses will be repeated in a population censored at the identification of the first event of interest. Therefore, multiple events per patients will not be allowed.

8.6. Study Size Considerations

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 2. 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 3. 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 4. 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

Power calculation

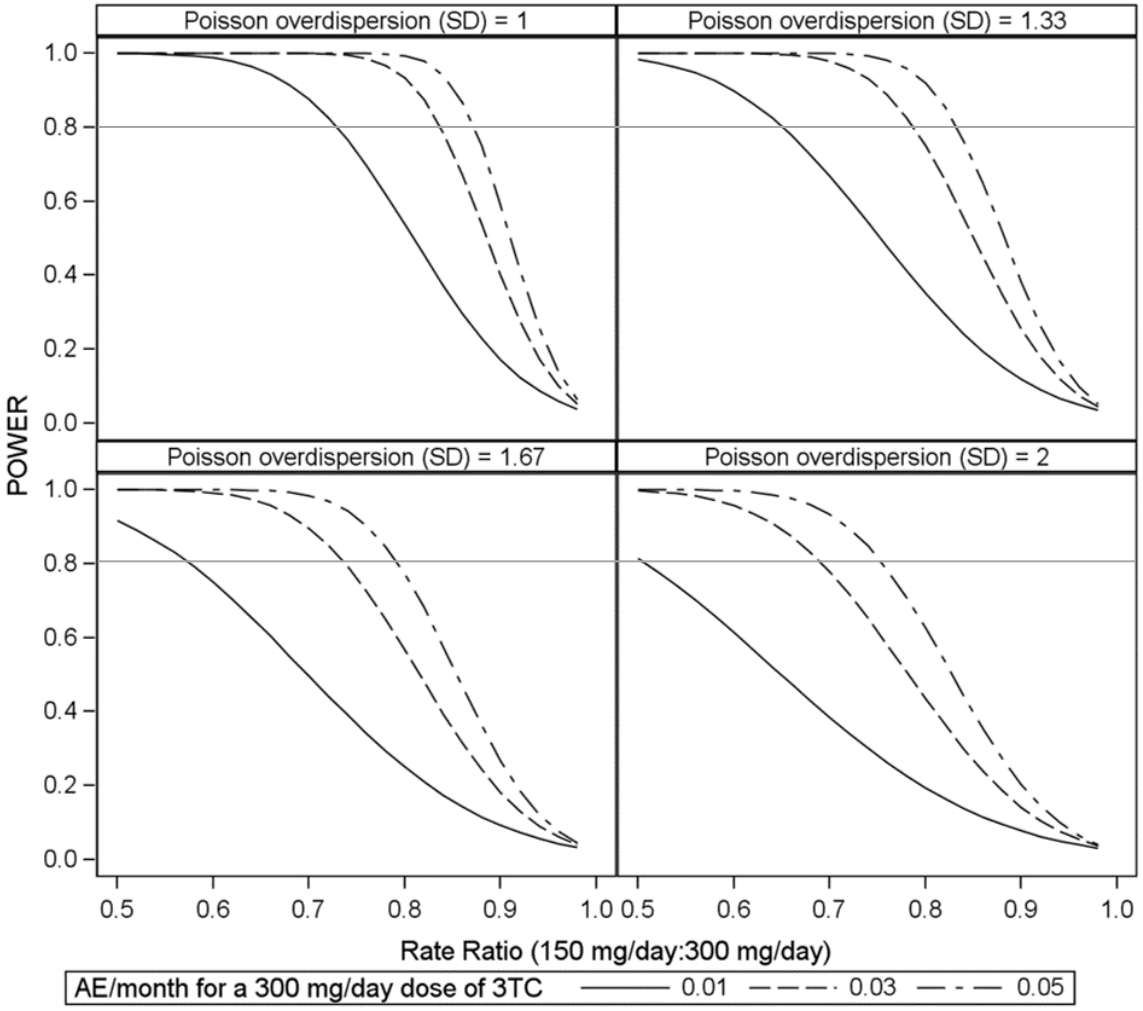
The power to detect a difference in rates of specific events of interest with 150 mg or 300 mg daily doses of 3TC has been computed under varying conditions, assuming a one-sided alternative hypothesis, and a sample size of 77 patients on 150 mg daily and 377 patients on 300 mg daily. A Wald test was 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 5. Power to detect a range of incidence rate ratio for a Poisson regression

Over-dispersion	3TC 150mg N	3TC 300mg N	3TC 150mg Incidence rate (events/month)	3TC 300mg 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 2. 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



8.7. Limitations

Like any observational study, this analysis could be biased by the presence of confounding. While we consulted with an HIV nephrologist to identify potential confounders which will be included in multivariate models, it is possible that residual confounding would remain. A careful assessment of label and risk management plans was conducted by the safety and clinical teams of the sponsor and a list of relevant outcomes is included in this analysis.

Although this study has adequate power to detect large effect sizes, it is limited by the small sample size to detect small effect sizes. The number of covariates that may be included in multivariable models based on the study size and expected incidence is also limited. Of note, the failure to detect statistically significant differences between groups does not imply the risks between groups are equivalent.

