CLINICAL STUDY PROTOCOL

Study Title:	A Randomized, Double-Blind Evaluation of the Antiviral Efficacy, Safety, and Tolerability of Tenofovir Disoproxil Fumarate Versus Placebo in Pediatric Patients with Chronic Hepatitis B Infection	
Sponsor:	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404 USA	
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Indication:	Chronic Hepatitis B	
Protocol ID:	GS-US-174-0144	
Gilead Sciences Medical Monitor/Study Director:	Name: Telephone: Fax: Mobile E-mail:	John Flaherty, PharmD PPD PPD PPD PPD
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PROTOCOL SYNOPSIS
Gilead Sciences, Inc.
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Study Title:	A Randomized, Double-Blind Evaluation of the Antiviral Efficacy, Safety, and Tolerability of Tenofovir Disoproxil Fumarate Versus Placebo in Pediatric Patients with Chronic Hepatitis B Infection
IND Number:	71,576
EudraCT Number:	2012-000586-20
Study Centers Planned:	Approximately 35 centers in North America, Europe, and Asia
Objectives:	The primary objective of this study is: • To evaluate the antiviral efficacy of tenofovir DF versus placebo in pediatric patients (aged 2 to < 12 years, at the time of enrollment) with chronic hepatitis B infection The key secondary objective is: • To evaluate the proportion of subjects with HBeAg seroconversion at Week 48 (in subjects with baseline HBeAg sero-positivity). Secondary objectives are: • To characterize the safety and tolerability profile of tenofovir DF in pediatric patients (aged 2 to < 12 years, at the time of enrollment) with chronic hepatitis B infection • To evaluate the biochemical and serological responses to tenofovir DF versus placebo • To evaluate the incidence of potential resistance mutations to tenofovir DF in the hepatitis B virus polymerase/reverse transcriptase (pol/RT) • To assess the pharmacokinetics of tenofovir in subjects receiving the tablet formulation and those receiving the oral powder formulation.

Study Design:

This is a Phase 3, randomized, prospective, double-blind study comparing the antiviral efficacy, safety, and tolerability of tenofovir DF to placebo in pediatric patients with chronic HBV infection. One hundred (100) tenofovir DF-naive pediatric patients aged 2 to < 12 years, at the time of enrollment, with chronic HBV infection (either HBeAg-positive or HBeAg-negative), HBV DNA $\geq 10^5$ copies/mL AND ALT $\geq 1.5 \times$ ULN at screening will be randomized in a 2:1 ratio to treatment arm A or B:

- Treatment A (n = 67): tenofovir DF PO once daily for 48 weeks
- Treatment B (n = 33): matching placebo PO once daily for 48 weeks

After 48 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for an additional 144 weeks. Subjects under Protocol Amendment 3 who are beyond Week 48 of blinded randomized treatment will switch to open-label tenofovir DF at the Week 72 visit and will continue on open-label treatment until Week 192.

After the completion of the study at Week 192, all subjects who have completed the study will be offered open label tenofovir DF under the Extension Phase of the protocol until it is commercially available in that country for treatment of Chronic HBV in patients of their age and weight. During the Extension Phase, subjects will return for study visits every 12 weeks to assess efficacy and safety, to conduct study drug accountability, and dispense study drug.

Randomization will be stratified by age (2 to < 6 and 6 to < 12, at the time of enrollment) and geographical location of study site (North America/Europe and Asia).

Randomization cannot occur until after the Baseline (pre-treatment) dual-energy X-ray absorptiometry (DEXA) scan has been performed.

Tenofovir DF tablets or oral powder, and their corresponding matching placebos will be provided by the Sponsor. During either the blinded randomized treatment or the open-label tenofovir DF treatment period, subjects whose body weight increases to ≥ 17 kg may be switched from the oral powder to the tenofovir DF tablet (or matching placebo tablet, if applicable).

Subjects must be naive to tenofovir DF, but could have received interferon-alfa and/or other oral anti-HBV nucleoside/nucleotide therapy. Subjects experienced on oral anti-HBV nucleoside/nucleotide therapy must have discontinued therapy ≥ 16 weeks prior to screening. Subjects must have discontinued interferon-alfa ≥ 6 months prior to screening.

