CLINICAL STUDY PROTOCOL

Study Title:	Pharmacoepidemiology study to define the long-term safety profile of tenofovir disoproxil fumarate (Tenofovir DF, Viread®) and describe the management of Tenofovir DF-associated renal and bone toxicity in Chronic Hepatitis B (CHB)-infected adolescents aged 12 to <18 years in Europe
Sponsor:	Gilead Sciences International Ltd., Flowers Building, Granta Park Cambridge, CB21 6GT, United Kingdom
IND Number:	This is a non-IND study
EudraCT Number:	2014-004939-39
Clinical Trials.gov Identifier:	Not Available
Indication:	Chronic Hepatitis B
Protocol ID:	GS-EU-174-1403
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PROTOCOL SYNOPSIS

Gilead Sciences International Ltd.
Flowers Building, Granta Park
Cambridge, CB21 6GT, United Kingdom

Study Title:	Pharmacoepidemiology study to define the long-term safety profile of tenofovir disoproxil fumarate (Tenofovir DF, Viread®) and describe the management of Tenofovir DF-associated renal and bone toxicity in Chronic Hepatitis B (CHB)-infected adolescents aged 12 to <18 years in Europe
IND Number:	This is a non-IND study
EudraCT Number:	2014-004939-39
Clinical Trials.gov Identifier:	Not Available
Study Centers Planned:	Approximately 25 centers in Europe.
Objectives:	The primary objective of this study is as follows: • To characterize the long term (i.e., 96 weeks of follow up) bone safety profile of open-label Tenofovir DF treatment in CHB-infected adolescents. This includes prospectively evaluating and comparing the bone mineral density (BMD) change between CHB- infected adolescents 12 to < 18 years of age treated with Tenofovir DF in European treatment centers who are assigned to one of two schedules for renal and bone laboratory monitoring and BMD measurement. Primary study outcome will be the percent changes in BMD from Baseline through study Week 96. The secondary objectives of this study are as follows: • To document all serious adverse drug reactions (SADR) and all renal- and bone-related adverse events (AEs), including renal and bone laboratory abnormalities • To determine the time to diagnosis of renal and bone AEs and document the resulting patient management and outcome(s) • To assess the clinical management and outcomes of renal- and bone-related ≥ Grade 3 laboratory markers and clinical SAEs

	• To assess the efficacy and tolerability of Tenofovir DF in adolescents with CHB infection • To assess the use of oral vitamin D, calcium and phosphate supplementation and explore the association between supplement use and rates of bone and renal AEs • To describe the demographics and disease characteristics of adolescents with CHB infection treated with Tenofovir DF. • To describe reasons for discontinuation of Tenofovir DF.
Study Design:	This is an interventional study involving two assigned monitoring groups of chronic HBV infected adolescents who will receive treatment with open-label, market-authorized Tenofovir DF and followed prospectively to a primary endpoint at 96 weeks. Subjects will be assigned to one of two monitoring groups (Group 1 or 2); subjects in both groups will follow pre-set schedules for bone and bone biomarker monitoring. Group 1 will receive more frequent BMD, bone biomarker, and renal function monitoring compared to Group 2 over the 96 week period. Subjects will be assigned but not blinded to one of the two monitoring groups.
Number of Subjects Planned:	100 subjects to receive treatment with Tenofovir DF and assigned to one of two monitoring groups (50 in each group)
Target Population:	Adolescents aged 12 to <16 years at the time of enrollment who are diagnosed with CHB and are Tenofovir DF treatment naïve
Duration of Treatment:	Subjects will be treated for 96 weeks with Tenofovir DF treatment
Diagnosis and Main Eligibility Criteria:	At screening, CHB subjects aged 12 to <16 years of age (e.g., HBsAg-positive for at least 6 months), weighing ≥35kg will be eligible for the study. Subjects must be naïve to Tenofovir DF, but could have received interferon or any oral anti-HBV nucleoside/nucleotide therapy. Subjects experienced on interferon must have discontinued therapy for a minimum of six months; treatment-experienced subjects receiving oral anti-HBV nucleoside/nucleotide treatment at screening must continue their current treatment regimen until Tenofovir DF is initiated; subjects previously treated with an oral anti-HBV nucleoside/nucleotide should initiate Tenofovir DF without a treatment washout period to prevent ALT flare. Pregnant or breastfeeding females will not be eligible for the study.

Study Procedures/ Frequency:

Screening measures for Groups 1 and 2 will include determining a subject's eligibility into the study with the following criteria: current age, documentation of chronic HBV infection (e.g., positive laboratory results for HBsAg for at least 6 months prior to screening, a medical assessment affirming CHB status), ability to swallow solid tablets, likelihood of adherence to treatment regimen, obtaining written informed consent from subject's parent or legal guardian or obtaining informed assent from the subject.

Baseline measures for Groups 1 and 2 will include laboratory assessments for HBV DNA, HBV serology, HCV, HDV, and HIV polymerase chain reaction (PCR) testing, HBV genotyping, serum renal and bone chemistries, liver function, hematology, urinalysis. Dual Energy X-ray Absorptiometry (DEXA) imaging and a physical exam, including current concomitant medications (including dietary supplements) and any renal or bone AEs, will also be collected.

FREQUENCY OF STUDY PROCEDURES (Appendix 2):

Subjects enrolled to either Group 1 or 2 will have the following measures assessed:

- HBV DNA: every 12 weeks from Baseline to Week 96
- HBV serology: every 24 weeks from Baseline to Week 96
- Liver chemistry (i.e., alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total and direct bilirubin, total protein, albumin, gamma glutamyl transpeptide (GGT), lactate dehydrogenase (LDH), creatine kinase (CK): every 24 weeks from Baseline to Week 96
- Hematology (i.e., complete blood count with differential and platelets, PT/INR): every 24 weeks from Baseline to Week 96
- Physical Examination and Drug Dispensing: every 12 weeks from Baseline to Week 96

Subjects enrolled to Group 1 will have the following additional monitoring;

- DEXA: every 24 weeks from Baseline to Week 96 (5 scans)
- Serum bone chemistry (i.e., calcium, phosphorus, vitamin D levels (25-hydroxy and 1,25 dihydroxyvitamin), parathyroid hormone (PTH), osteocalcin, bone-specific alkaline phosphatase, N and C telopeptides): every 24 weeks from Baseline to Week 96
- Serum renal chemistry (i.e., glucose, creatinine, calculated creatinine clearance, magnesium, bicarbonate, chloride, potassium, sodium): at 4 weeks and 12 weeks from Baseline and every 12 weeks thereafter to Week 96, according to SmPC

	• Urinalysis (i.e., protein, glucose, creatinine, phosphate, bicarbonate, blood, calcium): at 4 weeks and 12 weeks from Baseline and every 12 weeks thereafter to Week 96 Subjects enrolled to Group 2 will have the following monitoring: • DEXA: every 48 weeks from Baseline to Week 96 (3 scans) • Serum bone chemistry (i.e., calcium, phosphorus, vitamin D levels (25-hydroxy and 1,25 dihydroxyvitamin), PTH, osteocalcin, bone-specific alkaline phosphatase, N and C telopeptides): every 48 weeks from Baseline to Week 96 • Serum renal chemistry and urinalysis according local standards of care
Product, Dose, and Mode of Administration:	300mg oral Tenofovir disoproxil fumarate (Tenofovir DF), which is equivalent to 245mg Tenofovir disoproxil, following enhanced patient monitoring protocol for bone and renal biomarkers
Reference Therapy, Dose, and Mode of Administration:	300mg oral Tenofovir DF following local standards of care for patient monitoring for renal biomarkers

Criteria for Evaluation:

Safety:	The primary safety endpoint is the indication of a cumulative incidence of a ≥4% decrease from Baseline BMD of the spine or whole body through Week 96. Secondary endpoints include bone and renal AEs. Bone AEs may include reports of bone pain or fractures. Renal AEs may include reductions in creatinine clearance, and clinical and laboratory evidence of Grade 3 or greater renal tubulopathy or toxicity (e.g. proximal renal tubulopathy, Fanconi syndrome). Bone and renal AEs, concomitant medications (including dietary supplements) and specified clinical laboratory tests will be collected each time subjects from Group 1 or Group 2 return for scheduled monitoring by the study site over a 96 week period.
Efficacy:	An efficacy analysis will be conducted after the last assigned subject reaches Week 96. The analysis will evaluate the difference in the proportion of subjects (Group 1 and 2 data will be pooled, as subjects have exposure to a single mode of therapy) achieving a composite endpoint of HBV DNA < 400 copies/mL and ALT normal at Week 96.

Pharmacokinetics:	Not applicable
Statistical Methods:	The primary safety analysis will be performed after the last subject reaches Week 96 on Tenofovir DF treatment among both monitoring groups. The primary analysis will evaluate quantitative reductions in spine or whole body BMD compared to baseline assessments through DEXA scanning. A key secondary study objective is to assess within-group and between-group differences in renal biomarker changes (e.g., creatinine clearance using the Schwartz formula, glucosuria, proteinuria) suggestive of renal toxicity. To evaluate the time to detection of renal or bone AEs among the assigned monitoring groups, estimations of incidence rates (events over person time on Tenofovir DF) and time to event analysis will be determined. The evaluation of other clinically relevant endpoints such as sustained virologic response, serologic status, and development of Tenofovir DF resistance will also be assessed as secondary study outcomes for each monitoring group and as a combined cohort. Approximately 100 subjects are projected to enroll by the end of the study (50 in each group).

This study will be conducted in accordance with the guidelines of Good Clinical Practice (GCP) including archiving of essential documents.

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

ADR	Adverse drug reaction
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine transferase
AST	aspartate transaminase
BMC	bone mineral content
BMD	bone mineral density
CRF	case report form
CHB	chronic hepatitis b
CDC	U.S. Centers for Disease Control and Prevention
CHIPS	Collaborative HIV Pediatrics Study
CK	creatine kinase
CRO	Contract research organization
CSR	clinical study report
DEXA	Dual energy x-ray absorptiometry
DMC	Data Monitoring Committee
DSPH	Drug Safety & Public Health
DSUR	Development Safety Update Report
EDD	Estimated date of Delivery
EMA	European Medicines Agency
EU	European Union
FAS	Full Analysis Set
FDA	(United States) Food and Drug Administration
GPP	Good Pharmacoepidemiology Practices (guidelines for)
GSI	Gilead Sciences, Inc.
GVP	Good Pharmacovigilance Practices (guidelines for)
HAART	highly active anti-retroviral therapy
HCC	hepatocellular carcinoma
HDPE	high density polyethylene
HIPPA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug (Application)
INR	International Normalized Ratio
InVS	French Institute for Public Health Surveillance
IRB	institutional review board
LDH	lactate dehydrogenase
OBR	Optimized background regimen
PAS	Post-Authorization Study

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

PASS	Post-Authorization Safety Study
PhRMA	Pharmaceutical Research and Manufacturers of America
PI	protease inhibitor
PRT	proximal renal tubulopathy
REMS	Risk Evaluation and Mitigation Strategy
SADR	serious adverse drug reaction
SAE	serious adverse event
SAP	statistical analysis plan
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOP	standard operating procedure
SSR	Special situation report
STI	sexually transmitted infection
SUSAR	Serious Unexpected Suspected Adverse Reaction
TDF	Tenofovir disoproxil fumarate
US, USA	United States, United States of America

EPIDEMIOLOGIC TERMS AND DEFINITIONS

Analytical dataset	The minimum set of data required to perform the statistical analyses leading to the results of the primary objective(s) of the study
Bias	Systemic error in the design, conduct or analysis of a study that results in a mistaken estimate
Cases	Group of individuals with the condition of interest
Cohort	Group of people characterized by a common experience (e.g., occurrence of a specified disease, exposure to a given medication)
Confounder	Extraneous factor that accounts for a difference in disease frequency between the exposure groups; associated factors serving as surrogates for these factors are also commonly called confounders
Confounding by indication	A patient characteristic that is related to the outcome of interest and which influences treatment choice (exposure)
Controls	Group of individuals without the condition of interest but are otherwise similar to cases, or unexposed to or not treated with the agent of interest
Date at which a study commences	Date of the start of data collection
Effect modifier	If an effect measure varies within categories or levels of a variable, that variable is described as an effect-measure modifier
End of data collection	The date from which the analytical dataset is completely available
Exposure	A variable whose effect is of interest and is being studied
External validity	Whether or not the results from the study can be generalized to other populations
Internal validity	Whether or not the study provides an unbiased estimate of what it claims to estimate
Odds	The ratio of the probability that an event will happen to the probability that it will not happen
Outcome	An event (such as disease occurrence or death) that is studied in relation to exposure
Prevalence	Proportion of persons with the exposure/outcome at a specific point in time
Rate	A measure of event occurrence, calculated by dividing the total number of events by the total amount of person-time within an exposure category
Relative Risk (RR)	A general term that can refer to the ratio of 2 risks or the ratio of 2 rates
Risk	The proportion of a fixed cohort in which an outcome occurs during a specified period of time
Start of data collection	Date from which information on the first study subject is first recorded in the study dataset

1. INTRODUCTION

1.1. Background

Chronic hepatitis B (CHB) infection continues to be an important worldwide cause of morbidity and mortality. Worldwide, an estimated 2 billion people have been infected with HBV, and more than 350 million have chronic long-term liver infections {26857}. Up to 60% of the world's population lives in a high HBV endemicity region, such as China or other regions in Asia. Even after acute infection with HBV, approximately 3% to 5% of adults and up to 95% of children fail to clear the infection and develop CHB{1635}.

The incidence of HBV infection and patterns of transmission vary greatly throughout the world. HBV incidence rates have dramatically decreased {6666}, attributable in part to improved socio economic and sanitary conditions, but most importantly due to mass immunization programs, which have been recommended by the WHO since 1991. In Europe, Italy set an early example by offering a free HBV immunization program in 1984 and a mandatory program for infants and 12-year-olds beginning in 1991. Between 1987 and 2011, HBV incidence reported to Italy's longitudinal surveillance program declined from 10.4 per 100,000 inhabitants to 0.8 per 100,000{10698}, {26852}. Seroepidemiology studies in Italy have demonstrated that 56.1% of children 10 years old and under, and 53.8% of those aged 11 to 20 show vaccination-induced immunity to HBV, but only 11.3% of those above age 40 have vaccination-induced immunity. In France, the incidence of HBV infection appears to have been declining from 21 per 100,000 in 1991 to 6 per 100,000 in 1996 {10789}. Today, the French Institute for Public Health Surveillance (InVS) reports acute HBV infection at an incidence of 1.1 per 100,000 inhabitants among those 0 to 19 years, and 3.6 per 100,000 among 20 to 39 year olds {26853}. Despite the marked reductions in population-based HBV susceptibility through vaccination, the burden of HBV morbidity and related mortality continue to present significant public health challenges.

Western and northern Europe are areas of low endemicity (< 2% prevalence), while some countries of Eastern Europe are areas of high endemicity (> 8% prevalence) {19056}. In areas with high endemicity, such as Asia and sub-Saharan Africa, CHB infections are usually the result of perinatal (vertical) or child-to-child (horizontal) transmission. The mode of transmission has clinical implications because 90% of children infected in the first year of life and 30% to 50% of children infected between ages 1 and 4 years develop CHB.

Despite the availability of HBV vaccine programs in many countries, new HBV infections are still common, including areas of low endemicity such as the United States (US) and Europe {26406}. In Europe, 13.34 million people are chronically infected with HBV, with an estimated 36,000 deaths from HBV related causes annually {24854}, {25143}. Among 28 reporting EU countries, over 17,000 cases of HBV were reported in 2011; with over two-thirds of cases being newly identified CHB {26854}, {26858}. Challenges with reporting acute and chronic HBV cases mean that calculations of burden are very likely underestimated. CHB infection is the primary source of HBV-related morbidity and mortality, with up to 25% of early deaths associated with HBV cirrhosis or liver cancer {26858}. Those with CHB infection have a 12 to 300 times greater risk of hepatocellular carcinoma (HCC) than non-carriers {26858}.

There is a paucity of aggregated incidence data on HBV in children in Europe, partly due to the difficulty in identifying cases, as affected children are often virtually asymptomatic while Hepatitis B surface antigen (HBsAg) or Hepatitis B e (core) antigen (HBeAg) positive. A seroprevalence study conducted in 2007 within the Netherlands demonstrated an overall prevalence of 0.5% (95% CI 0.2-1.1) among children 0 to 14 years, and among indigenous children, the prevalence is only 0.1% (95% CI 0-1.0). The overall prevalence of HBV in the Netherlands is estimated at 3.5% {26855}. In a recent cross-sectional study among a large sample of UK schoolchildren aged 7 to 11 years old, 0.26% (95% CI 0.14-0.44) had persistent acute, or resolved HBV infection {26851}. Unlike infection during adulthood, children often exhibit immune tolerance during their initial infection with HBV, with only a very small proportion of children clearing HBsAg and either exhibiting HBeAg loss or seroconversion {27079}

Following acute hepatitis B infection, up to 90% of children become chronic carriers of the virus. Hepatitis B virus infection in children is usually asymptomatic, with a low rate of disease progression during the first 2 decades of life. There are four characteristic immunologic phases to this disease. In the first phase, the immune tolerant phase, patients have high HBV DNA levels and have detectable HBsAg and HBeAg. The hallmark of this phase is a normal ALT. The second phase that follows, known as the immune active stage, is characterized by persistently elevated ALT levels, an indicator of liver damage, even though children may show no signs or symptoms of disease. The third phase, the inactive HBsAg carrier stage, is characterized by undetectable or low levels of HBV DNA and the presence of anti-HBe antibodies. This third stage can evolve into a fourth stage, reactivation, in which ALT levels are abnormal and HBV DNA levels are increased.

The management of CHB in children and adolescents is still evolving {17786}. Current consensus is that no treatment is indicated in the immune tolerant (phase 1) or the inactive carrier state (phase 3). For children and adolescents in stages 2 or 4; however, treatment may be warranted in order to suppress viral replication and to prevent the emergence of complications, such as cirrhosis, decompensated liver disease, and HCC. Among infants and young children infected with HBV, 15-25% will die prematurely from these conditions {2826}. Although histological abnormalities are usually less severe in children than in adults, HBV infected infants and children are at higher risk for the eventual development of cirrhosis and primary HCC. In HBeAg-positive patients, treatment can lead to HBeAg loss and, more rarely, anti-HBe seroconversion, with the goal of subsequent HBsAg loss and seroconversion to anti-HBs. Achieving durable viral suppression in CHB infection usually requires long-term therapy, preferably utilizing potent oral regimens that limit the development of resistance.

1.2. Medicinal Product Name:

300mg Tenofovir disoproxil fumarate, Tenofovir DF (Viread®), which is equivalent to 245mg Tenofovir disoproxil.

1.2.1. General Information

Tenofovir disoproxil fumarate (Tenofovir DF; 9-[(R)-2-[[bis[[(isopropoxycarbonyl) oxy]methoxy]phosphi-nyl] methoxy]propyl] adenine fumarate (1:1); GS4331-05) is an oral prodrug (bisPOC-PMPA) of tenofovir (PMPA), an acyclic nucleoside phosphonate (nucleotide) analogue of adenosine 5'-monophosphate. Tenofovir DF has activity against HBV and HIV, and is indicated for use in HBV infection and in combination with other antiretroviral agents in the treatment of HIV-1 infection in adults. Tenofovir DF is primarily excreted by the kidney by both filtration and an active tubular transport system with approximately 70 to 80% of the dose excreted unchanged in urine following intravenous administration of Tenofovir DF. (VIREAD Company Core Data Sheet (CCDS), May 2013). Tenofovir DF was approved for the treatment of CHB in adults within the EU in April 2008.

On November 27, 2012, Tenofovir DF was approved by the European Commission for the treatment of HIV in children 2 to less than 18 years of age, and for the treatment of chronic HBV in children 12 to less than 18 years of age.