This study may also be potentially limited by an expected small number of severe events during follow-up. To alleviate this concern, a composite outcome was created, combining select clinically relevant diagnoses of interest and grade 3-4 laboratory abnormalities. Such a composite endpoint will likely increase the number of events, while only combining clinically relevant and severe events. The composite endpoint has been developed for this study and has not been used elsewhere. Baseline rates of adverse events from clinical trials were therefore used to inform power calculations with clinical judgement determining the magnitude of excess event rates to be ruled out.

eGFR is an estimation of the true GFR. As such, some level of misclassification of patients in the target range of renal impairment is likely. We selected the CKD-EPI equation because it is recommended by the 2016 European AIDS Clinical Society (EACS) Guidelines [19] and the Kidney Disease, Improving Global Outcomes (KDIGO) [20] and it performs well in HIV-infected populations. This eGFR equation is however limited by its reliance on serum creatinine. This can be problematic because certain antiretrovirals, including DTG, cobicistat, darunavir and rilpivirine are known to inhibit the tubular secretion of creatinine to different degrees, resulting in an artificially low eGFR which does not reflect true renal impairment [21]. While it is possible to calculate the eGFR using cystatin-C instead of creatinine, this option is not viable in a clinical cohort such as OPERA because cystatin-C is not routinely measured in clinical practice. Artificially low eGFRs caused by the inhibition of tubular secretion of creatinine will not result in confounding because renal disorders are not included in the composite outcome. However, this could affect selection into the study, which relies on eGFR levels for inclusion and censoring. The sensitivity analysis following patients even after they fall out of the target eGFR range has been designed to address this issue. In addition, this study will reflect clinical practice, as physicians must use the eGFR to decide whether a dose adjustment is necessary, despite knowing that some components of their patient's regimen can inhibit the tubular secretion of creatinine.

While OPERA represents a rich source of real-world clinical data, outcomes and covariates definitions are limited to diagnoses and symptoms that were recorded by the physician, as well as laboratory tests that were performed as part of routine clinical care. It is thus likely that symptoms of lower severity would be missing. In addition, the timing and frequency of clinical contact varies between patients and treating physicians.

8.8. Quality control and quality assurance

PPD has working practices & procedures governing the use of observational data, the development of analysis specifications and plans, the development of analytical programming and the analytical quality assurance (QA) process and the scientific review of reports as well as clinical advisory charters for the clinical review of output intended for public domain. Working practices for the development of analysis specifications include basic identifying information, background material, relevant definitions of key

study variables, population definitions, baseline definitions, specific requirements for dataset creation, and statistical requirements such as eligibility criteria, exposures, outcomes and model fitting. Working practices for programming include naming conventions, proper code documentation and commentary, content, appearance, efficiencies (i.e. use of macros), and organization of output, maintainability and generalizability. Working practices for programming QA include self-reviews of observational counts, missing data values, many-to-many merges, variable formatting, numeric-character & character-numeric conversions, uninitialized variables, unresolved macro references, report completeness and report-to-specification correspondence, and system errors and logs. The QA team review may include small sample spot-checking, coding log reviews, complete coding review, selected observations from intermediary dataset reviews, and/or independent programming to reproduce the results. Documentation of non-public domain reports includes market, scientific, statistical, and clinical review. Documentation of scientific protocols, reports and manuscripts intended for public domain follows two sequential steps: an internal-to-PPD epidemiological, statistical, and clinical review, followed by a clinical/epidemiological external advisory board review.

All analytical data, coding algorithms, QA documentation and report outputs will be retained per PPD standard practices.

9. PROTECTION OF HUMAN SUBJECTS

Clinical information is originally compiled into separate CHORUS databases for each clinic. This protected health information (PHI) is used in the creation of the CHORUS analytics and reporting used by each practice and its providers as part of Quality Improvement activities in an effort to improve care of patients. The data collection occurs via a secure and encrypted connection as part of PPD privacy and security policies and systems, which are routinely reviewed by a third-party privacy and security advisory organisation.

Subsequently, the clinical data in each CHORUS database is de-identified and aggregated into the OPERA® Database following the guidelines of the Health Insurance Portability and Accountability Act (HIPAA) and the Health Information Technology for Economic and Clinical Health Act (HITECH).

9.1. Ethical approval and subject consent

Business Associate Agreements (BAAs) in place between PPD and all medical practices govern, following the guidelines established in HIPAA and HITECH, the encryption, transportation, aggregation, de-identification and use of all clinical data in either the CHORUS reporting platform or the OPERA® Database. All medical practices are responsible for obtaining proper HIPAA consent for their patients. With BAAs in place and subsequent de-identification, a separate informed consent for each individual, non-interventional study is not required.

9.2. Subject confidentiality

All clinical data in CHORUS is PHI and managed as such according to HIPAA, HITECH and relevant state regulations. The CHORUS portal, as a Quality Improvement activity, is accessed securely by clinic staff to view PHI for only those patients seen at the practice. All clinical data is subsequently de-identified as per HIPAA and HITECH in OPERA® with all reports submitted at the aggregated population level in OPERA®. No personally identifiable information is available in the OPERA® Database. The OPERA® Epidemiology & Clinical Advisory Board provides clinical and methodological review & oversight.

10. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

There is no potential to collect serious and non-serious adverse events (AEs), pregnancy exposures, or incidents related to any ViiV Healthcare product during the conduct of this research, as the minimum criteria needed to report AEs, pregnancy exposures, and incidents are not present in the data source. Specifically, the data are insufficient to establish attribution between a potential safety event and an individual patient using a ViiV Healthcare product as the study design is to analyse the patient level information recorded in the OPERA database from electronic health records in an aggregate manner. Therefore, a study specific pharmacovigilance plan will not be developed.

11. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

11.1. Target Audience

Regulatory agencies, health care practitioners, health plan population based decision-makers.

11.2. Study reporting and publications

Final report to be submitted to regulatory agencies. Study results will be presented at a conference (CROI, IAS etc) and submitted to a peer reviewed journal.

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