Steady-state pharmacokinetics of Tenofovir DF were evaluated in 31 HIV-1 infected U.S. pediatric patients. Tenofovir DF exposure achieved in pediatric patients receiving oral daily doses of VIREAD 300 mg (tablet) or 8 mg/kg of body weight (powder) up to a maximum dose of 300 mg was similar to exposures achieved in adults receiving once-daily doses of 300 mg (GS-US-104-0352 Week 48 Clinical Study Report, GS-US-104-0321 Week 48 Clinical Study Report).

Table 1-1. Mean (± SD) Tenofovir Pharmacokinetic Parameters by Age Groups for Pediatric Patients

	300 mg Tablet	8 mg/kg Oral Powder
Dose and Formulation	**12 to < 18 Years (N = 8)**	**2 to < 12 Years (N = 23)**
C_{max} (µg/mL)	0.38 ± 0.13	0.24 ± 0.13
AUC_{tau} (µg•h/mL)	3.39 ± 1.22	2.59 ± 1.06

Tenofovir exposure in HBV infected pediatric patients (12 to < 18 years of age) receiving oral daily dose of VIREAD 300 mg tablet was similar to exposures achieved in adults receiving once-daily doses of 300 mg. (GS-US-174-0115 Week 72 Study Report)

For further information on Viread®, refer to the investigator's brochure for Viread®, 8th edition, dated 31 March 2014.

1.2.2. Preclinical Pharmacology and Toxicology

Not applicable

1.2.3. Studies in HIV-1 and HBV Infected Pediatric Subjects

1.2.3.1. Tenofovir DF Studies in HBV and HIV-1-Infected Pediatric and Adolescent Subjects

Study GS-US-174-0115 is an ongoing randomized, double-blind, double-dummy, multicenter study to evaluate the efficacy, safety, and tolerability of Tenofovir DF versus placebo in adolescents aged 12 to 17 years with CHB infection (hepatitis B early antigen $[HBeAg]^+$ or $HBeAg^-$; HBV DNA $\geq 10^5$ copies/ mL). Subjects had to be Tenofovir DF-naive, but could previously have received interferon or any other oral anti-HBV nucleoside/nucleotide therapy. Subjects were required to take a daily multivitamin that provided 100% of the recommended daily allowance of vitamin D.

The study consisted of two parts, an initial 72-week, randomized, double-blind period in which subjects (n = 106) were randomized 1:1 to receive TDF (n = 52; 300 mg once daily) or placebo (n = 54), following which subjects were eligible to receive open-label TDF for a further 120 weeks. This second part of the study is ongoing. DEXA scans of the spine and whole body were performed at baseline and at Weeks 24, 48, and 72, and will subsequently be performed at Week 96 and then annually until Week 192. Randomization was not performed until the baseline DEXA scan had been performed.

The primary safety endpoint was the cumulative incidence of at least a 6% decrease from baseline in BMD of the spine through Week 72. The cumulative incidence of at least a 6% decrease from baseline in BMD of the whole body through Week 72 was a secondary endpoint. Both of these proportions through Week 48 were also secondary endpoints, as were corresponding changes in lumbar spine and whole body Z-scores. Other safety endpoints included percent change from baseline in lumbar spine and whole body BMD.

Recent data from the GS-EU-174-0115 trial reported HBV DNA lower than 400 copies/mL in 88.5% of Tenofovir DF receiving patients after 72 weeks of treatment. At this same study milestone, no subjects met the primary safety endpoint of a 6% decrease in lumbar spine BMD. As expected for an adolescent population, both treatment arms experienced an overall increase in mean spine BMD. The mean (SD) change in lumbar spine BMD Z-score from baseline to Week 72 in the Tenofovir DF arm was -0.05 (0.310). The mean (SD) change in lumbar spine BMD Z score in the placebo arm was 0.07 (0.377). The mean change in whole body BMD Z score in the Tenofovir DF arm was -0.15 (0.379). The mean change in whole body BMD Z-score in the placebo arm was 0.06 (0.361). Using age and gender matched cohorts, the magnitude of the effect of Tenofovir DF on lumbar spine and whole body BMD also does not appear to be clinically relevant.

The mean (SD) change in creatinine from baseline to Week 72 was 0.1 (0.10) for the Tenofovir DF group and 0.1 (0.09) for the placebo group. No subject had a confirmed increase from the baseline serum creatinine concentration of at least 0.5 mg/dL, a confirmed creatinine clearance rate of < 50 mL/min, or a confirmed serum phosphorus concentration < 2 mg/dL. This study will continue to follow subjects to Week 192.

Two open-label, single-arm, single-center clinical studies have been performed to evaluate the safety and pharmacokinetics of Tenofovir DF in a small number of HIV-1-infected children. Studies 926 and 927 were 96 Week pharmacokinetic and safety studies, which enrolled treatment-experienced children with advanced HIV disease. All subjects received open-label Tenofovir DF as a component of a new antiretroviral regimen.

Study 926 was a Phase 1 study in which subjects received six days of Tenofovir DF monotherapy followed by the addition of individualized antiretroviral regimens. Monitoring for bone toxicity included measurement of lumbar spine bone mineral density by DEXA. Study 926 was conducted at the National Cancer Institute, National Institutes of Health. Eighteen subjects with a median (range) age of 11.9 years (6.2–16.2 years) were studied. Subjects received Tenofovir DF at a dose of ~ 175 mg/m^2 (administered in multiples of 75 mg tablets) once daily. Subjects had extensive treatment experience: median (range) duration of prior antiretroviral therapy was 9.7 years (4.8–13.5). At Week 48, median (range) decrease in $\log_{10}$ HIV RNA was –1.52 (–4.0 to 0.52). HIV RNA was < 50 copies/mL in 4 children (< 400 copies/mL in 6 children). Results from Study 926 demonstrated that Tenofovir DF-containing HAART regimens were effective and well tolerated in heavily treatment-experienced, HIV-infected children. However, five of 15 subjects evaluated at 48 weeks of treatment developed a > 6% decrease from baseline in bone mineral density. Of these subjects, two required discontinuation of Tenofovir DF, but decreases in BMD had partially or completed resolved by week 96. One additional subject developed a > 6% decrease from baseline in bone mineral density by Week 96. None of the six subjects experienced a bone fracture {8548},{9505}.

Study 927 was designed to assess safety and single-dose and steady-state pharmacokinetics (PK) of Tenofovir DF in HIV-1 infected, treatment-experienced children. Seven subjects were enrolled, including three children who were naive to Tenofovir DF and four patients who were Tenofovir DF experienced. Subjects received Tenofovir DF once daily at a dose of approximately 5 mg/kg (administered in multiples of 75 mg tablets). In the three children who were naive to Tenofovir DF, single dose PK studies were assessed over 48 hours after the first dose. In the four Tenofovir DF-experienced children, steady-state PK studies were assessed over 10 hours on Day 7, when Tenofovir DF was administered with an optimized background antiretroviral regimen.

The primary efficacy endpoint was virologic response, measured as a change from baseline in $\log_{10}$ HIV-1 RNA. Limited data available from these patients showed that, overall, treatment with Tenofovir DF resulted in a decline in mean plasma HIV-1 RNA of –0.95 $\log_{10}$ copies/mL from baseline to Week 72, and a decline in median plasma HIV-1 RNA of –0.39 $\log_{10}$ copies/mL from baseline at Week 108. These overall declines were seen despite high prior exposure and virological failure/intolerance to multiple antiretrovirals in all patients. Steady state PK parameters of Tenofovir DF in children and adolescents treated with Tenofovir DF-containing regimens were similar to those seen in HIV-infected adults treated with 300 mg once daily. Tenofovir DF was safe and well tolerated when given for a mean duration of 100 weeks in combination with other antiretrovirals. A renal tubular disorder and increase in urinary beta-2 microglobulin were the only events to occur that were considered possibly/probably related to Tenofovir DF. These events, in a subject who had a history of probable tubulopathy

while receiving Tenofovir DF before the study, resolved after withdrawal of Tenofovir DF. The limited available bone mineral density data in this study do not suggest a decline in BMD with increased exposure to Tenofovir DF. No bone fractures occurred during the study.

In a third study, 28 HIV-infected pediatric subjects (ages 5–17.9 years) receiving HAART therapy consisting of lamivudine, stavudine and a protease inhibitor (PI) with stable undetectable viral loads were randomized to switch the PI to efavirenz and stavudine to tenofovir at baseline (Group 1) or at Week 24 (Group 2). At 96 weeks, virological suppression and unchanged CD4 counts were maintained in all subjects {11526}. Tenofovir DF therapy was well tolerated. Through 96 weeks, no child experienced a Grade 1 or higher increase in serum creatinine or phosphorus, and mean serum creatinine, phosphorus and bicarbonate values were unchanged {11445}. The authors concluded that no evidence of impaired glomerular filtration or tubular renal function was observed in HIV-infected children treated with Tenofovir DF for 96 weeks. The authors also evaluated bone mineral content (lumbar spine and whole-body bone mineral content (BMC) and BMD via DEXA scan in 16 subjects in this study through 12 months of Tenofovir DF treatment {10417}. DEXA scans were obtained 12 months prior to therapy switch, at baseline, and 12 months after switch to Tenofovir DF. The authors calculated expected changes in bone measurements from data obtained from 166 healthy children. The BMC and BMD increments observed before and after switching to Tenofovir DF did not differ significantly from those calculated in healthy controls. Although the sample size is small, results from this study suggest that 12 months of Tenofovir DF treatment does not impair bone mineral accrual in HIV-infected children.

Most recently, a clinical trial of Tenofovir DF in pediatric and/or adolescent HIV-1-infected subjects is ongoing. Study GS-US-104-352 is evaluating 100 HIV-infected children and adolescents (2 to < 16 years of age) who are virologically suppressed (HIV-1 RNA < 400 copies/mL) on their current antiretroviral regimen containing either stavudine or zidovudine. Subjects are randomized 1:1 to continue on the same stavudine- or zidovudine-containing regimen, or to replace stavudine or zidovudine with Tenofovir DF while continuing their other background antiretroviral agents. The study will assess the efficacy, safety (including BMD) and tolerability of switching to Tenofovir DF, compared to continuing stavudine or zidovudine therapy. The study duration is 48 weeks. Enrollment is ongoing, with a total of 97 subjects enrolled to date.

1.3. Information about Comparator Product:

Not applicable.

1.4. Rationale for This Study

Tenofovir DF-associated bone toxicities have not been well understood in children. One theory is that Tenofovir DF may stimulate bone reabsorption which can be detected with increases in bone markers and calcium excretion. Among children and adolescents, skeletal growth increases and these same indicators may be expected to be present in children without Tenofovir DF exposure. Bone events or abnormalities, such as fractures, related to both proximal renal tubulopathy (PRT) and loss of BMD have been documented in both control and

Tenofovir DF-receiving arms in adult HIV trials. Among HIV-1 positive adolescents (GS-US-104-0321), the Tenofovir DF-receiving arm reported osteopenia in 6.7% of subjects (3/45) and 4.8% (2/42) in the placebo group, and among HBV infected adolescents, there were no reported bone events meeting the safety criteria of ≥6% BMD loss (GS-US-174-0115). In pediatric clinical trials of HIV-1 infected children (2-11 years), no bone events associated with BMD loss were reported up through 144 weeks of follow up time (GS-US-104-0352).

In a limited study of six pediatric subjects taking Tenofovir DF, two children developed BMD decreases of greater than 6% over the course of treatment. Contrary to these findings was a cross over trial from Italy where no notable decreases in BMD were detected when pediatric patients taking d4T and protease inhibitor HIV treatment regimens switched to Tenofovir DF/3TC/EFV. The Collaborative HIV Paediatric study (CHIPS) based in Ireland and the UK, reported no BMD decreases in their longitudinal cohort {15423}. Among HIV infected adults taking Tenofovir DF, the observed pattern is that BMD loss is sustained early in the therapeutic course [TIME] and stabilizes with no further notable BMD loss; this pattern has not been established in the limited registered studies on either HIV or HBV infected children.

Renal toxicity associated with Tenofovir DF has been previously identified in small subsets of study participants across randomized controlled clinical trials. In an NIH systematic review of Tenofovir DF's renal safety profile, the mean difference in reduction of creatinine clearance among subjects on Tenofovir DF was 3.92 mL/min (2.13-5.70 mL/min 95% confidence interval) compared to controls, and no clear associations between treatment and proteinuria, hypophosphatemia, nor fractures. A modest increase in risk of acute renal injury (0.7%, 0.2-1.2% 95% confidence interval) was noted. The clinical impact of these findings is unknown {16177}. Among registered studies the detection on renal toxicities has been very low, ranging between 1 to 2%. Detailed information and sequelae as a result of serious renal toxicities, such as Fanconi syndrome, come from case series or single reports. A recent NIH report documented 7 of 51 (14%) adult patients treated for CHB with Tenofovir DF (and/or adefovir) who developed renal tubular dysfunction after 22 to 94 months on therapy. Subjects with renal tubular dysfunction had an average age of 58, and 6 of the 7 were male. All affected subjects had hypophosphatemia, with others having one or more indications of hypouricemia, increased serum creatinine, proteinuria, or glycosuria {20768}.

The available data to characterize the incidence of renal toxicities in children is lacking, and collected primarily within registration studies of CHB and Tenofovir DF. As Tenofovir DF also has the indication for treatment of pediatric HIV, the drug's safety profile has some overlay to both the HIV and the HBV-infected populations. Among pediatric subjects completing 144 weeks of treatment in clinical trial GS-US-104-0352, 4 of 89 (4.5%) patients discontinued Tenofovir DF treatment for HIV-1 as a result of new onset adverse reactions suggestive of PRT (e.g., glycosuria, hypophosphatemia).

Data from Collaborative HIV Pediatrics Study (CHIPS) in Ireland and the UK reported that among the 159 HIV-1 positive children taking Tenofovir DF (since 2001) of a cohort of 1253 participants, 6 (3.8%) developed renal toxicities including PRT, Fanconi syndrome, and acute renal failure. Five of the six were also taking concurrent NRTI and PI combinations, and

were receiving a higher concentration of Tenofovir DF (two were greater than 120% of the recommended Tenofovir DF dosing regimen), likely due to advanced disease {13569}. A study derived from the same CHIPS cohort evaluated renal toxicities and of those 456 participants on antiretroviral therapy, 20 developed hypophosphatemia (4.4%), 10 of whom were on Tenofovir DF (2.2%). Incidence of hypophosphatemia associated with Tenofovir DF was 4.3/100 person-years and among controls (not exposed to Tenofovir DF) it was 0.9/100 person-years. Recommendations from these studies include longitudinal monitoring of renal function {15423}.

Independent and sponsored studies in recent years have shown that Tenofovir DF use among adults with diagnoses of HBV or HIV carries a modest risk of BMD decreases or acute renal injury compared to placebo or other non-Tenofovir DF containing treatments. Reported decreases in bone mineral density among patients on Tenofovir DF therapy compared to age-and height adjusted bone density scores are rare across all age strata seen in clinical trials and observational studies (GS-US-104-0321, GS-US-104-0352, GS-US-174-0115). Among HIV- and HBV-infected adolescents and children, understanding the longitudinal risk of changes in bone mineral density is critical for managing Tenofovir DF-treatment plans weighed against any possible alterations to bone development in children.

Adverse event data from Gilead-sponsored trials have shown increases in creatinine among 0.2% of subjects, and renal failure in 0.06%. In the Viread® Expanded Access Program, 0.04% of subjects were diagnosed with new onset PRT (including Fanconi syndrome). In pediatric HIV study GS-US-104-0352, 0.05% of patients between 2 and 12 years of age developed PRT; there were no reports of PRT in the parallel trial among adolescents with HIV. Some mediating factors identified in past studies have included advancing age, pre-existing renal insufficiencies, use of known nephrotoxic medications, and low weight. Renal injuries while on Tenofovir DF treatment have been reversible after discontinuation of Tenofovir DF with complete resolution within 4 months for the majority of subjects. The follow up period and sample size of study subjects reporting renal injury have been limited and it is prudent to evaluate these potential risks over a longer timeframe and under real world conditions.

Among adolescent patients with CHB, available interim 72 Week clinical trial data indicate that 5.8% (95% confidence band of 1.2-15.9%) of subjects experienced a ≥4% decrease in BMD; by comparison 3.7% (95% CI 0.4-12.5%) of placebo controls had a BMD loss of ≥4%. In this same trial, no renal adverse drug reactions were detected by Week 72. Detecting renal or bone AEs in CHB adolescents taking Tenofovir DF are infrequent events, and the pool of Tenofovir DF-naïve subjects within the EU suitable for enrollment is limited. With a fixed sample size of 100 (50 in each monitoring group) one would expect to detect 4-5 renal or bone adverse drug reactions across both real-world groups (both receive equivalent treatment) over 96 weeks.

1.5. Compliance

This study will be conducted in compliance with this protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements.

2. OBJECTIVES

The primary objective of this study is as follows:

- To characterize the long term (i.e., 96 weeks of follow up) bone safety profile of open-label Tenofovir DF treatment in CHB-infected adolescents. This includes prospectively evaluating and comparing the bone mineral density (BMD) change between CHB- infected adolescents 12 to < 18 years of age treated with Tenofovir DF in European treatment centers who are assigned to one of two schedules for renal and bone laboratory monitoring and BMD measurement. Primary study endpoint will be the percent changes in BMD from Baseline through study Week 96.

The secondary objectives of this study are as follows:

- To document all serious adverse drug reactions (SADR) and all renal- and bone-related adverse events (AEs), including renal and bone laboratory abnormalities

- To determine the time to diagnosis of renal and bone AEs and document the resulting patient management and outcome(s)

- To assess the clinical management and outcomes of renal- and bone-related ≥ Grade 3 laboratory markers and clinical SAEs.

- To assess the efficacy and tolerability of Tenofovir DF in adolescents with CHB infection

- To assess the use of oral vitamin D, calcium and phosphate supplementation and explore the association between supplement use and rates of bone and renal AEs

- To describe the demographics and disease characteristics of adolescents with CHB infection treated with Tenofovir DF.

3. STUDY DESIGN

3.1. Endpoints

The primary endpoint of this study is:

- The identification of bone AEs occurring in subjects taking Tenofovir DF between Baseline and Week 96 of treatment including the identification of ≥4% reduction in BMD within subjects and between monitoring groups from Baseline.

The secondary endpoints of this study are:

- Documentation of renal or bone AEs and outcomes among subjects receiving Tenofovir DF, including time to medication withdrawal or discontinuation
- Subjects' cumulative exposure time on Tenofovir DF at the time renal or bone AEs are detected up to and including Week 96 or to study discontinuation (subjects without renal or bone AEs will contribute cumulative exposure time on Tenofovir DF from Baseline to Week 96 or to study discontinuation). The cumulative time to renal or bone AEs will be expressed as incident rates.
- Documentation of the medical management and classification of detected renal or bone laboratory abnormalities
- Analysis of subjects' virological and immunological status from Baseline to Week 96 under real world treatment practices
- Report provider recommendations and subject adherence to dietary supplements (e.g., Vitamin D, calcium)
- Documentation of subject demographics, new medical issues, medications, biometrics, treatment adherence
- Reasons for discontinuation of Tenofovir DF

3.2. Study Design

The study will be a prospective cohort comprised of HBV infected adolescents who initiate Tenofovir DF therapy in clinics across Europe. The subjects will be assigned to one of two monitoring groups using a validated computer-generated tool for randomization. Subjects will be assigned to Group 1 or 2 upon enrollment into the study but prior to Baseline laboratory and DEXA imaging assessments.

DEXA scans are non-invasive tests to measure bone mineral density as well as skeletal maturity and bone mass. DEXA scans emit an average effective dose of 0.001 milli Sieverts (mSv) in adults {22238}. These scans emit very minor radiation (i.e., in comparison, one mammogram has an effective dose of 0.4 mSv), and with such low doses of radiation do not pose concerning health hazards. Data are based on adult studies and may have a higher relative effective dose, particularly in very young children. Repeated DEXA scans should be conducted using the same model of equipment, if possible, and scans should be read in a standardized manner.

Group 1 will receive Tenofovir DF for the treatment of CHB infection, followed over 96 weeks using an enhanced monitoring protocol which includes more frequent laboratory bone biomarker testing and lumbar spine and whole-body DEXA scans than specified for Group 2. With the exception of an enhanced monitoring protocol for bone and renal outcomes, subjects will be managed according to local standards of care.

Group 2 will be the comparator group of subjects receiving Tenofovir DF for the treatment of CHB infection and with the exception of pre-specified bone monitoring, managed according to local standards of care. Group 2 will receive bone biomarker testing, lumbar spine and whole-body DEXA at Baseline, Weeks 48 and 96.

Both groups will be monitored for 96 weeks on Tenofovir DF during clinic visits for response to treatment, AEs, adherence to supplementary vitamins and mineral intake assessments.

Both groups will be required to have DEXA (whole body and spine) scans for BMD at Baseline (week 0) and prior to receipt of Tenofovir DF, and at the end of the study period (Week 96). Group 2 will be a comparator cohort to Group 1 in evaluating the hypothesis of whether enhanced monitoring for renal and BMD changes and AEs provides a net benefit in preventing renal- or bone-related adverse outcomes to adolescents receiving Tenofovir DF therapy for HBV.

For this study design and limited age groupings, matching at enrollment on other factors such as demographics, would likely have a marginal benefit on the strength of the results; matching may occur (e.g., age, gender, ethnicity, duration of Tenofovir DF exposure) during the analysis phase as specified in the statistical analysis plan (SAP).

The strengths of the study include a focus on long term outcomes related to renal and bone adverse event reporting in a vulnerable population. Use of two real world cohorts will permit the evaluation of whether a schedule of more frequent clinical assessments of adolescent patients on Tenofovir DF benefit from early detection of potential AEs. Previous studies across multiple HIV and HBV infected patient populations have demonstrated a modest risk for both bone and renal AEs related to Tenofovir DF-containing medications. Understanding whether changes in monitoring frequency for serum and urine biomarkers and use of more frequent DEXA may provide early detection to subtle changes in bone or renal dynamics and be beneficial in enhancing patient outcomes.

The feasibility of study design implementation has been assessed using clinical trials data from GS-US-174-0115. Demographic, adverse event reporting, clinical and laboratory markers, and DEXA screen results at baseline and at regular intervals from clinical trials show a range of 90-100% reporting adherence for key variables, when applicable.

The primary safety analysis will be conducted after the last assigned subject reaches Week 96 of Tenofovir DF treatment.

Subjects with the following characteristics will be eligible for enrollment: 12 to <16 years of age with chronic HBeAg-positive or HBeAg-negative HBV infection (e.g., HBsAg-positive for at least 6 months)

Subjects must be naïve to Tenofovir DF, but could have received prior treatment with other anti-HBV nucleoside/nucleotide therapy or interferon (e.g., treatment failure). Previous treatment with interferon should have ended at least six months prior to the Screening Visit.

Screening measures for Monitoring Groups 1 and 2 will include determining a subject's eligibility into the study with the following criteria: current age, documentation of chronic HBV infection, prior Tenofovir DF exposure, weight, laboratory assessments, ability to swallow solid tablets, subject's ability to adhere to treatment regimen, obtaining written informed consent from subject's parent or legal guardian or obtain informed assent from the subject

Baseline measures for Monitoring Groups 1 and 2 will include laboratory assessments for HBV DNA, HBV serology, HCV, HDV, and HIV polymerase chain reaction (PCR) testing, HBV genotyping, serum renal and bone chemistries, liver function, hematology, urinalysis. DEXA imaging, a physical exam (including pregnancy testing for females), current concomitant medications and any reported prior bone and renal AEs, will also be collected.

FREQUENCY OF STUDY PROCEDURES (Appendix 2):

Subjects enrolled to either Group 1 or 2 will have the following measures assessed:

- HBV DNA: every 12 weeks
- HBV serology: every 24 weeks
- Liver chemistry (i.e., alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total and direct bilirubin, total protein, albumin, gamma glutamyl transpeptide (GGT), lactate dehydrogenase (LDH), creatine kinase (CK) every 24 weeks
- Hematology (i.e., complete blood count with differential and platelets, PT/INR): every 24 weeks
- Physical Examination and Drug Dispensing: every 12 weeks
- Documentation of bone or renal AEs since the subject's last visit
- Documentation of medical management in the event of bone or renal AEs

Subjects enrolled to Group 1 will have the following additional monitoring;

- DEXA: every 24 weeks from Baseline to Week 96 (5 scans)
- Serum bone chemistry (i.e., calcium, phosphorus, vitamin D levels (25-hydroxy and 1, 25 dihydroxyvitamin), parathyroid hormone (PTH), osteocalcin, bone-specific alkaline phosphatase, N and C telopeptides): every 24 weeks
- Serum renal chemistry (i.e., glucose, creatinine, calculated creatinine clearance, magnesium, bicarbonate, chloride, potassium, sodium): at 4 weeks and 12 weeks from Baseline and every 12 weeks thereafter, according to SmPC
- Urinalysis (i.e., protein, glucose, creatinine, phosphate, bicarbonate, blood, calcium): at 4 weeks and 12 weeks from Baseline and every 12 weeks thereafter

Subjects enrolled to Group 2 will have the following monitoring:

- DEXA: every 48 weeks from Baseline to Week 96 (3 scans)
- Serum bone chemistry (i.e., calcium, phosphorus, vitamin D levels (25-hydroxy and 1,25 dihydroxyvitamin), PTH, osteocalcin, bone-specific alkaline phosphatase, N and C telopeptides): every 48 weeks
- Serum renal chemistry and urinalysis according to local standards of care

Subjects who permanently discontinue Tenofovir DF will be asked to return for an end of treatment visit within 72 hours of the last dose of Tenofovir DF. Subjects who permanently discontinue Tenofovir DF will be followed for 24 weeks off treatment or up to initiation of local standard of care treatment, whichever occurs first.

3.3. Study Treatments

The proposed study is an open-label trial of 300mg oral Tenofovir DF which is equivalent to 245mg Tenofovir disoproxil, taken once daily. Tenofovir DF is packaged in white, high-density polyethylene (HDPE) bottles with a white child resistant cap. There are 30 tablets per bottle. Each bottle also contains silica gel as a desiccant to protect the product from humidity and fiber packing to protect the product during handling and shipping.

During Weeks 0 to 96, subjects will be provided with sufficient supplies for 12 weeks of dosing. At a minimum, each bottle will be labeled with a lot number, the protocol number, administration instructions, storage instructions, expiration date, Sponsor name and address. Additional information will be included according to the requirements of the protocol and local law.

Tenofovir DF will be supplied by Gilead as per its current commercial tablet formulation for the EU (300mg Tenofovir DF) for patients greater than 35 kg. There will be no difference in

medication formulation or frequency as per Tenofovir DF SmPC among subjects assigned to either Group 1 or Group 2.

3.4. Duration of Treatment

Subjects will be treated with Tenofovir DF therapy for 96 weeks (each subject will receive a total of 96 weeks of Tenofovir DF treatment). Tenofovir DF is currently approved for use in patients across all specified study countries with participating study clinics aged 12 years of age and up.

3.5. Discontinuation Criteria

Treatment medication may be discontinued in the following instances:

- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree
- Unacceptable toxicity, as defined in the toxicity management section of the protocol, or toxicity that, in the judgment of the investigator, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest
- Therapeutic failure
- Subject or legal guardian requests to discontinue for any reason
- Subject non-compliance with taking Tenofovir DF or sustained inability to adhere to Tenofovir DF treatment plan
- Pregnancy during the study
- Discontinuation of the study at the request of Gilead Sciences, regulatory agency or an IEC

3.6. Source Data

Source data will include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender)
- Documentation that subject meets eligibility criteria, i.e., history, physical examination and confirmation of diagnosis to support inclusion and exclusion criteria
- Participation in study (including study number)
- Study discussed and date of informed consent
- Dates of all visits

- Documentation that protocol specific procedures were performed
- Results of monitored parameters, as required by the protocol
- Start and end date of Tenofovir DF (preferably with drug dispensing information)
- Record of all AEs and other safety parameters (start and end date, and preferably including causality and intensity);
- Concomitant medications (including start and end dates, dose if relevant; dose changes)
- Date of study completion and reason for early discontinuation, if applicable

All clinical study documents must be retained by the investigator until at least 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if required by applicable regulatory requirements, by local regulations, or by an agreement with Gilead Sciences. The investigator must notify Gilead Sciences before destroying any clinical study records.

Should the investigator wish to assign the study records to another party or move them to another location, Gilead Sciences must be notified in advance. If the investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Gilead Sciences to store these in sealed containers outside of the site so that they can be returned sealed to the investigator in case of a regulatory audit. When source documents are required for the continued care of the subject, appropriate copies should be made for storage outside of the site.

4. SUBJECT POPULATION

4.1. Number of Subjects and Subject Selection

100 subjects to receive treatment with Tenofovir DF and assigned to one of two monitoring groups (50 in each group). The subjects will be assigned to one of two monitoring groups using a validated computer-generated tool for randomization. Subjects will be assigned to Group 1 or 2 upon enrollment into the study but prior to Baseline laboratory and DEXA imaging assessments.

4.2. Inclusion Criteria

Subjects must meet *all* of the following inclusion criteria to be eligible for participation in this study.

1) 12 to <16 years of age

2) Documented chronic HBV infection (e.g. positive serum HBsAg≥ 6 months)

3) Weight ≥ 35 kg

4) Able to swallow oral tablets

5) Negative serum β-HCG pregnancy test (for females of childbearing potential, see Appendix 5)

6) Estimated glomerular filtration rate (creatinine clearance) ≥ 80 mL/min/1.73m^2

   Estimated creatinine clearance using Schwartz Formula
   (mL/min/1.73m^2) = k × L/Scr

   [(k is a proportionality constant: for adolescent females ≥ 12 years old, k = 0.55, and for adolescent males ≥ 12 years, k = 0.70); L is height in centimeters (cm); and Scr is serum creatinine (mg/dL)]

7) Parent or legal guardian of potential study subjects able to provide written informed consent prior to any screening evaluations and willing to comply with study requirements or subject able to provide written assent as determined by IEC/local requirements and at the Investigator's discretion.

4.3. Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria are not to be enrolled in this study.

1) Prior Tenofovir DF therapy; subjects may have received prior interferon or oral anti-HBV nucleoside/nucleotide therapy (subjects experienced on interferon must have discontinued

therapy for a minimum of six months; treatment-experienced subjects receiving oral anti-HBV nucleoside/nucleotide treatment at screening should continue their current treatment regimen until Tenofovir DF is initiated (i.e., to prevent ALT flare))

2) Sexually-active males or females of reproductive potential who are not willing to use an effective method of contraception during the study. (see Appendix 5 for further details).

3) Females who are pregnant or breastfeeding, or females who wish to become pregnant during the course of the study.

4) Known hypersensitivity to Tenofovir DF, the metabolites or formulation excipients

5) Any condition (including alcohol or substance abuse) or prior therapy that, in the opinion of the Investigator, would make the subject unsuitable for the study or unable to comply with treatment requirements

5. ADMINISTRATION OF TENOFOVIR DF TREATMENT

5.1. Randomization, Blinding and Treatment Codes

After screening and once eligibility has been confirmed, subjects will be assigned a subject number and monitoring arm (i.e., Group 1 or Group 2). A centralized procedure will be used, to assign subjects to the respective monitoring arm. For the 96 weeks of the study, Tenofovir DF will be dispensed to the subject in numbered bottles from supplies stored at the study site. Tenofovir DF monitoring groups will be randomly allocated, but the allocation to one or the other group will not be blinded. Subjects will be informed of the two monitoring groups at the time of study screening and informed of their assignment to one of the two selected CHB management monitoring groups after consent is obtained at the subject's screening appointment.

All Baseline tests and procedures must be completed prior to the administration of the first dose of Tenofovir DF. Initiation of treatment with Tenofovir DF should take place within 24 hours of the Baseline Visit.

For duration of the study, Tenofovir DF 300mg will be supplied by Gilead.

5.1.1. Procedures for Breaking Treatment Codes:

Not applicable

5.2. Description and Handling of Tenofovir DF

5.2.1. Formulation

Tenofovir DF tablets are light blue, almond-shaped, film-coated tablets containing 300mg of Tenofovir disoproxil fumarate (equivalent to 245mg Tenofovir disoproxil). Each tablet contains the following inactive ingredients: microcrystalline cellulose, lactose monohydrate, pregelatinized starch, croscarmellose sodium, and magnesium stearate. The Tenofovir DF tablets are film-coated to mask taste. Each tablet is film-coated with a mixture of lactose monohydrate, hypromellose (hydroxypropyl methylcellulose), glycerol triacetate, titanium dioxide, and indigo carmine aluminum lake.

5.2.2. Packaging and Labeling

Tenofovir DF is packaged in white, high-density polyethylene (HDPE) bottles with a white child resistant cap. There are 30 tablets per bottle. Each bottle also contains silica gel as a dessicant to protect the product from humidity and fiber packing to protect the product during handling and shipping.

During Weeks 0 to 96, subjects will be provided with sufficient supplies for 12 weeks of dosing. At a minimum, each bottle will be labeled with a lot number, the protocol number, administration instructions, storage instructions, expiration date, Sponsor name and address. Additional information will be included according to the requirements of the protocol and local law.

5.2.3. Storage and Handling

Tenofovir DF tablets should be stored at 25°C (77°F); excursions are permitted to 15°-30°C (59°–86°F).

To ensure product stability, Tenofovir DF should not be dispensed in a container other than the one supplied.

5.3. Dosage and Administration of Tenofovir DF

Subjects will be assigned to receive one of the following treatments in an unblinded fashion:

Group 1: open-label Tenofovir DF 300mg PO once daily, enhanced monitoring

Group 2: open-label Tenofovir DF 300mg PO once daily, Tenofovir DF, local standards of care for monitoring

Subjects will be instructed to take one tablet by mouth daily. Tenofovir DF should be taken with food.

After 96 weeks of protocol-guided monitoring, each subject will switch to medical management for CHB as per local standards of care.

Active study subjects will be dispensed Tenofovir DF at baseline and through the end of the study (until each subject reaches Week 96). Subjects will be instructed to return empty containers as well as unused study medication in the original container at each study visit. The investigator will be responsible for maintaining accurate records for Tenofovir DF and Tenofovir DF bottles dispensed and returned. The inventory must be available for inspection by the study monitor. Study medication supplies, including partially used or empty bottles, must be accounted for and the dispensing logs must be verified by the study monitor prior to destruction or return.

5.4. Prior and Concomitant Medications

5.4.1. Prior to Study Entry

Refer to Exclusion Criteria in Section 4.3.

5.4.2. During the Study

Use of the following medications is prohibited while subjects are taking Tenofovir DF, and subjects should be followed closely by their providers:

- Antiviral agents with anti-HBV activity, including lamivudine, emtricitabine, entecavir, adefovir, telbivudine, clevudine, or others
- Antiviral agents associated with potential renal impairment (e.g., cidofovir, acyclovir, valacyclovir, ganciclovir, valgancicyclovir)

- Interferon and pegylated interferon
- Nephrotoxic agents (e.g., aminoglycoside antibiotics, cisplatin, foscarnet, IV amphotericin B, IV pentamidine, cyclosporine, tacrolimus, chronic daily non-steroidal anti-inflammatory drugs)
- Hepatotoxic agents (e.g., anabolic steroids, isoniazid, itraconazole, ketoconazole, lovastatin, rifabutin, rifampin, simvastatin)
- Competitors of renal excretion, such as probenecid
- Systemic chemotherapeutic agents
- Interleukin-2 [IL-2] and other immunomodulating agents
- Systemic corticosteroids
- Investigational agents, except with written approval of the Sponsor

All concomitant medications, including vitamin supplements, herbal remedies and hormonal contraception, must be recorded in the appropriate section of the Case Report Forms.

5.5. Devices

Not applicable

5.6. Accountability for Tenofovir DF

The investigator is responsible for ensuring adequate accountability of all used and unused Tenofovir DF. This includes acknowledgement of receipt of each shipment of Tenofovir DF (quantity and condition). All used and unused Tenofovir DF dispensed to subjects must be returned to the site.

Tenofovir DF accountability records will be provided to each study site to:

- Record the date received and quantity of Tenofovir DF
- Record the date, subject number, subject initials, the Tenofovir DF lot numbers dispensed
- Record the date, quantity of used and unused Tenofovir DF returned, along with the initials of the person recording the information.

5.6.1. Tenofovir DF Return or Disposal

At study initiation, the monitor will evaluate the site's standard operating procedure for drug disposal/destruction in order to ensure that it complies with Gilead requirements. Drug may be returned or destroyed on an ongoing basis during the study if appropriate. At the end of the study, following final drug inventory reconciliation by the monitor, the study site will dispose of and/or destroy all unused drug supplies, including empty containers, according these procedures.

All drug supplies and associated documentation will be periodically reviewed and verified by the study monitor over the course of the study.

6. STUDY PROCEDURES

The study procedures to be conducted for each subject enrolled in the study are presented in tabular form in Appendix 2 and described in the text that follows.

The investigator must document any deviation from protocol procedures and notify the study sponsor or contract research organization (CRO).

6.1. Subject Enrollment and Treatment Assignment

The study population will consist of HBV-infected adolescents who are between 12 to <16 years of age at the commencement of the study and receiving Tenofovir DF for the treatment of CHB during study treatment. The study population will also be enrolled through one of the approximately 25 participating European clinics across several countries (eg., Bulgaria, Poland, Romania, Italy, France, Spain, and the United Kingdom) projected to commence in June 2015.

Eligibility criteria will include a confirmed diagnosis of HBV infection in subjects who are Tenofovir DF treatment naïve. At screening, adolescent subjects (12 to<16 years of age) with documented chronic hepatitis B (e.g HBsAg-positive for ≥6 months), weighing ≥ 35 kg will be eligible for the study.

Subjects must be naïve to Tenofovir DF, but could have received interferon or any oral anti-HBV nucleosides/nucleotide therapy. Subjects experienced on oral anti-HBV nucleoside/nucleotide therapy must have discontinued therapy prior to screening. Subjects must have discontinued interferon at least six months prior to screening.

Data will be collected from subjects prospectively from initial enrollment into the study (baseline) and up to 96 weeks, study completion, loss to follow-up or death, whichever occurs first. Subjects who discontinue Tenofovir DF as a consequence of an AE will be monitored for up to 6 months from the date of the observed AE.

The data will contain medical records, demographics, diagnoses, procedures, medications, and laboratory tests (including DEXA).

6.2. Pre-treatment Assessments

6.2.1. Screening Visit

Subjects will be screened within 30 days before assignment to determine eligibility for participation in the study. The following will be performed and documented at screening:

1) Obtain written informed consent

2) Obtain medical history

3) Complete physical examination including, vital signs, body weight, and height

4) Screen for pregnancy (serum screen) and contraceptive use

5) Conduct preliminary liver chemistry tests (ALT, ALP, AST, total and direct bilirubin, total protein, albumin, GGT, LDH, CK), serum renal chemistry (glucose, creatinine, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium) and urinalysis (glucose, protein, creatinine, phosphate, bicarbonate, blood, calcium) (to be averaged with Baseline renal chemistry values), fasting required

6) HBV serology (HBeAg and HBsAg; anti-HBe and anti-HBs (if Ag negative))

Subjects meeting all of the inclusion criteria and none of the exclusion criteria will return to the clinic within 30 days after screening for commencement into the study.

From the time of obtaining informed consent through the first administration of Tenofovir DF, record all renal and bone AEs and any SAEs (not limited to bone or renal) on the adverse events case report form (CRF/eCRF). All other untoward medical occurrences observed during the screening period, including exacerbation or changes in medical history are to be captured on the medical history CRF/eCRF. See Section 7 Adverse Events and Toxicity Management for additional details.

6.3. Baseline Visit

The Baseline Visit should occur within 30 (± 3) days of the initial Screening Visit. All baseline tests and procedures should be completed prior to the receipt of the first dose of Tenofovir DF. Subjects will be dispensed Tenofovir DF medication at their Baseline Visit. Tenofovir DF should be initiated within 24 hours of the Baseline Visit.

6.3.1. Assignment

Once eligibility has been confirmed, subjects will be assigned a subject number and monitoring group.

6.3.2. Baseline Assessments

The following assessments and procedures will be performed and recorded on CRFs for Groups 1 and 2 at the Baseline Visit and prior to the subject's first dose of Tenofovir DF:

- Changes in medical status since screening
- Physical exam including body weight and height
- Blood samples for:
  - HBV DNA and HBV genotyping (A-H)
  - HBV serology (HBeAg and HBsAg; anti-HBe and anti-HBs (if Ag negative))

  - HCV, HDV, HIV testing
  - Hematology (CBC with differential and platelets,PT/INR)
  - Serum renal chemistry (glucose, creatinine, creatinine clearance (calculated) , magnesium, bicarbonate, chloride, potassium, sodium), fasting required
  - Liver chemistry tests (ALT, ALP, AST, total and direct bilirubin, total protein, albumin, GGT, LDH, CK), fasting required
  - Serum bone chemistry (calcium, phosphorus, 1,25 hydroxy vitamin D, PTH, osteocalcin, bone alkaline phosphatase, N and C telopeptides), fasting required

- Urinalysis (glucose, protein, creatinine, phosphate, bicarbonate, blood, calcuim), fasting required
- DEXA scan of spine and whole body (DEXA may be performed between Screening and Baseline Visits, but must be performed no later than the Baseline Visit), and prior to receipt of Tenofovir DF.
- Review of bone and renal AEs and any associated medical management
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)
- Tenofovir DF dispensing and instructions on appropriate dosing and administration
- Screening for pregnancy (urine screen)

6.4. Treatment Assessments

Unless fasting is required (i.e., serum renal chemistry and urinalysis, serum bone chemistry, liver chemistry) for the study visit, subjects should take their dose of Tenofovir DF prior to the visit with food.

6.4.1. Group 1: Week 4 Assessments

The following evaluations will be performed at Week 4 unless otherwise specified. Study visits are to be completed ± 7 days of the protocol-specified visit date, based on the Baseline Visit. Week 4 assessments are outlined in the following section (Section 6.4.4). Group 2 subjects will follow local standard of care for renal monitoring and AE collection.

The following assessments and procedures will be performed and recorded on CRFs:

- Changes in medical status or symptoms from prior visit

- Body weight and height
- Blood samples for:
  - Serum renal chemistry (creatinine, glucose, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium), fasting required
- Urinalysis (protein, glucose, creatinine, phosphate, bicarbonate, blood, calcium) , fasting required
- Assessment of medication adherence
- Review of bone and renal AEs and any associated medical management
- Tenofovir DF dispensing and instructions on appropriate dosing and administration
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)

6.4.2. Group 1: Week 12 and 36 Assessments

Study Visit for Weeks 12 and 36 should be completed ± 7 days of the protocol-specified visit date, based on the Baseline Visit. Group 2 subjects will follow local standard of care for renal monitoring and AE collection. The following assessments and procedures will be performed and recorded on CRFs:

- Complete physical examination including, vital signs, body weight and height
- Group 1: Urinalysis (protein, glucose, creatinine, phosphate, bicarbonate, blood, calcium), fasting required
- Blood samples for:
  - Group 1: Serum renal chemistry (creatinine, glucose, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium), fasting required
  - Plasma HBV DNA (PCR method)
- Review of bone and renal AEs and any associated medical management
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)
- Assessment of medication adherence
- Tenofovir DF dispensing and instructions on appropriate dosing and administration

- Screening for pregnancy (urine screen)

Subjects who discontinue the study prior to the Week 12 or 36 Visit will complete all Week 12 or 36 assessments and procedures at an Early Drug Discontinuation Visit, to be completed within 72 hours of last dose of Tenofovir DF. Subjects who have received at least one dose of Tenofovir DF and permanently discontinue treatment will be followed for 24 weeks off treatment or up to initiation of an alternative treatment regimen, whichever occurs first.

6.4.3. Groups 1 and 2: Week 24 and 72 Assessments

Study Visit for Weeks 24 and 72 should be completed ± 7 days of the protocol-specified visit date, based on the Baseline Visit. Group 2 subjects will follow local standard of care for renal monitoring. The following assessments and procedures will be performed and recorded on CRFs:

- Complete physical exam including vital signs, body weight and height
- Blood samples for:
  - Plasma HBV DNA (for PCR)
  - HBV serology (HBeAg and HBsAg; anti-HBe and anti-HBs (if HBsAg negative))
  - Hematology (CBC with differential and platelets, PT/INR)
  - Group 1: Serum renal chemistry (glucose, creatinine, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium), fasting required
  - Liver chemistry (ALT, ALP, AST, total and direct bilirubin, total protein, albumin, GGT, LDH, CK), fasting required
  - Group 1 Only: Serum bone chemistry (calcium, phosphorus, 1,25 hydroxy vitamin D, PTH, osteocalcin, bone alkaline phosphatase, N and C telopeptides), fasting required
- Group 1 Only: DEXA (+/- 14 days of the Week 24 or Week 72 visit) scan of spine and whole body
- Group 1: Urinalysis (glucose, protein, creatinine, phosphate, bicarbonate, blood, calcium), fasting required
- Review of bone and renal AEs and any associated medical management
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)
- Assessment of medication adherence

- Tenofovir DF dispensing and instructions on appropriate dosing and administration
- Screening for pregnancy (urine screen)

Subjects who discontinue the study prior to the Week 24 or 72 Visit will complete all Week 24 or 72 assessments and procedures at an Early Drug Discontinuation Visit, to be completed within 72 hours of last dose of Tenofovir DF. Subjects who have received at least one dose of Tenofovir DF and permanently discontinue treatment will be followed for 24 weeks off treatment or up to initiation of an alternative treatment regimen, whichever occurs first.

6.4.4. Groups 1 and 2: Week 48 Assessments

Study Visit for Weeks 48 should be completed ± 7 days of the protocol-specified visit date, based on the Baseline Visit. Group 2 subjects will follow local standard of care for renal monitoring. The following assessments and procedures will be performed and recorded on CRFs:

- Complete physical exam including vital signs, body weight and height
- Blood samples for:
  - Plasma HBV DNA (for PCR)
  - HBV serology (HBeAg and HBsAg; anti-HBe and anti-HBs (if HBsAg negative))
  - Hematology (CBC with differential and platelets, PT/INR)
  - Group 1: Serum renal chemistry (glucose, creatinine, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium), fasting required
  - Liver chemistry (ALT, ALP, AST, total and direct bilirubin, total protein, albumin, GGT,LDH, CK), fasting required
  - Groups 1 and 2: Serum bone chemistry (calcium, phosphorus, 1,25 hydroxy vitamin D, PTH, osteocalcin, bone alkaline phosphatase, N and C telopeptides), fasting required
- Group 1: Urinalysis (glucose, protein, creatinine, phosphate, bicarbonate, blood, calcuim), fasting required
- Groups 1 and 2: DEXA scan (+/- 14 days of the Week 48 visit) of spine and whole body
- Review of bone and renal AEs and any associated medical management
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)

- Assessment of medication adherence
- Tenofovir DF dispensing and instructions on appropriate dosing and administration
- Screening for pregnancy (urine screen)

Subjects who discontinue the study prior to the Week 48 Visit will complete all Week 48 assessments and procedures at an Early Drug Discontinuation Visit, to be completed within 72 hours of last dose of Tenofovir DF. Subjects who have received at least one dose of Tenofovir DF and permanently discontinue treatment will be followed for 24 weeks off treatment or up to initiation of an alternative treatment regimen, whichever occurs first.

6.4.5. Groups 1 and 2: Week 60 and 84 Assessments

Study Visit for Weeks 60 and 84 should be completed ± 7 days of the protocol-specified visit date, based on the Baseline Visit. Group 2 subjects will follow local standard of care for renal monitoring. The following assessments and procedures will be performed and recorded on CRFs:

- Complete physical exam including vital signs, body weight and height
- Blood samples for:
  - Plasma HBV DNA (for PCR)
  - Group 1: Serum renal chemistry (glucose, creatinine, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium), fasting required
- Group 1: Urinalysis (glucose, protein, creatinine, phosphate, bicarbonate, blood, calcium), fasting required
- Review of bone and renal AEs and any associated medical management
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)
- Assessment of medication adherence
- Tenofovir DF dispensing and instructions on appropriate dosing and administration
- Screening for pregnancy (urine screen)

Subjects who discontinue the study prior to the Week 60 or 84 Visit will complete all Week 60 or 84 assessments and procedures at an Early Drug Discontinuation Visit, to be completed within 72 hours of last dose of Tenofovir DF. Subjects who have received at least one dose of

Tenofovir DF and permanently discontinue treatment will be followed for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first.

6.4.6. Groups 1 and 2: Week 96 Assessments/End of Treatment Visit

The Study Visit for Week 96/End of Treatment should be completed ± 7 days of the protocol-specified visit date, based on the Baseline Visit. The following assessments and procedures will be performed and recorded on CRFs:

- Complete physical exam including vital signs, body weight and height
- Blood samples for:
  - Plasma HBV DNA (for PCR)
  - HBV serology (HBeAg and HBsAg; anti-HBe and anti-HBs (if Ag negative))
  - Hematology (CBC with differential and platelets, total and direct bilirubin, total protein, albumin, GGT, PT/INR)
  - Serum renal chemistry (glucose, creatinine, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium), fasting required
  - Liver chemistry (ALT, ALP, AST, total and direct bilirubin, total protein, albumin, GGT, LDH, CK), fasting required
  - Serum bone chemistry (calcium, phosphorus, 1,25 hydroxy vitamin D, PTH, osteocalcin, bone alkaline phosphatase, N and C telopeptides), fasting required
- Urinalysis (glucose, protein, creatinine, phosphate, bicarbonate, blood, calcium), fasting required
- Groups 1 and 2: DEXA scan (+/- 14 days of the Week 96 visit) of spine and whole body
- Review of bone and renal AEs and any associated medical management
- Concomitant medications (including dietary supplements such as calcium, vitamin D, phosphorus)
- Assessment of medication adherence
- Tenofovir DF accountability
- Screening for pregnancy (urine screen)

Subjects who discontinue the study prior to the Week 96 Visit will complete all Week 96 assessments and procedures at an Early Drug Discontinuation Visit, to be completed within 72 hours of last dose of Tenofovir DF. Subsequent off-study therapy, if any, is at the discretion of the subject/physician and will not be provided by Gilead Sciences. Subjects who have received at least one dose of Tenofovir DF and permanently discontinue Tenofovir DF treatment will be followed for 24 weeks off treatment or up to initiation of standard of care treatment, whichever occurs first.

The study treatment will end after the each subject reaches Week 96.

6.5. Assessments for Premature Discontinuation from Study

If a subject discontinues Tenofovir DF (for example, as a result of an AE), every attempt should be made to keep the subject in the study and continue to perform the required study-related follow-up and procedures (see Section 6.6, Criteria for Discontinuation of Study Treatment). If this is not possible or acceptable to the subject or investigator, the subject may be withdrawn from the study.

6.6. Criteria for Discontinuation of Study Treatment

Study medication (Tenofovir DF) may be discontinued in the following instances:

- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree
- Unacceptable toxicity, as defined in the toxicity management section of this protocol, or toxicity that, in the judgment of the investigator, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest
- Therapeutic failure
- Subject request to discontinue for any reason
- Subject non-compliance with taking Tenofovir DF or sustained inability to adhere to Tenofovir DF treatment plan
- Pregnancy during the study; refer to Appendix 5
- Discontinuation of the study at the request of Gilead, a regulatory agency or independent ethics committee (IEC)

6.7. End of Study

The end of study is defined as the completion of Tenofovir DF therapy and close-out monitoring by the last enrolled subject (e.g., last enrolled subject completes 96 Weeks of treatment and monitoring).

7. ADVERSE EVENTS AND TOXICITY MANAGEMENT

7.1. Definitions of Adverse Events, Adverse Reactions, and Serious Adverse Events

7.1.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study subject administered a medicinal product, which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. AEs may also include pre- or post-treatment complications that occur as a result of protocol specified procedures, lack of efficacy, overdose, drug abuse/misuse reports, or occupational exposure. Preexisting events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs.

An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an adverse event and must be reported.
- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the screening visit that do not worsen
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions)
- Overdose without clinical sequelae, unless the subject is hospitalized (see Section 7.6.1)
- Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented on the medical history CRF.
- Uncomplicated pregnancy
- An induced elective abortion to terminate a pregnancy without medical reason

7.1.2. Serious Adverse Events

A **serious adverse event** (SAE) is defined as an event that, at any dose, results in the following:

- Death
- Life-threatening (Note: The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)

- In-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- A medically important event or reaction: such events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes constituting SAEs. Medical and scientific judgment must be exercised to determine whether such an event is a reportable under expedited reporting rules.

Examples of medically important events include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; and development of drug dependency or drug abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product will be considered a medically important event and subject to expedited reporting requirements.

The purposes of this study, in addition to the above criteria, the following must be reported as an SAE:

7.1.3. Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Adverse Events

Laboratory or imaging abnormalities without clinical significance are usually not recorded as AEs or SAEs. However, laboratory abnormalities (eg, renal or bone lab values, hematology, liver chemistry, urinalysis) or imaging abnormalities (eg, DEXA) that require medical or surgical intervention or lead to Tenofovir DF interruption, modification, or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (eg, electrocardiogram, x-rays, vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE or SAE as described in Sections 7.1.1 and 7.1.2. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (eg, anemia), not the laboratory result (ie, decreased hemoglobin).

For specific information on handling of clinical laboratory abnormalities in this study, please refer to Appendix 4.

7.2. Assessment of Adverse Events and Serious Adverse Events

The investigator or qualified sub investigator is responsible for assessing AEs and SAEs for causality and severity, and for final review and confirmation of accuracy of event information and assessments.

7.2.1. Assessment of Causality for Tenofovir DF Treatment and Procedures

The investigator or qualified sub investigator is responsible for assessing the relationship to Tenofovir DF therapy using clinical judgment and the following considerations:

- **No**: Evidence exists that the adverse event has an etiology other than Tenofovir DF. For SAEs, an alternative causality must be provided (eg, pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).
- **Yes**: There is reasonable possibility that the event may have been caused by the medicinal product (i.e., Tenofovir DF).

The site investigator or sub-investigator may further classify renal or bone AEs as an ADR or SADR, if an association of the clinical event to Tenofovir DF and the degree of AE seriousness can be ascertained. All SAEs (i.e., not limited to renal or bone events) will be reported.

It should be emphasized that ineffective treatment should not be considered as causally related in the context of adverse event reporting.

The relationship to study procedures (e.g., invasive procedures such as venipuncture or biopsy) should be assessed using the following considerations:

- **No:** Evidence exists that the adverse event has an etiology other than the study procedure.
- **Yes:** The adverse event occurred as a result of protocol procedures, (eg., venipuncture)

7.2.2. Assessment of Severity

Severity should be recorded and graded according to the GSI Grading Scale for Severity of Adverse Events and Laboratory Abnormalities (Appendix 4). For AEs associated with laboratory abnormalities, the event should be graded on the basis of the clinical severity in the context of the underlying conditions; this may or may not be in agreement with the grading of the laboratory abnormality.

7.3. Investigator Requirements and Instructions for Reporting Adverse Events and Serious Adverse Events to Gilead

Requirements for collection prior to Tenofovir DF initiation:

After informed consent, but prior to initiation of Tenofovir DF, the following types of events should be reported on the case report form (CRF/eCRF): all SAEs and AEs related to protocol-mandated procedures.

Adverse Events

Following initiation of Tenofovir DF, collect all bone and renal AEs, regardless of cause or relationship, until 30 days after last administration of Tenofovir DF must be reported to the CRF/eCRF database as instructed.

All bone and renal AEs should be followed up until resolution or until the adverse event is stable, if possible. Gilead Sciences may request that certain AEs be followed beyond the protocol defined follow up period.

Serious Adverse Events

All bone and renal SAEs, regardless of cause or relationship, that occurs after the subject first consents to participate in the study (i.e., signing the informed consent) and throughout the duration of the study, including the protocol-required post treatment follow-up period, must be reported to the CRF/eCRF database and Gilead Drug Safety and Public Health (DSPH) as instructed. This also includes any bone and renal SAEs resulting from protocol-associated procedures performed after informed consent is signed.

Any SAEs and deaths that occur after the post treatment follow-up visit but within 30-days of the last dose of Tenofovir DF, regardless of causality, should also be reported.

Investigators are not obligated to actively seek bone and renal SAEs after the protocol defined follow up period, however, if the investigator learns of any SAEs that occur after study participation has concluded and the event is deemed relevant to the use of Tenofovir DF, he/she should promptly document and report the event to Gilead DSPH.

- All bone and renal AEs and (S)ADRs and all SAEs (not limited to bone and renal events) will be recorded in the CRF/eCRF database within the timelines outlined in the CRF/eCRF completion guideline.

- At the time of study start, SAEs will be reported using a paper serious adverse event reporting form. During the study conduct, sites may transition to an electronic SAE (eSAE) system. Gilead will notify sites in writing and provide training and account information prior to implementing an eSAE system.

<u>Serious Adverse Event Paper Reporting Process</u>

- All pre-specified SAEs will be recorded on the serious adverse event report form and submitted by faxing or emailing the report form within 24 hours of the investigator's knowledge of the event to the attention of Gilead DSPH or to the designated CRO.

CRO (TBD)	Contacts (TBD)	
Gilead Sciences DSPH Representative:	Fax:	+1 650 522 5477
	E-mail:	safety_fc@gilead.com

<u>Electronic Serious Adverse Event (eSAE) Reporting Process</u>

- Site personnel record all SAE data in the eCRF database and from there transmit the SAE information to Gilead DSPH within 24 hours of the investigator's knowledge of the event. Detailed instructions can be found in the eCRF completion guidelines.

- If for any reason it is not possible to record the SAE information electronically, i.e, the eCRF database is not functioning, record the SAE on the paper serious adverse event reporting form and submit within 24 hours as described above.

- As soon as it is possible to do so, any SAE reported via paper must be transcribed into the eCRF Database according to instructions in the eCRF completion guidelines.

- If an SAE has been reported via a paper form because the eCRF database has been locked, no further action is necessary.

- For fatal or life-threatening events, copies of hospital case reports, autopsy reports, and other documents are also to be submitted by e-mail or fax when requested and applicable. Transmission of such documents should occur without personal subject identification, maintaining the traceability of a document to the subject identifiers.

- Additional information may be requested to ensure the timely completion of accurate safety reports.

- Any medications necessary for treatment of the SAE must be recorded onto the concomitant medication section of the subject's CRF/eCRF and the event description section of the SAE form.

7.4. Gilead Reporting Requirements

Depending on relevant local legislation or regulations, including the EU Clinical Trials Directive (2001/20/EC) and relevant updates, and other country-specific legislation or regulations, Gilead may be required to expedite to worldwide regulatory agencies reports of SAEs, SADRs, or suspected unexpected serious adverse reactions (SUSARs). In accordance with the EU Clinical Trials Directive (2001/20/EC), Gilead or a specified designee will notify worldwide regulatory agencies and the relevant IEC in concerned Member States of applicable SUSARs as outlined in current regulations.

Assessment of expectedness for SAEs will be determined by Gilead using reference safety information specified in the investigator's brochure or relevant local label as applicable.

All investigators will receive a safety letter notifying them of relevant SUSAR reports associated with any Tenofovir DF treatment. The investigator should notify the IEC of SUSAR reports as soon as is practical, where this is required by local regulatory agencies, and in accordance with the local institutional policy.

7.5. Toxicity Management

All clinical toxicities and/or abnormal laboratory findings should be investigated for etiology and graded according to the uniform guidelines detailed in Appendix 5. The Gilead Sciences Medical Monitor is available for consultation on all medical and toxicity-related issues and may be contacted as shown below:

Gilead Sciences Medical Monitor	Name:	Benedetta Massetto, MD
	Telephone:	PPD
	Mobile:	PPD
	Fax:	PPD
	Email:	PPD

- All clinical and clinically significant laboratory toxicities will be managed according to uniform guidelines detailed in Appendix 5.
- Grade 3 and 4 clinically significant laboratory abnormalities should be confirmed by repeat testing within 3 calendar days of receipt of results and before drug discontinuation, unless such a delay is not consistent with good medical practice.
- Clinical events and clinically significant laboratory abnormalities will be graded according to the GSI Grading Scale for Severity of Adverse Events and Laboratory Abnormalities (Appendix 4).
- When restarting Tenofovir DF following resolution of the adverse event, the drug should be restarted at full dose or modified dose that is dependent upon discussion with the Gilead Sciences Medical Monitor.
- Any recurrence of the Tenofovir DF-related Grade 3 or 4 clinical or clinically significant laboratory adverse event following dose interruption mandates permanent discontinuation of drug.
- Any questions regarding toxicity management should be directed to the Gilead Sciences Medical Monitor.

7.5.1. Grades 1 and 2 Laboratory Abnormality or Clinical Event

- Continue Tenofovir DF at the discretion of the investigator.

7.5.2. Grade 3 Laboratory Abnormality or Clinical Event

- For Grade 3 clinically significant laboratory abnormality or clinical event, Tenofovir DF may be continued if the event is considered to be unrelated to drug.
- For a Grade 3 clinical event, or clinically significant laboratory abnormality confirmed by repeat testing, that is considered to be related to Tenofovir DF, drug should be withheld until the toxicity returns to ≤ Grade 2.

- If a laboratory abnormality recurs to ≥ Grade 3 following restarting Tenofovir DF and is considered related to Tenofovir DF, then Tenofovir DF should be permanently discontinued and the subject managed according to local practice. Recurrence of laboratory abnormalities considered unrelated to Tenofovir DF may not require permanent discontinuation.

7.5.3. Grade 4 Laboratory Abnormality or Clinical Event

For a Grade 4 clinical event or clinically significant Grade 4 laboratory abnormality confirmed by repeat testing that is considered related to Tenofovir DF, Tenofovir DF should be permanently discontinued and the subject managed according to local practice. The subject should be followed as clinically indicated until the laboratory abnormality returns to baseline or is otherwise explained, whichever occurs first. A clinically significant Grade 4 laboratory abnormality that is not confirmed by repeat testing should be managed according to the algorithm for the new toxicity grade.

Tenofovir DF may be continued without dose interruption for a clinically non-significant Grade 4 laboratory abnormality (e.g., Grade 4 CPK after strenuous exercise, or triglyceride elevation that is non-fasting or that can be medically managed) or a clinical event considered unrelated to Tenofovir DF.

7.5.4. Management of Elevated Serum Creatinine and Decreased Creatinine Clearance

- Glomerular filtration rate (estimated creatinine clearance) will be calculated at each visit using the Schwartz Formula for subjects ages 12–17 as follows:

  Schwartz Formula (mL/min/1.73 m^2) = k × L/Scr

  [(k is a proportionality constant, for adolescent females ≥ 12 years old is 0.55; and for adolescent males ≥ 12 years old is 0.70); L is height in centimeters (cm); and Scr is serum creatinine (mg/dL)]

- If creatinine clearance decreases to < 50 mL/min/1.73 m^2 (confirmed) at any time during the study, Tenofovir DF should be permanently discontinued, followed by 24 weeks of monthly monitoring in treatment-free follow up if alternative HBV therapy is not initiated.

- Subjects with estimated creatinine clearance < 70 mL/min/1.73 m^2 and serum creatinine increased ≥ 0.5 mg/dL above baseline should have the serum creatinine and creatinine clearance confirmed by repeating testing within three calendar days of receipt of results and before Tenofovir DF discontinuation, unless such a delay is not consistent with good medical practice.

- Subjects with confirmed estimated creatinine clearance < 70 mL/min/1.73 m^2 and serum creatinine increased ≥ 0.5 mg/dL above baseline should have Tenofovir DF discontinued, and the subject should be followed weekly for two weeks. After two weeks, if the estimated creatinine clearance is ≥ 70 mL/min/1.73/m^2, Tenofovir DF may be resumed, with monitoring as described below. If the estimated clearance remains < 70 mL/min/1.73 m^2, Tenofovir DF should be permanently discontinued, followed by 24 weeks of monthly monitoring in treatment-free follow up if alternative HBV therapy is not initiated.

- The serum creatinine and estimated creatinine clearance should be rechecked within two weeks after restarting treatment to ensure that the subject has stabilized. Once this has been determined, the subject should be evaluated at regularly scheduled study visits.

- If creatinine clearance decreases to < 70 mL/min/1.73 m^2 (confirmed) following restarting Tenofovir DF treatment, Tenofovir DF should be permanently discontinued, followed by 24 weeks of monthly monitoring in treatment-free follow up if alternative HBV therapy is not initiated.

7.5.5. Management of Reduced Serum Phosphate

If serum phosphate is confirmed to be < 3.0 mg/dl (0.96 mmol/l) in any paediatric patient receiving Tenofovir DF, renal function should be re-evaluated within one week, including measurements of blood glucose, blood potassium and urine glucose concentrations. If renal abnormalities are suspected or detected then consultation with a nephrologist should be obtained to consider interruption of Tenofovir DF treatment.

7.5.6. Management of Bone Abnormalities

Among Group 1 subjects, if bone abnormalities are detected or suspected in paediatric patients, consultation with an endocrinologist and/or nephrologist should be obtained. Group 2 subjects with detected or suspected bone abnormalities should be managed as per the local standard of care.

7.5.7. Management of ALT Flares

Flares on treatment: Spontaneous exacerbations in CHB are relatively common and are characterised by transient increases in serum ALT. After initiating antiviral therapy, serum ALT may increase in some patients. In patients with compensated liver disease, these increases in serum ALT are generally not accompanied by an increase in serum bilirubin concentrations or hepatic decompensation. Patients with cirrhosis may be at a higher risk for hepatic decompensation following hepatitis exacerbation, and therefore should be monitored closely during therapy.

Flares after treatment discontinuation: Acute exacerbation of hepatitis has also been reported in patients who have discontinued HBV therapy. Post-treatment exacerbations are usually associated with rising HBV DNA, and the majority appears to be self-limited. However, severe exacerbations, including fatalities, have been reported. Hepatic function should be monitored at repeated intervals with both clinical and laboratory follow-up for at least 6 months after

discontinuation of HBV therapy. If appropriate, resumption of HBV therapy may be warranted. In patients with advanced liver disease or cirrhosis, treatment discontinuation is not recommended since post-treatment exacerbation of hepatitis may lead to hepatic decompensation. Patients should be placed on commercially available HBV therapy following study drug discontinuation. Liver flares are especially serious, and sometimes fatal in patients with decompensated liver disease.

On-Treatment ALT Flare and Post-Treatment Exacerbation of Hepatitis are defined as:

- Serum ALT > 2 × baseline and > 10 × ULN, with or without associated symptoms OR
- Confirmed ALT elevation (defined as 1-grade shift or 2 × previous value) associated with confirmed changes outside of the normal range in other laboratory parameters suggestive of worsening hepatic function (total bilirubin ≥ 2 mg/dL above baseline, abnormal PT ≥ 2 seconds or INR ≥ 0.5 over baseline, abnormal serum albumin ≥ 1 g/dL below baseline or elevated serum lactate levels (if available), defined as 2 × ULN per the Adult AIDS Clinical Trials Group (AACTG) guidelines).

7.5.7.1. Management of ALT Flare in Subjects Receiving Tenofovir DF

If laboratory results indicate (1) elevation of ALT > 2 × baseline and > 10 × ULN OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (abnormal bilirubin ≥ 2mg/dL above baseline, abnormal PT ≥ 2 sec above baseline, INR ≥ 0.5 above baseline, abnormal albumin ≥ 1 g/dL decrease from baseline or elevated serum lactate levels > 2 × ULN along with any ALT elevation (i.e., grade shift or 2 × previous value), the following is recommended:

- Schedule the subject to return to the clinic as soon as possible and ideally no later than one week after the initial labs were drawn. During the visit, perform a clinical assessment of the subject. The assessment should include a physical examination and evaluation of the subject's mental status.
- Draw blood samples, request lactate testing and send for confirmation of elevated serum transaminases (ALT/AST), total bilirubin and PT/INR, and albumin. [Note: If, in the investigator's judgment, the central laboratory cannot provide adequate turn around time, the confirmation test may also be performed at a local laboratory. However, the central laboratory results are considered definitive].

If the elevations are confirmed, request the central clinical laboratory to conduct reflex testing for serum HBV DNA, HBV serology (HBeAg, HBeAb, and HBsAg), HDV, HAV IgM, and HCV serology.

Based on the results of the confirmatory tests, the following treatment modifications are recommended:

Elevated Liver Enzymes, Normal Bilirubin, Normal PT/INT, Normal Albumin, Normal Lactate

If ALT and/or AST levels are elevated (i.e., > 2 × baseline and > 10 × ULN) but total bilirubin and PT/INR, albumin and lactate are normal, the subject may remain on Tenofovir DF and should be monitored every week until ALT/AST return to normal or baseline levels. During monitoring:

- If ALT/AST levels decline within 4 weeks, the subject should remain on study and return to the clinic per protocol.

- If after 4 weeks of monitoring, ALT/AST values remain elevated (e.g., > 2 × baseline and > 10 × ULN) or have worsened, with bilirubin ≤ 2.5 × ULN, PT ≤ 1.5 × ULN, or abnormal albumin or lactate levels, the investigator should consult with the Gilead Medical Monitor.

- If ALT remains > 2 × baseline and > 10 × ULN and the bilirubin or PT values are confirmed at > 2.5 × ULN or > 1.5 × ULN, respectively, the investigator should consider discontinuing Tenofovir DF and initiating alternative HBV therapy (see below). However, prior to initiating alternative therapy, medical management of the subject should be discussed with the Gilead Medical Monitor. (Note: Once a subject has started alternative therapy, s/he must be discontinued from the study.)

Elevated Liver Enzymes, Elevated Bilirubin and PT/INR (> Grade 2) Symptomatic Elevated Lactate (> 2 × ULN) or Asymptomatic Elevated Lactate (> 4 × ULN)

If ALT/AST values are elevated (i.e., > 2 × baseline and > 10 × ULN) and bilirubin or PT values are confirmed at > 2.5 × ULN or > 1.5 × ULN , respectively, or lactate levels are increased (symptomatic and > 2 × ULN or asymptomatic and > 4 × ULN) the investigator should consider discontinuing Tenofovir DF and initiating alternative HBV treatment. The subject must be monitored weekly for as long as enzyme levels and bilirubin and PT/INR remain elevated or above baseline values. Refer to Appendix 6

for specific guidelines for the management of symptomatic and asymptomatic hyperlactatemia.

- If the ALT/AST levels return to the baseline level and/or Grade 2 or lower during the first 8 weeks of monitoring, Tenofovir DF may be resumed.

- If the ALT/AST levels, bilirubin, PT/INR or lactate levels remain elevated up through Week 8 or deteriorate at any point, the investigator should consult with the Gilead Medical Monitor.

7.5.7.2. Management of Exacerbation of Hepatitis in Subjects Who Have Discontinued Study Medication (Tenofovir DF)

If laboratory results indicate (1) an ALT elevation > 2 × baseline and > 10 × ULN OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (bilirubin ≥ 2 mg/dL above baseline, abnormal PT ≥ 2 secs above baseline, abnormal albumin ≥ 1 g/dL below baseline or elevated lactate levels > 2 × ULN) along with any ALT elevation (i.e., 1 grade shift or 2 × previous value) and the subject is on no post-study therapy for HBV, the following is recommended:

- Schedule the subject to return to the clinic as soon as possible and ideally no later than 1 week after the initial labs were drawn. During the visit, perform a clinical assessment of the subject. The assessment should include a physical examination and evaluation of the subject's mental status.

- Draw blood samples and request lactate testing and confirmation of elevated serum transaminases (ALT/AST), bilirubin, PT/INR, and albumin. [Note: If, in the investigator's judgment, the central lab cannot provide adequate turn around time, the confirmation test may also be performed at a local lab. However, the central lab results are considered definitive].

- If the elevations are confirmed (e.g., ALT > 2 × baseline and > 10 × ULN) OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (abnormal bilirubin ≥ 2 mg/dL above baseline, abnormal PT ≥ 2 secs above baseline, abnormal albumin ≥ 1 g/dL below baseline, or elevated lactate levels > 2 × ULN) along with any ALT elevation (i.e., 1 grade shift or 2 × previous value), request the clinical laboratory to conduct reflex testing for serum HBV DNA, HBV serology (HBeAg, HBeAb, and HBsAg), HDV, HAV IgM and HCV. If serum HBV DNA is increasing, the investigator should consider immediate initiation of approved therapy.

The subject should be followed until the abnormal ALT/AST values, bilirubin, PT/INR, albumin or lactate laboratory parameters return to normal or baseline up to a maximum of 6 months after the initial occurrence of the event. Refer to Appendix 6

for specific guidelines for the management of symptomatic and asymptomatic hyperlactatemia.

7.6. Special Situations Reports

7.6.1. Definitions of Special Situations

Special situation reports include reports of pregnancy; medication error, abuse, misuse, or overdose; lack of effect; adverse reactions in infants following exposure from breastfeeding; and adverse reactions associated with product complaints and occupational exposure.

Medication error is any unintentional error in the prescribing, dispensing, or administration of a medicinal product while in the control of the health care provider, subject, or consumer.

Abuse is defined as persistent or sporadic intentional excessive use of a medicinal product by a subject.

Misuse is defined as any intentional and inappropriate use of a medicinal product that is not in accordance with the protocol instructions or the local prescribing information.

An overdose is defined as an accidental or intentional administration of a quantity of a medicinal product given per administration or cumulatively which is above the maximum recommended dose as per protocol or in the product labelling (as it applies to the daily dose of the subject in question). In cases of a discrepancy in drug accountability, overdose will be established only when it is clear that the subject has taken the excess dose(s). Overdose cannot be established when the subject cannot account for the discrepancy except in cases in which the investigator has reason to suspect that the subject has taken the additional dose(s).

Product complaint is defined as complaints arising from potential deviations in the manufacture, packaging, or distribution of the medicinal product.

Lack of effect is defined as the failure of the expected or intended pharmacologic action or therapeutic effect as described in the pharmacology and/or indications section of the current product labeling.

Occupational exposure is defined as exposure to a medicinal product as a result of one's professional or non-professional occupation.

7.6.2. Instructions for Reporting Special Situations

7.6.2.1. Instructions for Reporting Pregnancies

The investigator should report pregnancies in female study subjects that are identified after initiation of study medication (i.e., Tenofovir DF) and throughout the study to the CRO or Gilead DSPH Gilead DSPH using the pregnancy report form within 24 hours of becoming aware of the pregnancy.

Refer to Section 7.3 and the CRF/eCRF completion guidelines for full instructions on the mechanism of pregnancy reporting.

The pregnancy itself is not considered an AE nor is an induced elective abortion to terminate a pregnancy without medical reasons.

Any premature termination of pregnancy (eg, a spontaneous abortion, an induced therapeutic abortion due to complications or other medical reasons) must be reported within 24 hours as an SAE. The underlying medical reason for this procedure should be recorded as the AE term.

A spontaneous abortion is always considered to be an SAE and will be reported as described in Sections 7.1.1 and 7.1.2. Furthermore, any SAE occurring as an adverse pregnancy outcome post study must be reported to Gilead DSPH.

The subject should receive appropriate monitoring and care until the conclusion of the pregnancy. The outcome should be reported to CRO [TBD] or Gilead DSPH using the pregnancy outcome report form. If the end of the pregnancy occurs after the study has been completed, the outcome should be reported directly to Gilead DSPH. Gilead DSPH contact information is as follows: Email: Safety_FC@gilead.com and Fax: +1 (650) 522-5477.

Refer to Appendix 5 for Pregnancy Precautions, Definition for Female of Childbearing Potential, and Contraceptive Requirements.

7.6.2.2. Reporting Other Special Situations

All other special situation reports must be reported on the special situations report form and forwarded to Gilead DSPH within 24 hours of the investigator becoming aware of the situation. These reports must consist of situations that involve Tenofovir DF and/or Gilead concomitant medications, but do not apply to non-Gilead concomitant medications.

Special situations involving non-Gilead concomitant medications donot need to be reported on the special situations report form; however, for special situations that result in renal or bone AEs due to a non-Gilead concomitant medication, the AE should be reported on the AE form.

Any inappropriate use of concomitant medications prohibited by this protocol should not be reported as “misuse,” but may be more appropriately documented as a protocol deviation.

Refer to Section 7.3 and the CRF/eCRF completion guidelines for full instructions on the mechanism of special situations reporting.

All clinical sequelae in relation to these special situation reports will be reported as AEs or SAEs at the same time using the AE CRF/eCRF and/or the SAE report form. Details of the symptoms and signs, clinical management, and outcome will be reported, when available.

8. STATISTICAL CONSIDERATIONS

8.1. Analysis Objectives and Endpoints

8.1.1. Analysis Objectives

The primary objective of this study is:

- To characterize the long term (i.e., 96 weeks of follow up) bone safety profile of open-label Tenofovir DF treatment in CHB-infected adolescents. This includes prospectively evaluating and comparing the BMD change between CHB infected adolescents 12 to < 18 years of age treated with Tenofovir DF in European treatment centers who are assigned to one of two schedules for renal and bone laboratory monitoring and BMD measurement. The primary study endpoint will be the percent changes in BMD from Baseline through study Week 96.

The secondary objectives of this study are as follows:

- To document all SADRs and all renal- and bone-related AEs including renal and bone laboratory abnormalities, as applicable
- To determine the time to diagnosis of renal and bone AEs and document the resulting patient management and outcome(s)
- To assess the clinical management and outcomes of any renal- or bone related ≥ Grade 3 laboratory markers and clinical SAEs
- To assess the efficacy and tolerability of Tenofovir DF 300mg once daily in adolescents aged 12 to <18 years with CHB infection
- To assess the use of oral vitamin D, calcium and phosphate supplementation and explore the association between supplement use and rates of bone and renal AEs
- To describe the demographic characteristics, the disease characteristics, of adolescents with CHB infection treated with Tenofovir DF
- To describe reasons for discontinuation of Tenofovir DF.

8.1.2. Primary Endpoint

The primary endpoint of this study is:

- The identification of bone AEs occurring in subjects taking Tenofovir DF between Baseline and Week 96 of treatment, including the identification of ≥4% percent reduction in BMD within subjects and between monitoring groups from Baseline.

8.1.3. Secondary Endpoints

- Documentation of renal or bone AEs and outcomes among subjects receiving Tenofovir DF, including rates of medication withdrawal or drug discontinuation
- Subjects' cumulative exposure time on Tenofovir DF at the time renal or bone AEs are detected up to and including Week 96 or to study discontinuation (subjects without renal or bone AEs will contribute cumulative exposure time on Tenofovir DF from Baseline to Week 96 or to study discontinuation). The cumulative time to renal or bone AEs will be expressed as incident rates.
- Documentation of the medical management and classification of detected renal or bone laboratory abnormalities
- Analysis of subjects' virological and immunological status from Baseline to Week 96 under real world treatment practices
- Report provider recommendations and subject adherence to dietary supplements (e.g., Vitamin D, calcium)
- Documentation of subject demographics, new medical issues, medications, biometrics, treatment adherence
- Reasons for discontinuation of Tenofovir DF

8.1.4. Other Endpoints of Interest

Not applicable

8.2. Analysis Conventions

All individual subject data will be listed as measured. All statistical summaries and analyses will be performed using SAS® software (SAS Institute, Cary, North Carolina, USA) or other standard software tools including the STATA software (STATACorp LP, College Station, TX, USA).

8.2.1. Analysis Sets

The analysis set will be the Full Analysis Set (FAS), which includes all subjects enrolled in the study.

8.2.1.1. Efficacy

An efficacy analysis will be conducted after the last assigned subject reaches Week 96. The analysis will evaluate the difference in the proportion of subjects (Group 1 and 2 data will be pooled, as subjects have exposure to a single mode of therapy) achieving a composite endpoint of HBV DNA < 400 copies/mL and ALT normal at Week 72, using a two-sided Fisher exact test with a non-completer equals failure approach.

The primary analysis set for safety analyses will include all randomized subjects who received at least one dose of Tenofovir DF. All data collected during the course of the study (on treatment and during the treatment-free follow up window) will be included in the safety summaries. Normalized bone biomarker ranges (i.e., calcium, phosphorus, vitamin D levels, PTH, osteocalcin, bone alkaline phosphatase, N and C telopeptides) in pediatric populations have not been established. Bone serum biomarkers will be collected in parallel to DEXA scan schedules for Groups 1 and 2 and summarized and compared to DEXA z-score findings.

8.2.1.2. Interim Analysis

An analysis for collected renal and bone adverse event data will be conducted after the first 50 patients are enrolled and complete Week 48 of the study. Final analyses will be conducted after all subjects have completed the study. All data collected during treatment plus 30 days will be included in the safety summaries**.**

8.2.1.3. Pharmacokinetics

Not applicable

8.2.1.4. Biomarkers

Not applicable

8.3. Data Handling Conventions

Missing data can have an impact upon the interpretation of the trial data. In general, values for missing data will not be imputed.

For the primary endpoint and categorical secondary efficacy endpoints, missing data will be detailed in a separate statistical analysis plan.

Sensitivity analyses will be performed if warranted.

8.4. Demographic Data and Baseline Characteristics

Demographic and baseline measurements will be summarized using standard descriptive methods

Demographic summaries will include sex, race/ethnicity, randomization stratification group, and age.

Baseline data will include a summary of body weight, height, body mass index, $\log_{10}$ (HBV DNA) level, HBV serology, liver tests, creatinine clearance calculations using the Schwartz formula, hematology, previous nucleoside and interferon exposure, genotype, bone mineral density (via DEXA scan), and serum bone biochemical markers.

For categorical demographic and baseline characteristics, a two-sided Fisher exact test may be used to compare monitoring groups. Similarly, for continuous demographic and baseline characteristics, a Wilcoxon rank sum test may be used to compare monitoring groups.

8.5. Efficacy Analysis

8.5.1. Primary Analysis

An efficacy analysis will be conducted after the last assigned subject reaches Week 96. The analysis will evaluate the difference in the proportion of subjects (Group 1 and 2 data will be pooled, as subjects have exposure to a single mode of therapy) achieving a composite endpoint of HBV DNA < 400 copies/mL and ALT normal at Week 96, using a two-sided Fisher exact test with a non-completer equals failure approach.

8.5.2. Secondary Analyses

Not applicable

8.6. Safety Analysis

All safety data collected on or after the date that Tenofovir DF was first dispensed up to the date of last dose of Tenofovir DF will be summarized by monitoring group. Data for any pre-treatment and treatment-free follow up periods will be included in data listings.

The primary safety analysis will be performed after the last assigned subject reaches Week 96. The analysis will evaluate the difference between renal or bone AEs recorded within each of the monitoring groups with respect to the primary safety endpoint, estimating the cumulative incidence proportions using time-to-event methodology and performing comparisons using 95% confidence intervals for differences in proportions.

The proportion of subjects in each monitoring group with renal or bone AE leading to permanent discontinuation of Tenofovir DF through Week 96 will be summarized. Descriptive statistics such as n, mean, standard deviation, median, Q1, Q3, minimum and maximum) by monitoring group will be used to report demographics and time to event summaries, and a Wilcoxon rank sum test may be used to compare AEs between monitoring groups.

For categorical safety data including incidence of AEs and categorizations of laboratory data, a Fisher's exact test will be used to compare monitoring groups. For continuous safety data including laboratory data, a Wilcoxon Rank sum test will be used to compare monitoring groups. Subgroup analyses of safety endpoints will include analyses by assigned monitoring group, age groups, and geographical location of study site.

8.6.1. Extent of Exposure

A subject's extent of exposure to drug treatment will be generated from the Tenofovir DF drug administration page of the CRF. Exposure data will be summarized by monitoring group.

8.6.2. Adverse Events

Clinical and laboratory AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). System Organ Class (SOC), High-Level Group Term (HLGT), High-Level Term (HLT), Preferred Term (PT), and Lower-Level Term (LLT) will be attached to the clinical database.

Events will be summarized on the basis of the date of onset for the event. A treatment-emergent adverse event will be defined as any adverse event that begins on or after the date of first dose up to the date of last dose of Tenofovir DF plus 30 days.

Summaries (number and percentage of subjects) of treatment-emergent AEs related to **bone or renal SOCs** (hierarchically ordered by SOC, HLT, and PT) will be provided by treatment group:

- all treatment-emergent bone or renal AEs,
- all related treatment-emergent bone or renal AEs,
- combined Grade 2, 3, and 4 treatment-emergent bone or renal AEs,
- combined Grade 3 and 4 treatment-emergent bone or renal AEs,
- combined Grade 2, 3, and 4 related treatment-emergent bone or renal AEs,
- combined Grade 3 and 4 related treatment-emergent bone or renal AEs,
- all AEs that caused permanent discontinuation from Tenofovir DF,
- all AEs that caused permanent discontinuation from study,
- all AEs that caused change in dose or temporary interruption of Tenofovir DF,
- all serious AEs (all SOCs, i.e., not limited to bone or renal SOCs), and
- all serious bone or renal related AEs.

Events will be summarized based on the date of onset for the event. A treatment-emergent adverse event will be defined as any adverse event that begins on or after the date of first dose of Tenofovir DF. Events that occur prior to the first dose of Tenofovir DF or after the last dose of Tenofovir DF will be included in data listings.

PPD

8.6.3. Laboratory Evaluations

Selected laboratory data (using conventional units) will be summarized using only observed data. Data and change from baseline at all scheduled time points will be summarized.

Selected laboratory data (using conventional units) will be summarized by the observed data and by the change from baseline across time.

Graded laboratory abnormalities will be defined using the grading scheme defined in Appendix 4. Grading of laboratory abnormalities for analysis purposes will be performed by the central laboratory.

Incidence of treatment-emergent laboratory abnormalities, defined as values that increase at least one toxicity grade from baseline at any time post baseline, will be summarized by monitoring group. If baseline data are missing, then any graded abnormality (i.e., at least a Grade 1) will be considered treatment-emergent.

Laboratory abnormalities that occur before the first dose of Tenofovir DF or after the last dose of Tenofovir DF plus 30 days will be included in a data listing.

8.6.4. Other Safety Evaluations: Analyses of BMD

Cumulative incidence of at least a 4% decrease from baseline in BMD (spine BMD and whole body BMD) using time-to-event methodology will be summarized and compared between treatment groups using 95% confidence intervals for difference in person time rates.

The percent change from baseline in BMD (spine BMD and whole body BMD) will be summarized over time and compared between treatment groups using the Generalized Linear Model. Lumbar spine and whole body z-scores (derived from DEXA-derived BMD assessments) and the changes in z-scores from Baseline will be summarized.

BMD (via DEXA scan) and serum bone biochemical markers will be summarized over time. Changes from study baseline in bone biochemical markers will also be summarized after adjusting for potential confounders such as age and gender..

8.7. Pharmacokinetic Analysis

Not applicable

8.8. Biomarker Analysis

Not applicable

8.9. Sample Size

Pediatric patients receiving Tenofovir DF may exhibit renal toxicities (e.g., proximal renal tubulopathy) among 4.5% of subjects (95% confidence band of 0.4-8.6%). Among adolescent patients with CHB, available interim 72 Week clinical trial data indicate that 5.8% (95% confidence band of 1.2-15.9%) of subjects experienced a ≥4% decrease in BMD; by comparison 3.7% (95% CI 0.4-12.5%) of placebo controls had a BMD loss of ≥4% . In this same trial, no renal adverse drug reactions were detected by Week 72. Detecting renal or bone AEs in CHB adolescents taking Tenofovir DF are infrequent events, and the pool of Tenofovir DF-naïve subjects within the EU suitable for enrollment is limited. With a fixed sample size of 100 (50 in each monitoring group) we would expect to detect 4-5 renal or bone adverse drug reactions across both real-world groups (both receive equivalent treatment) over 96 weeks.

The study employs two monitoring protocols which specify the same laboratory and imaging tests for bone and renal markers, but differ in the frequency of testing. Group 1 will receive more frequent monitoring which is protocol-defined or SmPC specified, and Group 2 will be monitored according to a less frequent DEXA schedule for BMD and will follow the local standard of care for renal monitoring. It is hypothesized (H_0) that there will be no difference in the timing, management, or subject outcomes resulting from renal or bone AEs among the two monitoring regimens. With a one-tailed test of α=0.05, and assuming a non-inferiority margin of 0.10, the study has at least 80% power to detect a difference between the monitoring regimens beyond the specified margin of non-inferiority.

8.10. Data Monitoring Committee

Not applicable

8.11. Endpoint Adjudication Committee

Not applicable

8.12. Investigator Responsibilities

8.12.1. Good Clinical Practice

The investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), International Conference on Harmonisation (ICH) guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject. These standards are consistent with the European Union Clinical Trials Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC.

The investigator and all applicable sub investigators will comply with 21 CFR, Part 54, 1998, providing documentation of their financial interest or arrangements with Gilead, or proprietary interests in the drug under study. This documentation must be provided prior to the investigator's (and any sub investigator's) participation in the study. The investigator and sub investigator agree to notify Gilead of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date when the last subject completes the protocol-defined activities.

8.12.2. Independent Ethics Committee (IEC) Review and Approval

The investigator (or sponsor as appropriate according to local regulations) will submit this protocol, informed consent form, and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) to an IEC. The investigator will not begin any study subject activities until approval from the IEC has been documented and provided as a letter to the investigator.

Before implementation, the investigator will submit to and receive documented approval from the IEC any modifications made to the protocol or any accompanying material to be provided to the subject after initial IEC approval, with the exception of those necessary to reduce immediate risk to study subjects.

8.12.3. Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. Informed consent will be obtained by the parent or legal guardian of the subject, or informed assent will be obtained by the individual aged <18 years with the provision that the subject fully understands the terms of the study and understands their right to withdraw assent from the study at any time. The investigator must use the most current IEC approved consent form for documenting written informed consent. Each informed consent (or assent as applicable) will be appropriately signed and dated by the subject or the subject's legally authorized representative and the person conducting the consent discussion, and also by an impartial witness if required by IEC local requirements.

8.12.4. Confidentiality

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, another unique identifier (as allowed by local law) and an identification code will be recorded on any form or biological sample submitted to the Sponsor, IEC, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject. Refer to specific laboratory instructions. NOTE: The investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial. Subject data will be processed in accordance with all applicable regulations.

The investigator agrees that all information received from Gilead, including but not limited to the investigator brochure, this protocol, CRF/eCRF, Tenofovir DF, and any other study information, remain the sole and exclusive property of Gilead during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Gilead. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

8.12.5. Study Files and Retention of Records

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data should include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender);
- Documentation that subject meets eligibility criteria, i.e., history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);
- Documentation of the reason(s) a consented subject is not enrolled
- Participation in study (including study number);
- Study discussed and date of informed consent;
- Dates of all visits;
- Documentation that protocol specific procedures were performed;
- Results of efficacy parameters;
- Start and end date (including dose regimen) of Tenofovir DF, including dates of dispensing and return;
- Record of all AEs and other safety parameters (start and end date, and including causality and severity);
- Concomitant medication (including start and end date, dose if relevant; dose changes);
- Date of study completion and reason for early discontinuation, if it occurs.

All clinical study documents must be retained by the investigator until at least 2 years or according to local laws, whichever is longer, after the last approval of a marketing application in an ICH region (ie, United States, Europe, or Japan) and until there are no pending or planned marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory

authorities have been notified. Investigators may be required to retain documents longer if specified by regulatory requirements, by local regulations, or by an agreement with Gilead. The investigator must notify Gilead before destroying any clinical study records.

Should the investigator wish to assign the study records to another party or move them to another location, Gilead must be notified in advance.

If the investigator cannot provide for this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Gilead to store these records securely away from the site so that they can be returned sealed to the investigator in case of an inspection. When source documents are required for the continued care of the subject, appropriate copies should be made for storage away from the site.

8.12.6. Case Report Forms

For each subject consented, an eCRF will be completed by an authorized study staff member whose training for this function is documented according to study procedures. eCRF should be completed on the day of the subject visit to enable the sponsor to perform central monitoring of safety data. Subsequent to data entry, a study monitor will perform source data verification within the EDC system. Original entries as well as any changes to data fields will be stored in the audit trail of the system. Prior to database lock (or any interim time points as described in the clinical data management plan), the investigator will use his/her log in credentials to confirm that the forms have been reviewed, and that the entries accurately reflect the information in the source documents. The eCRF capture the data required per the protocol schedule of events and procedures. System-generated or manual queries will be issued to the investigative site staff as data discrepancies are identified by the monitor or internal Gilead staff, who routinely review the data for completeness, correctness, and consistency. The site coordinator is responsible for responding to the queries in a timely manner, within the system, either by confirming the data as correct or updating the original entry, and providing the reason for the update (e.g. data entry error). At the conclusion of the trial, Gilead will provide the site with a read-only archive copy of the data entered by that site. This archive must be stored in accordance with the records retention requirements outlined in Section 8.12.5.

8.12.7. Drug and Supplies Accountability and Return

Gilead recommends that used and unused Tenofovir DF and supplies be returned to the shipping facility from which it came for eventual destruction. The study monitor will provide instructions for return. If return is not possible, the study monitor will evaluate each study center's drug disposal procedures and provide appropriate instruction for destruction of unused Tenofovir DF and supplies. If the site has an appropriate standard operating procedure (SOP) for drug destruction as determined by Gilead QA, the site may destroy used (empty or partially empty) and unused drug and supplies in accordance with that site's approved SOP. A copy of the site's approved SOP will be obtained for central files.

If Tenofovir DF is destroyed on site, the investigator must maintain accurate records for all drug destroyed. Records must show the identification and quantity of each unit destroyed, the method of destruction, and the person who disposed of Tenofovir DF. Upon study completion, copies of drug accountability records must be filed at the site. Another copy will be returned to Gilead.

The study monitor will review Tenofovir DF and supplies and associated records at periodic intervals.

8.12.8. Inspections

The investigator will make available all source documents and other records for this trial to Gilead's appointed study monitors, to IECs, or to regulatory authority or health authority inspectors.

8.12.9. Protocol Compliance

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

8.13. Sponsor Responsibilities

8.13.1. Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Gilead. All protocol modifications must be submitted to the EC in accordance with local requirements. Approval must be obtained before modifications can be implemented.

8.13.2. Study Report and Publications

A clinical study report (CSR) will be prepared and provided to the regulatory agency(ies)). Gilead will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after the following conditions have been met:

The results of the study in their entirety have been publicly disclosed by or with the consent of Gilead in an abstract, manuscript, or presentation form or the study has been completed at all study sites for at least 2 years

The investigator will submit to Gilead any proposed publication or presentation along with the respective scientific journal or presentation forum at least 30 days before submission of the publication or presentation.

No such communication, presentation, or publication will include Gilead's confidential information (see Section 8.12.4).

The investigator will comply with Gilead's request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days in order to obtain patent protection if deemed necessary.

8.14. Joint Investigator/Sponsor Responsibilities

8.14.1. Payment Reporting

Investigators and their study staff may be asked to provide services performed under this protocol, e.g. attendance at Investigator's Meetings. If required under the applicable statutory and regulatory requirements, Gilead will capture and disclose to Federal and State agencies any expenses paid or reimbursed for such services, including any clinical trial payments, meal, travel expenses or reimbursements, consulting fees, and any other transfer of value.

8.14.2. Access to Information for Monitoring

In accordance with regulations and guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the accuracy of the data recorded in the CRF/eCRF.

The monitor is responsible for routine review of the CRF/eCRF at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The monitor should have access to any subject records needed to verify the entries on the CRF/eCRF. The investigator agrees to cooperate with the monitor to ensure that any problems detected through any type of monitoring (central, on site) are resolved.

8.14.3. Access to Information for Auditing or Inspections

Representatives of regulatory authorities or of Gilead may conduct inspections or audits of the clinical study. If the investigator is notified of an inspection by a regulatory authority the investigator agrees to notify the Gilead medical monitor immediately. The investigator agrees to provide to representatives of a regulatory agency or Gilead access to records, facilities, and personnel for the effective conduct of any inspection or audit.

8.14.4. Study Discontinuation

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies) and IECs. In terminating the study, Gilead and the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

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10. APPENDICES

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Appendix 1. Investigator Signature Page

GILEAD SCIENCES INTERNATIONAL LTD.
FLOWERS BUILDING, GRANTA PARK
CAMBRIDGE, CB21 6GT, UNITED KINGDOM

STUDY ACKNOWLEDGEMENT

Pharmacoepidemiology study to define the long-term safety profile of tenofovir disoproxil fumarate (Tenofovir DF, Viread®) and describe the management of Tenofovir DF-associated renal and bone toxicity in CHB-infected adolescents aged 12 to <18 years in Europe

GS-EU-174-1403 Amendment 2, 10 March 2015

This protocol has been approved by Gilead Sciences International Ltd. The following signature documents this approval.

PPD

Name Study Director (Printed)
Author

PPD

Signature

March 29, 2015

Date

Gilead EU QPPV (Printed)
Author

Signature

Date

Appendix 1. Investigator Signature Page

GILEAD SCIENCES INTERNATIONAL LTD.
FLOWERS BUILDING, GRANTA PARK
CAMBRIDGE, CB21 6GT, UNITED KINGDOM

STUDY ACKNOWLEDGEMENT

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Name Study Director (Printed) *Author*	Signature
Date	
PPD Gilead EU QPPV (Printed) ~~Author~~	PPD Signature
26 Mar 2015 Date	

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Gilead Sciences International Ltd. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

Principal Investigator Name (Printed)	Signature
Date	Site Number

Appendix 2. Study Procedures Table

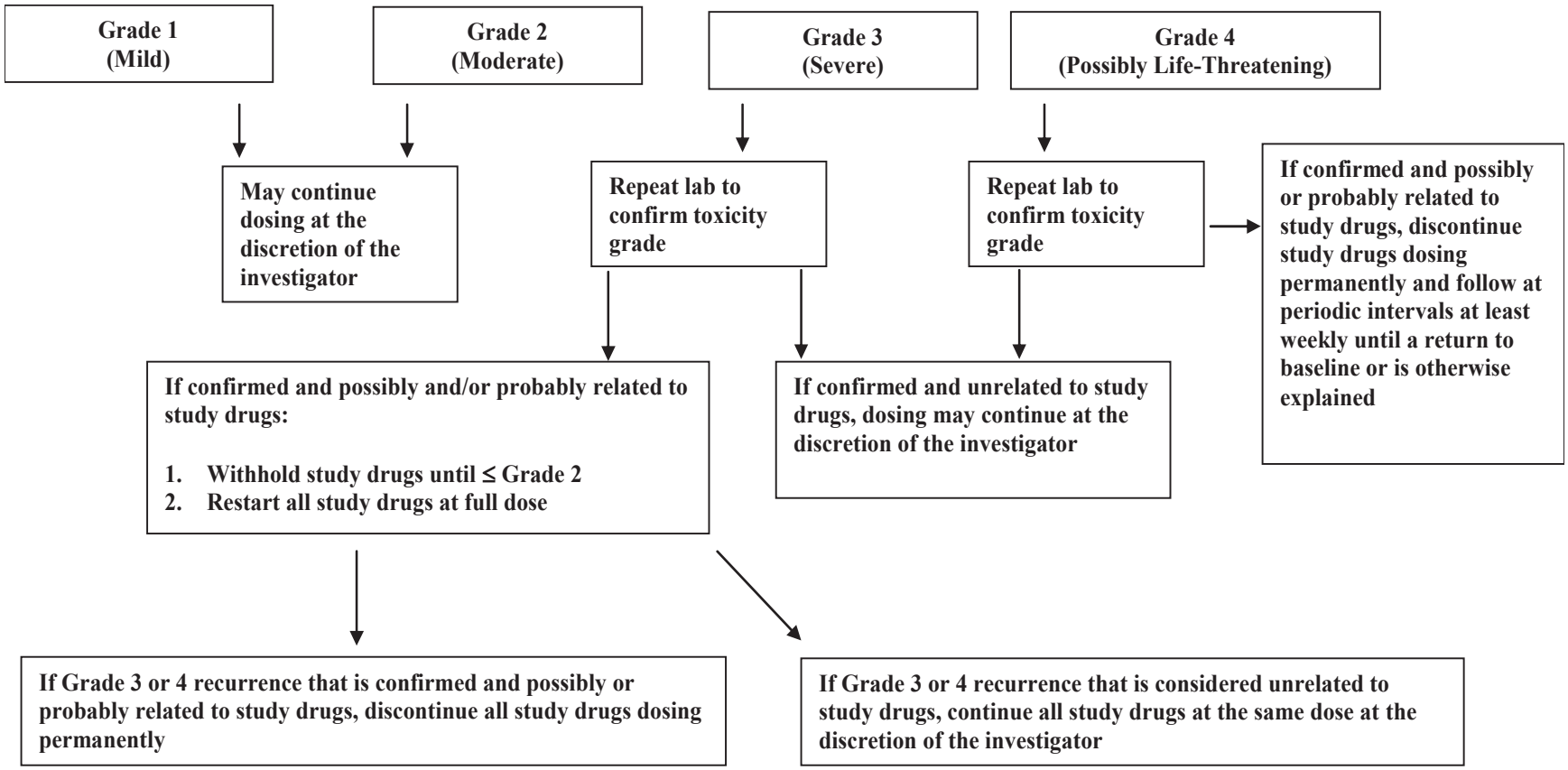
Study Procedures		Study Week										
	Screening	Baseline	4	12	24	36	48	60	72	84	96	30d Follow-Up
Written Informed Consent	x											
Medical History	x											
HBV DNA		x		x	x	x	x	x	x	x	x	
HBV serology	x	x			x		x		x		x	
HCV, HDV, HIV testing		x										
HBV genotype		x										
DEXA, Group 1		x			x		x		x		x	
DEXA, Group 2		x					x				x	
Serum bone chemistry, Group 1		x			x		x		x		x	
Serum bone chemistry, Group 2		x					x				x	
Serum renal chemistry, Group 1	x	x	x	x	x	x	x	x	x	x	x	
Serum renal chemistry, Group 2*	x	x									x	
Liver chemistry	x	x			x		x		x		x	
Hematology		x			x		x		x		x	
Urinalysis, Group 1 (see * for Group 2)	x	x	x	x	x	x	x	x	x	x	x	
Complete physical examination	x	x		x	x	x	x	x	x	x	x	
Changes in medical status or symptoms, Group 1 (see † for Group 2)		x	x									
Drug dispensed and accountability/Concomitant medications		x	x	x	x	x	x	x	x	x	x	
Assess for AE, including renal and bone events (see † for Group 2)		x	x	x	x	x	x	x	x	x	x	x
Pregnancy test	x	x		x	x	x	x	x	x	x	x	

* Group 2 will follow local standard of care for renal monitoring, which may include the Tenofovir DF SmPC recommended screening intervals
ł Group 2 subjects may have changes in medical status or symptoms and new AE assessed upon return for renal monitoring, as per standard of care and within the 12 week intervals of scheduled physical examinations

Definitions:

HBV serology	HBeAg, HBsAg, anti-Hbe, anti-HBs
Serum bone chemistry	calcium, phosphorus, 25-hydroxy and 1, 25 dihydroxyvitamin, PTH, osteocalcin, bone alkaline phosphatase, N and C telopeptides
Serum renal chemistry	glucose, creatinine, creatinine clearance (calculated), magnesium, bicarbonate, chloride, potassium, sodium
Liver chemistry	alanine aminotransferase (ALT), alkaline aminophosphatase (ALP), aspartate aminotransferase (AST), total and direct bilirubin, total protein, albumin, gamma glutamyl transpeptide (GGT), LDH, CK
Hematology	PT/INR, complete blood count (CBC) with differential, platelets
Urinalysis	glucose, protein, creatinine, phosphate, bicarbonate, blood, calcium
Complete physical exam	includes vital signs, body weight and height

Appendix 3. Management of Clinical and Laboratory Adverse Events



Appendix 4. GSI Grading Scale for Severity of Adverse Events and Laboratory Abnormalities

Version: 18Junel2012

HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hemoglobin HIV POSITIVE **Adult and Pediatric ≥ 57 Days**	8.5 to 10.0 g/dL 85 to 100 g/L	7.5 to < 8.5 g/dL 75 to < 85 g/L	6.5 to < 7.5 g/dL 65 to < 75 g/L	< 6.5 g/dL < 65 g/L
HIV NEGATIVE **Adult and Pediatric ≥ 57 Days**	10.0 to 10.9 g/dL 100 to 109 g/L OR Any decrease from Baseline 2.5 to < 3.5 g/dL 25 to < 35 g/L	9.0 to < 10.0 g/dL 90 to < 100 g/L OR Any decrease from Baseline 3.5 to < 4.5 g/dL 35 to < 45 g/L	7.0 to < 9.0 g/dL 70 to < 90 g/L OR Any decrease from Baseline ≥ 4.5 g/dL ≥ 45 g/L	< 7.0 g/dL < 70 g/L
Infant, 36–56 Days (HIV POSITIVE OR NEGATIVE)	8.5 to 9.4 g/dL 85 to 94 g/L	7.0 to < 8.5 g/dL 70 to < 85 g/L	6.0 to < 7.0 g/dL 60 to < 70 g/L	< 6.0 g/dL < 60 g/L
Infant, 22–35 Days (HIV POSITIVE OR NEGATIVE)	9.5 to 10.5 g/dL 95 to 105 g/L	8.0 to < 9.5 g/dL 80 to < 95 g/L	7.0 to < 8.0 g/dL 70 to < 80 g/L	< 7.0 g/dL < 70 g/L
Infant, 1–21 Days (HIV POSITIVE OR NEGATIVE)	12.0 to 13.0 g/dL 120 to 130 g/L	10.0 to < 12.0 g/dL 100 to < 120 g/L	9.0 to < 10.0 g/dL 90 to < 100 g/L	< 9.0 g/dL < 90 g/L

HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Absolute Neutrophil Count (ANC) **Adult and Pediatric, > 7 Days** **Infant, 2 – ≤ 7 Days** **Infant, 1 Day**	1000 to 1300/mm^3 1.00 to 1.30 GI/L 1250 to 1500/mm^3 1.25 to 1.50 GI/L 4000 to 5000/mm^3 4.00 to 5.00 GI/L	750 to < 1000/mm^3 0.75 to < 1.00 GI/L 1000 to < 1250/mm^3 1.00 to < 1.25 GI/L 3000 to < 4000/mm^3 3.00 to < 4.00 GI/L	500 to < 750/mm^3 0.50 to < 0.75 GI/L 750 to < 1000/mm^3 0.75 to < 1.00 GI/L 1500 to < 3000/mm^3 1.50 to < 3.00 GI/L	< 500/mm^3 < 0.50 GI/L < 750/mm^3 < 0.75 GI/L < 1500/mm^3 < 1.50 GI/L
Absolute CD4+ Count HIV NEGATIVE ONLY **Adult and Pediatric > 13 Years**	300 to 400/mm^3 300 to 400/µL	200 to < 300/mm^3 200 to < 300/µL	100 to < 200/mm^3 100 to < 200/µL	< 100/mm^3 < 100/µL
Absolute Lymphocyte Count HIV NEGATIVE ONLY **Adult and Pediatric > 13 Years**	600 to 650/mm^3 0.60 to 0.65 GI/L	500 to < 600/mm^3 0.50 to < 0.60 GI/L	350 to < 500/mm^3 0.35 to < 0.50 GI/L	< 350/mm^3 < 0.35 GI/L
Platelets	100,000 to < 125,000/mm^3 100 to < 125 GI/L	50,000 to < 100,000/mm^3 50 to < 100 GI/L	25,000 to < 50,000/mm^3 25 to < 50 GI/L	< 25,000/mm^3 < 25 GI/L
WBCs	2000/mm^3 to 2500/mm^3 2.00 GI/L to 2.50 GI/L	1,500 to < 2,000/mm^3 1.50 to < 2.00 GI/L	1000 to < 1,500/mm^3 1.00 to < 1.50 GI/L	< 1000/mm^3 < 1.00 GI/L

HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hypofibrinogenemia	100 to 200 mg/dL 1.00 to 2.00 g/L	75 to < 100 mg/dL 0.75 to < 1.00 g/L	50 to < 75 mg/dL 0.50 to < 0.75 g/L	< 50 mg/dL < 0.50 g/L
Hyperfibrinogenemia	> ULN to 600 mg/dL > ULN to 6.0 g/L	> 600 mg/dL > 6.0 g/L	— —	— —
Fibrin Split Product	20 to 40 µg/mL 20 to 40 mg/L	> 40 to 50 µg/mL > 40 to 50 mg/L	> 50 to 60 µg/mL > 50 to 60 mg/L	> 60 µg/mL > 60 mg/L
Prothrombin Time (PT)	> 1.00 to 1.25 × ULN	> 1.25 to 1.50 × ULN	> 1.50 to 3.00 × ULN	> 3.00 × ULN
International Normalized Ratio of prothrombin time (INR)	1.1 to 1.5 x ULN	>1.5 to 2.0 x ULN	>2.0 to 3.0 x ULN	>3.0 x ULN
Activated Partial Thromboplastin Time (APTT)	> 1.00 to 1.66 × ULN	> 1.66 to 2.33 × ULN	> 2.33 to 3.00 × ULN	> 3.00 × ULN
Methemoglobin	5.0 to 10.0%	> 10.0 to 15.0%	> 15.0 to 20.0%	> 20.0%

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hyponatremia	130 to <LLN mEq/L 130 to <LLN mmol/L	125 to < 130 mEq/L 125 to < 130 mmol/L	121 to < 125 mEq/L 121 to < 125 mmol/L	< 121 mEq/L < 121 mmol/L
Hypernatremia	146 to 150 mEq/L 146 to 150 mmol/L	> 150 to 154 mEq/L > 150 to 154 mmol/L	> 154 to 159 mEq/L > 154 to 159 mmol/L	> 159 mEq/L > 159 mmol/L
Hypokalemia	3.0 to 3.4 mEq/L 3.0 to 3.4 mmol/L	2.5 to < 3.0 mEq/L 2.5 to < 3.0 mmol/L	2.0 to < 2.5 mEq/L 2.0 to < 2.5 mmol/L	< 2.0 mEq/L < 2.0 mmol/L
Hyperkalemia	5.6 to 6.0 mEq/L 5.6 to 6.0 mmol/L	> 6.0 to 6.5 mEq/L > 6.0 to 6.5 mmol/L	> 6.5 to 7.0 mEq/L > 6.5 to 7.0 mmol/L	> 7.0 mEq/L > 7.0 mmol/L
Hypoglycemia **Adult and Pediatric ≥ 1 Month** **Infant, < 1 Month**	55 to 64 mg/dL 3.03 to 3.58 mmol/L 50 to 54 mg/dL 2.8 to 3.0 mmol/L	40 to < 55 mg/dL 2.20 to < 3.03 mmol/L 40 to < 50 mg/dL 2.2 to < 2.8 mmol/L	30 to < 40 mg/dL 1.64 to < 2.20 mmol/L 30 to < 40 mg/dL 1.7 to < 2.2 mmol/L	< 30 mg/dL < 1.64 mmol/L < 30 mg/dL < 1.7 mmol/L
Hyperglycemia, Nonfasting	116 to 160 mg/dL 6.42 to 8.91 mmol/L	> 160 to 250 mg/dL > 8.91 to 13.90 mmol/L	> 250 to 500 mg/dL > 13.90 to 27.79 mmol/L	> 500 mg/dL > 27.79 mmol/L
Hyperglycemia, Fasting	110 to 125 mg/dL 6.08 to 6.96 mmol/L	>125 to 250 mg/dL >6.96 to 13.90 mmol/L	>250 to 500 mg/dL >13.90 to 27.79 mmol/L	>500 mg/dL >27.79 mmol/L

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hypocalcemia (corrected for albumin if appropriate*) **Adult and Pediatric ≥ 7 Days**	7.8 to 8.4 mg/dL 1.94 to 2.10 mmol/L	7.0 to < 7.8 mg/dL 1.74 to < 1.94 mmol/L	6.1 to < 7.0 mg/dL 1.51 to < 1.74 mmol/L	< 6.1 mg/dL < 1.51 mmol/L
Infant, < 7 Days	6.5 to 7.5 mg/dL 1.61 to 1.88 mmol/L	6.0 to < 6.5 mg/dL 1.49 to < 1.61 mmol/L	5.5 to < 6.0 mg/dL 1.36 to < 1.49 mmol/L	< 5.5 mg/dL < 1.36 mmol/L
Hypercalcemia (corrected for albumin if appropriate*) **Adult and Pediatric ≥ 7 Days**	>ULN to 11.5 mg/dL >ULN to 2.88 mmol/L	> 11.5 to 12.5 mg/dL > 2.88 to 3.13 mmol/L	> 12.5 to 13.5 mg/dL > 3.13 to 3.38 mmol/L	> 13.5 mg/dL > 3.38 mmol/L
Infant, < 7 Days	11.5 to 12.4 mg/dL 2.86 to 3.10 mmol/L	> 12.4 to 12.9 mg/dL > 3.10 to 3.23 mmol/L	> 12.9 to 13.5 mg/dL > 3.23 to 3.38 mmol/L	> 13.5 mg/dL > 3.38 mmol/L
Hypocalcemia (ionized)	3.0 mg/dL to < LLN 0.74 mmol/L to < LLN	2.5 to < 3.0 mg/dL 0.62 to < 0.74 mmol/L	2.0 to < 2.5 mg/dL 0.49 to < 0.62 mmol/L	< 2.0 mg/dL < 0.49 mmol/L
Hypercalcemia (ionized)	> ULN to 6.0 mg/dL > ULN to 1.50 mmol/L	> 6.0 to 6.5 mg/dL > 1.50 to 1.63 mmol/L	> 6.5 to 7.0 mg/dL > 1.63 to 1.75 mmol/L	> 7.0 mg/dL > 1.75 mmol/L
Hypomagnesemia	1.40 to <LLN mg/dL 1.2 to <LLN mEq/L 0.58 to <LLN mmol/L	1.04 to < 1.40 mg/dL 0.9 to < 1.2 mEq/L 0.43 to < 0.58 mmol/L	0.67 to < 1.04 mg/dL 0.6 to < 0.9 mEq/L 0.28 to < 0.43 mmol/L	< 0.67 mg/dL < 0.6 mEq/L < 0.28 mmol/L

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hypophosphatemia **Adult and Pediatric > 14 Years**	2.0 to < LLN mg/dL 0.63 to < LLN mmol/L	1.5 to < 2.0 mg/dL 0.47 to < 0.63 mmol/L	1.0 to < 1.5 mg/dL 0.31 to < 0.47 mmol/L	< 1.0 mg/dL < 0.31 mmol/L
Pediatric 1 Year–14 Years	3.0 to 3.5 mg/dL 0.96 to 1.12 mmol/L	2.5 to < 3.0 mg/dL 0.80 to < 0.96 mmol/L	1.5 to < 2.5 mg/dL 0.47 to < 0.80 mmol/L	< 1.5 mg/dL < 0.47 mmol/L
Pediatric < 1 Year	3.5 to 4.5 mg/dL 1.12 to 1.46 mmol/L	2.5 to < 3.5 mg/dL 0.80 to < 1.12 mmol/L	1.5 to < 2.5 mg/dL 0.47 to < 0.80 mmol/L	< 1.5 mg/dL < 0.47 mmol/L
Hyperbilirubinemia **Adult and Pediatric > 14 Days**	> 1.0 to 1.5 × ULN	> 1.5 to 2.5 × ULN	> 2.5 to 5.0 × ULN	> 5.0 × ULN
Infant, ≤ 14 Days (non-hemolytic)	NA	20.0 to 25.0 mg/dL 342 to 428 µmol/L	> 25.0 to 30.0 mg/dL > 428 to 513 µmol/L	> 30.0 mg/dL > 513 µmol/L
Infant, ≤ 14 Days (hemolytic)	NA	NA	20.0 to 25.0 mg/dL 342 to 428 µmol/L	> 25.0 mg/dL > 428 µmol/L
Blood Urea Nitrogen	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
Hyperuricemia	>ULN to 10.0 mg/dL >ULN to 597 µmol/L	> 10.0 to 12.0 mg/dL > 597 to 716 µmol/L	> 12.0 to 15.0 mg/dL > 716 to 895 µmol/L	> 15.0 mg/dL > 895 µmol/L
Hypouricemia	1.5 mg/dL to < LLN 87 µmol/L to < LLN	1.0 to < 1.5 mg/dL 57 to < 87 µmol/L	0.5 to < 1.0 mg/dL 27 to < 57 µmol/L	< 0.5 mg/dL < 27 µmol/L
Creatinine	> 1.50 to 2.00 mg/dL > 133 to 177 µmol/L	> 2.00 to 3.00 mg/dL > 177 to 265 µmol/L	> 3.00 to 6.00 mg/dL > 265 to 530 µmol/L	> 6.00 mg/dL > 530 µmol/L

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Bicarbonate	16.0 mEq/L to < LLN 16.0 mmol/L to < LLN	11.0 to < 16.0 mEq/L 11.0 to < 16.0 mmol/L	8.0 to < 11.0 mEq/L 8.0 to < 11.0 mmol/L	< 8.0 mEq/L < 8.0 mmol/L
Triglycerides (Fasting)	NA	500 to 750 mg/dL 5.64–8.47 mmol/L	> 750 to 1200 mg/dL > 8.47–13.55 mmol/L	> 1200 mg/dL > 13.55 mmol/L
LDL (Fasting)	130 to 160 mg/dL 3.35 to 4.15 mmol/L	>160 to 190 mg/dL >4.15 to 4.92 mmol/L	> 190 mg/dL >4.92 mmol/L	NA
Pediatric >2 to <18 years	110 to 130 mg/dL 2.84 to 3.37 mmol/L	>130 to 190 mg/dL >3.37 to 4.92 mmol/L	> 190 mg/dL >4.92 mmol/L	NA
Hypercholesterolemia (Fasting)	200 to 239 mg/dL 5.16 to 6.19 mmol/L	> 239 to 300 mg/dL > 6.19 to 7.77 mmol/L	> 300 mg/dL > 7.77 mmol/L	NA
Pediatric < 18 Years	170 to 199 mg/dL 4.39 to 5.15 mmol/L	> 199 to 300 mg/dL > 5.15 to 7.77 mmol/L	> 300 mg/dL > 7.77 mmol/L	NA
Creatine Kinase	3.0 to < 6.0 × ULN	6.0 to < 10.0 × ULN	10.0 to < 20.0 × ULN	≥ 20.0 × ULN

*Calcium should be corrected for albumin if albumin is < 4.0 g/dL

ENZYMES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
AST (SGOT)	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
ALT (SGPT)	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
GGT	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
Alkaline Phosphatase	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
Total Amylase	> 1.0 to 1.5 × ULN	> 1.5 to 2.0 × ULN	> 2.0 to 5.0 × ULN	> 5.0 × ULN
Pancreatic Amylase	> 1.0 to 1.5 × ULN	> 1.5 to 2.0 × ULN	> 2.0 to 5.0 × ULN	> 5.0 × ULN
Lipase	> 1.0 to 1.5 × ULN	> 1.5 to 3.0 × ULN	> 3.0 to 5.0 × ULN	> 5.0 × ULN
Albumin	3.0 g/dL to < LLN 30 g/L to < LLN	2.0 to < 3.0 g/dL 20 to < 30 g/L	< 2.0 g/dL < 20 g/L	NA

URINALYSIS				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hematuria (Dipstick)	1+	2+	3-4+	NA
Hematuria (Quantitative) See Note below Females	>ULN - 10 RBC/HPF	> 10-75 RBC/HPF	> 75 RBC/HPF	NA
Males	6-10 RBC/HPF	> 10-75 RBC/HPF	> 75 RBC/HPF	NA
Proteinuria (Dipstick)	1+	2–3+	4+	NA
Proteinuria, 24 Hour Collection **Adult and Pediatric ≥ 10 Years**	200 to 999 mg/24 h	>999 to 1999 mg/24 h	>1999 to 3500 mg/24 h	> 3500 mg/24 h
Pediatric > 3 Mo to < 10 Years	201 to 499 mg/m^2/24 h	>499 to 799 mg/m^2/24 h	>799 to 1000 mg/m^2/24 h	> 1000 mg/ m^2/24 h
Glycosuria (Dipstick)	1+	2-3+	4+	NA

Notes:
Toxicity grades for Quantitative and Dipstick Hematuria will be assigned by Covance Laboratory, however for other laboratories, toxicity grades will only be assigned to Dipstick Hematuria.
With the exception of lipid tests, any graded laboratory test with a result that is between the LLN and ULN should be assigned Grade 0.
If the severity of a clinical AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE.

CARDIOVASCULAR				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Cardiac Arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic, non-life-threatening AND Non-urgent medical intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Cardiac-ischemia/Infarction	NA	NA	Symptomatic ischemia (stable angina) OR Testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Hemorrhage (significant acute blood loss)	NA	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≤ 2 units packed RBCs (for children ≤ 10 cc/kg) indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs indicated (for children ≤ 10 cc/kg) indicated
Hypertension (with repeat testing at same visit)	140–159 mmHg systolic OR 90–99 mmHg diastolic	> 159–179 mmHg systolic OR > 99–109 mmHg diastolic	> 179 mmHg systolic OR > 109 mmHg diastolic	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization (other than ER visit) indicated
Pediatric ≤ 17 Years (with repeat testing at same visit)	NA	91st–94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure

CARDIOVASCULAR				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Pericardial Effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no intervention	Effusion with non-life-threatening physiologic consequences OR Effusion with nonurgent intervention indicated	Life-threatening consequences (eg, tamponade) OR Urgent intervention indicated
Prolonged PR Interval	PR interval 0.21 to 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec	Complete AV block
Pediatric ≤ 16 Years	1st degree AV block (PR > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block	Complete AV block
Prolonged QTc	Asymptomatic, QTc interval 0.45 to 0.47 sec OR Increase interval < 0.03 sec above baseline	Asymptomatic, QTc interval 0.48 to 0.49 sec OR Increase in interval 0.03 to 0.05 sec above baseline	Asymptomatic, QTc interval ≥ 0.50 sec OR Increase in interval ≥ 0.06 sec above baseline	Life-threatening consequences, eg, Torsade de pointes or other associated serious ventricular dysrhythmia
Pediatric ≤ 16 Years	Asymptomatic, QTc interval 0.450 to 0.464 sec	Asymptomatic, QTc interval 0.465 to 0.479 sec	Asymptomatic, QTc interval ≥ 0.480 sec	Life-threatening consequences, eg, Torsade de pointes or other associated serious ventricular dysrhythmia
Thrombosis/Embolism	NA	Deep vein thrombosis AND No intervention indicated (eg, anticoagulation, lysis filter, invasive procedure)	Deep vein thrombosis AND Intervention indicated (eg, anticoagulation, lysis filter, invasive procedure)	Embolic event (eg, pulmonary embolism, life-threatening thrombus)
Vasovagal Episode (associated with a procedure of any kind)	Present without loss of consciousness	Present with transient loss of consciousness	NA	NA

CARDIOVASCULAR				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Ventricular Dysfunction (congestive heart failure, CHF)	NA	Asymptomatic diagnostic finding AND intervention indicated	New onset with symptoms OR Worsening symptomatic CHF	Life-threatening CHF

RESPIRATORY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Bronchospasm (acute)	FEV1 or peak flow reduced to 70% to 80%	FEV1 or peak flow 50% to 69%	FEV1 or peak flow 25% to 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or Respiratory Distress	Dyspnea on exertion with no or minimal interference with usual social & functional activities	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities	Dyspnea at rest causing inability to perform usual social & functional activities	Respiratory failure with ventilatory support indicated
Pediatric < 14 Years	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90% to 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilatory support indicated

OCULAR/VISUAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Uveitis	Asymptomatic but detectable on exam	Symptomatic anterior uveitis OR Medical intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Visual Changes (from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)

SKIN				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Alopecia	Thinning detectable by study participant or caregiver (for disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	NA
Cutaneous Reaction – Rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	NA	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA

GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [eg, tube feeding or total parenteral nutrition]
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (eg, diuretics or therapeutic paracentesis)	Symptomatic despite intervention	Life-threatening consequences
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (eg, sepsis or perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (eg, obstruction)
Diarrhea **Adult and Pediatric ≥ 1 Year**	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline/24 hr	Persistent episodes of unformed to watery stools OR Increase of 4–6 stools over baseline per 24 hrs.	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (eg, hypotensive shock)
Pediatric < 1 Year	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Liquid stools resulting in severe dehydration with aggressive rehydration indicated OR Hypotensive shock
Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated	Life-threatening reduction in oral intake

GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Mucositis/Stomatitis (clinical exam) See also Proctitis, Dysphagia-Odynophagia	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences (eg, aspiration, choking)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24–48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (eg, IV fluids)	Life-threatening consequences (eg, hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than ER visit)	Symptomatic AND Hospitalization indicated (other than ER visit)	Life-threatening consequences (eg, sepsis, circulatory failure, hemorrhage)
Proctitis (functional-symptomatic) Also see Mucositis/ Stomatitis for Clinical Exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social/ functional activities OR Operative intervention indicated	Life-threatening consequences (eg, perforation)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated	Life-threatening consequences (eg, hypotensive shock)

NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Alteration in Personality-Behavior or in Mood (eg, agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with usual social & functional activities	Alteration causing inability to perform usual social & functional activities	Behavior potentially harmful to self or others (eg, suicidal/homicidal ideation or attempt, acute psychosis) OR Causing inability to perform basic self-care functions
Altered Mental Status For Dementia, see Cognitive and Behavioral/Attentional Disturbance (including dementia and ADD)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions
Cognitive and Behavioral/Attentional Disturbance (including dementia and Attention Deficit Disorder)	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
CNS Ischemia (acute)	NA	NA	Transient ischemic attack	Cerebral vascular accident (CVA, stroke) with neurological deficit

NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Developmental delay – **Pediatric ≤ 16 Years**	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than ER visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social/functional activities	Difficulty sleeping causing inability to perform usual social & functional activities	Disabling insomnia causing inability to perform basic self-care functions
Neuromuscular Weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal interference with usual social & functional activities	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory Alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions

NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Seizure: (new onset)	NA	1 seizure	2–4 seizures	Seizures of any kind that are prolonged, repetitive (eg, status epilepticus), or difficult to control (eg, refractory epilepsy)
Seizure: (pre-existing) For Worsening of Existing Epilepsy the Grades Should Be Based on an Increase from Previous Level of Control to Any of These Levels	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR infrequent breakthrough seizures while on stable meds in a previously controlled seizure disorder	Change in seizure character from baseline either in duration or quality (eg, severity or focality)	Seizures of any kind that are prolonged, repetitive (eg, status epilepticus), or difficult to control (eg, refractory epilepsy)
Seizure – Pediatric < 18 Years	Seizure, generalized onset with or without secondary generalization, lasting < 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5–20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Syncope (not associated with a procedure)	NA	Present	NA	NA
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions

MUSCULOSKELETAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss	BMD t-score or z-score –2.5 to –1.0	BMD t-score or z-score < –2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Pediatric < 21 Years	BMD z-score –2.5 to –1.0	BMD z-score < –2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	Asymptomatic with radiographic findings AND No operative intervention indicated	Symptomatic bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions

SYSTEMIC				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Acute Systemic Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions
Fever (nonaxillary)	37.7°C to 38.6°C 99.8°F to 101.5°F	38.7°C to 39.3°C 101.6°F to 102.8°F	39.4°C to 40.5°C 102.9°F to 104.9°F	> 40.5°C > 104.9°F
Pain- Indicate Body Site See also Injection Site Pain, Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than ER visit) indicated
Unintentional Weight Loss	NA	5% to 9% loss in body weight from baseline	10% to 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [eg, tube feeding or total parenteral nutrition]

INJECTION SITE REACTION				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Injection Site Pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than ER visit) indicated for management of pain/tenderness
Injection Site Reaction (Localized), > 15 Years **Pediatric ≤ 15 Years**	Erythema OR Induration of 5 × 5 cm to 9 × 9 cm (or 25–81 × cm^2) **Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter**	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2) **Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (eg, upper arm/thigh)**	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage **Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (eg, upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage**	Necrosis (involving dermis and deeper tissue) **Necrosis (involving dermis and deeper tissue)**
Pruritis Associated with Injection See also Skin: Pruritis (itching—no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 h treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 h treatment	Generalized itching causing inability to perform usual social & functional activities	NA

ENDOCRINE/METABOLIC				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Lipodystrophy (eg, back of neck, breasts, abdomen)	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	NA
Diabetes Mellitus	NA	New onset without need to initiate medication OR Modification of current meds to regain glucose control	New onset with initiation of indicated med OR Diabetes uncontrolled despite treatment modification	Life-threatening consequences (eg, ketoacidosis, hyperosmolar non-ketotic coma)
Gynecomastia	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (eg, thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (eg, myxedema coma)
Lipoatrophy (eg, fat loss from the face, extremities, buttocks)	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA

GENITOURINARY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Intermenstrual Bleeding (IMB)	Spotting observed by participant OR Minimal blood observed during clinical or colposcopic exam	Intermenstrual bleeding not greater in duration or amount than usual menstrual cycle	Intermenstrual bleeding greater in duration or amount than usual menstrual cycle	Hemorrhage with life-threatening hypotension OR Operative intervention indicated
Urinary Tract obstruction (eg, stone)	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences

INFECTION				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Infection (any other than HIV infection)	Localized, no systemic antiμbial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antiμbial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antiμbial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (eg, septic shock)

Basic Self-care Functions: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.
Usual Social & Functional Activities: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Appendix 5. Pregnancy Precautions, Definition for Female of Childbearing Potential

1. Pregnancy and Contraception Requirements for Males and Females of Childbearing Potential

The risks of treatment with Tenofovir DF during pregnancy have not been evaluated. Pregnancy must be excluded before the start of treatment with Tenofovir DF and prevented thereafter by reliable contraceptive methods. Pregnancy tests will be performed regularly throughout this study. Data available at this time suggest that this drug does not have a drug-drug interaction (DDI) with hormones used for contraception. Please refer to the latest version of the investigator's brochure for additional information.

2. Definition of Female of Childbearing Potential

For the purposes of this study, a female subject of childbearing potential is a woman who has not had a hysterectomy, bilateral oophorectomy, or medically documented ovarian failure. This definition includes pubertal females regardless of whether or not she has had a menses (premenarchal, Tanner Stage 3) and perimenopausal women who have had a spontaneous menses in the last 12 months. A woman who has had a tubal sterilization is considered to be of childbearing potential.

Women ≤ 54 years of age with amenorrhea of any duration will be considered to be of childbearing potential unless they have had a hysterectomy, bilateral oophorectomy, or medically documented ovarian failure.

Women > 54 years of age with cessation (for ≥12 months) of previously occurring menses due to ovarian failure will not be considered to be of childbearing potential.

3. Contraceptive Requirements for Females

Female subjects of childbearing potential must agree to use protocol specified method(s) of contraception from the screening/enrollment visit throughout the study period and for 30 days following the last dose of Tenofovir DF or choose continuous heterosexual abstinence as a lifestyle choice. The investigator should counsel subjects on the protocol specified method(s) for avoiding pregnancy during the study. These methods are recommended due to the low failure rate (ie, less than 1% per year). See Appendix Table 1 for the protocol specified contraceptive methods. Protocol specified contraceptive methods are as follows: (1) a combination of one hormonal method and one barrier method; (2) two barrier methods where one method is the male condom; or (3) use of an intrauterine device (IUD) or tubal sterilization; see below. Acceptable hormonal methods include injectable progesterone, progesterone implants, combination oral contraceptives, transdermal contraceptive patch, and vaginal ring. Acceptable barrier methods include diaphragm with spermicide, cervical cap with spermicide, and the male condom. Female subjects must use either a hormonal method or a barrier method if the partner has a vasectomy. If a subject has undergone tubal sterilization or has had a Copper T 380A IUD or LNg 20 IUD inserted, no other contraception is needed.

Complete abstinence from intercourse. Abstinence is an acceptable method of contraception only when it is in line with the subject's preferred and usual lifestyle. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Female study subjects who are not heterosexually active must have periodic confirmation of continued abstinence from heterosexual intercourse and regular pregnancy testing while taking Tenofovir DF. The investigator should counsel subjects on the protocol specified method(s) for avoiding pregnancy in case the subject chooses to engage in heterosexual intercourse.

Female subjects of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test at baseline (Day 1) prior to receiving the first dose of Tenofovir DF. Lactating females must discontinue nursing before Tenofovir DF administration.

Appendix Table 1. Protocol Specified Contraceptive Methods

	Combination Methods	
Methods to Use by Themselves	**Hormone Methods** (choose one and use with a barrier method)	**Barrier Methods** (use both OR choose one and use with a hormone method)
Intrauterine Devices (IUDs) Copper T 380A IUD LNg 20 IUD **Tubal Sterilization**	**Estrogen and Progesterone** Oral contraceptives Transdermal patch Vaginal ring **Progesterone** Injection Implant	Diaphragm with spermicide OR Cervical cap with spermicide Male condom (with or without spermicide)
	Partner's vasectomy must be used with a hormone or barrier method.	

If tubal sterilization is via the Essure procedure, verification of tubal blockage by hysterosalpingogram (HSP) must be performed approximately 3 months after microinsertion. Prior to verification, Essure is not considered a reliable form of contraception and another contraception method described above should be used.

4. Contraceptive Requirements for Males

Male subjects must agree to use condoms and avoid sperm donation from the screening/enrollment visit throughout the study period and for 30 days after administration of the last dose of Tenofovir DF.

Use of condoms (except for lambskin) has been proven to decrease the risk of transmission of HIV and other sexually transmitted diseases. The use of spermicide is not recommended if the subject or subject's partner is infected with HIV.

5. Procedures to be Followed in the Event of Pregnancy

Subjects should be instructed to notify the investigator if they become pregnant at any time during the study, or if they become pregnant within 30 days of last Tenofovir DF dose. Subjects who become pregnant or who suspect that they are pregnant during the study must report the information to the investigator. The investigator should report all pregnancies to the Gilead DSPH using the pregnancy report form within 24 hours of becoming aware of the pregnancy.

Instructions for reporting pregnancy, and pregnancy outcome are outlined in Section 7.6.2.1.

Appendix 6. Lactic Acidosis Guidelines

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogues alone or in combination with other antiretrovirals. A majority of these cases have been in women. Obesity and prolonged nucleoside exposure may be risk factors; however, cases have also been reported in subjects with no known risk factors.

Guidelines for management of symptomatic hyperlactatemia and asymptomatic hyperlactatemia are outlined in Section A and B below and are derived from the AIDS Clinical Trials Group (ACTG) Lactic Acidosis Guidelines. Section C outlines venous lactate collection techniques.

Section A. Symptomatic Hyperlactatemia

Symptomatic hyperlactatemia is defined as a clinical suspicion of hyperlactatemia characterized by new, otherwise unexplained and persistent (≥ 2 weeks) occurrence of 1 or more of the following symptoms:

- Nausea and vomiting
- Abdominal pain or gastric discomfort
- Abdominal distention
- Increased LFTs
- Unexplained fatigue
- Dyspnea

AND

- Venous lactate level greater than twice the upper normal limit (ULN) confirmed by repeat venous lactate analysis within 1 week and, if persistently elevated, arterial lactate with blood gas analysis.

If the repeat venous lactate is elevated confirmation with an arterial lactate specimen and arterial blood gas (pH, PO_2, PCO_2, bicarbonate, oxygen saturation) should be performed within 48 hours. If the arterial specimen contains lactate at a level more than two times the upper limit of normal, the patient should be discontinued from the study and alternative therapy instituted. Subjects should be monitored weekly until signs and symptoms resolve. Hyperlactatemia should be followed until levels return to below two times the ULN and the patient.

An elevated anion gap in a patient with metabolic acidosis suggests the diagnosis of lactic acidosis. It can be suspected when the sum of cations minus the sum of anions [$(Na^+ + K^+) - (Cl^- + HCO_3^-)$] exceeds 18 mEq/L (18 mmol/L) in the absence of other causes of increased anion gap such as renal failure, salicylate ingestion or other poisoning, or significant ketonemia (e.g., diabetic ketoacidosis, alcohol).

Management of symptomatic subjects with lactate levels of 1 to 2 times the ULN is left to the discretion of the Investigator. As some of the symptoms are sufficiently vague (e.g., fatigue) to be present in everyone, serial repeat testing is encouraged with plans to modify the regimen if the lactate level rises to greater than two times the ULN as outlined above.

Section B. Asymptomatic Hyperlactatemia

In ASYMPTOMATIC subjects, lactic acidosis will be defined as hyperlactatemia greater than four times the ULN. Any patient with a lactate level more than two times the ULN but less than or equal to four times the ULN, should be questioned closely for symptoms (described above) and have a repeat venous sample obtained in 1 week, and, if confirmed, subsequently at monthly intervals.

If the patient fulfills the definition for ASYMPTOMATIC hyperlactatemia, repeat venous lactate should be obtained within a week with confirmation of a more than 4-fold venous elevation in lactate by arterial lactate measurement and arterial blood gas (pH, PO_2, PCO_2, bicarbonate, oxygen saturation) within 48 hours. If confirmed, the patient should be discontinued from the study and alternative therapy instituted. Hyperlactatemia should be followed until levels return to below two times the ULN.

Section C. Specimen Collection

Venous lactate levels are highly dependent on collection techniques. It is therefore recommended that the instructions below be followed closely. High lactate levels should be repeated for verification. If carefully collected, venous lactate level is equivalent to an arterial collection in most clinical situations. If it is not possible to collect the specimen without hand clenching or prolonged tourniquet time, an arterial lactate should be considered, as this will help exclude falsely elevated lactate levels.

1. Have subject sit, relaxed for 5 minutes prior to venipuncture.

2. Instruct subject to not clench the fist before or during the procedure and to relax the hand as much as possible.

3. If possible, do not use a tourniquet. If a tourniquet is necessary, then apply tourniquet lightly and draw lactate first before the other samples with the tourniquet still in place.

4. Collect the blood in a chilled gray-top (sodium fluoride-potassium oxalate) tube.

5. Place the specimen immediately on ice and send to the laboratory for immediate processing, preferably within 30 minutes of collection.

6. If random lactate is elevated, then repeat as above with the following additional patient instructions: no alcohol within 24 hours, no exercise within 8 hours, and no food or drink except water within 4 hours of the draw.