	Subjects who experience a Grade 4 ALT elevation while on blinded study medication will be evaluated weekly with serum chemistry and liver function test monitoring. In the event that any subject has a sustained Grade 4 ALT for ≥ 16 weeks (i.e., failure to resolve ALT to grade ≤ 3 or baseline), the serial HBV DNA values on study will be provided to the investigator, and the subject can be offered open-label tenofovir DF after discussion with the Gilead Medical Monitor. Such events should be recorded as an adverse event (AE) leading to blinded study drug discontinuation. All subjects will take a daily multivitamin with no additional mineral components that contain a minimum of 400 IU of vitamin D or higher per country specific regulations. The primary analysis will be conducted at the completion of double-blinded treatment, after the last randomized subject reaches Week 48. Subsequently, efficacy and safety analyses of all subjects continuing on open-label tenofovir DF will be performed after the last randomized subject reaches Weeks 144 and 192, respectively. During the blinded portion of the study, HBV DNA results will not be distributed to investigators, subjects, or clinical research personnel involved with the clinical conduct of the study. The only exception, as mentioned earlier, will be in the event of a subject having Grade 4 ALT values that persist for 16 weeks and in the case of an ALT flare, both of which are considered situations of medical need, such that the patient's serial HBV DNA values from the Screening visit through the time of the event will be made available to the investigator. An external independent multidisciplinary Data Monitoring Committee (DMC) will review the progress and safety of this study approximately every 24 weeks following the time of randomization of the first subject. During the open-label duration of the study, the DMC will convene approximately every 52 weeks. At each meeting, the DMC will review routine safety and DEXA data and will make recommendations regarding modification of study treatment.
Number of Subjects Planned:	One hundred (100) subjects, randomized in a 2:1 ratio to receive treatment with tenofovir DF or placebo.
Target Population:	Pediatric subjects, aged 2 to < 12, at the time of enrollment, with chronic HBV infection

Duration of Treatment:	Subjects will be treated with blinded randomized therapy for 48 weeks (approximately 1 year), followed by an additional 144 weeks (approximately 3 years) of open-label tenofovir DF therapy. After the completion of the study at Week 192, all subjects who have completed the study will be offered open label tenofovir DF under the Extension Phase of the protocol until it is commercially available in that country for treatment of chronic HBV in patients of their age and weight. During the Extension Phase, subjects will return for study visits every 12 weeks to assess efficacy and safety, to conduct study drug accountability, and dispense study drug.
Diagnosis and Main Eligibility Criteria:	At screening, pediatric subjects (2 to < 12 years of age, at the time of enrollment) with chronic HBeAg-positive or HBeAg-negative HBV infection (HBsAg-positive for at least 6 months; with HBV DNA $\geq 10^5$ copies/mL, ALT $\geq 1.5 \times$ ULN and creatinine clearance ≥ 80 mL/min/1.73 m^2 at screening) will be eligible for the study. Subjects must be naive to tenofovir DF, but could have received interferon-alfa and/or other oral anti-HBV nucleoside/nucleotide therapies. Subjects experienced on oral anti-HBV nucleoside/nucleotide therapy must have discontinued therapy ≥ 16 weeks prior to screening. Subjects must have discontinued interferon-alfa ≥ 6 months prior to screening. Subjects must be without serological evidence of co-infection with HIV, HCV, HDV or acute HAV. Subjects with a history of significant renal disease, bone disease, decompensated liver disease, evidence of hepatocellular carcinoma (i.e., α-fetoprotein > 50 ng/mL), or any chronic liver disease not related to HBV-infection will not be eligible for the study.
Study Procedures/ Frequency:	Plasma HBV DNA levels, laboratory analyses (serum chemistry, liver tests, hematology, and urinalysis), pregnancy test (females of childbearing potential only), vital signs, adverse events and concomitant medications will be measured or assessed at screening, baseline, Weeks 4, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, then every 12 weeks thereafter until the end of the study (and at Early Discontinuation or Extension Phase, if applicable). HBV serology (HBsAg, HBeAg, and reflex HBeAb and HBsAb if Ag negative) will be conducted at Screening, Baseline, Weeks 16, 32, 48, 64, 72, 80, and 96, then every 12 weeks through the end of study (and at Early Discontinuation or during the Extension Phase, if applicable). DEXA scans of the spine and whole body will be performed at Baseline, and Weeks 24, 48, 72, and 96, then annually until completion of the study (and at Early Discontinuation or during the Extension Phase, if applicable). Bone biochemical markers will be

	measured at Screening, Baseline, every 24 weeks through Week 96, then annually until the end of study (and at Early Discontinuation or during the Extension Phase, if applicable). DEXA and bone biochemical markers will also be required at time of switching from placebo to tenofovir DF, if the last measurement was performed > 12 weeks prior to switch. Complete physical examinations (including Tanner Staging starting at Baseline) will be performed at Screening, Baseline, Week 24 and then every 24 weeks through the end of study (and at Early Discontinuation, if applicable), and every 48 weeks during the Extension Phase, if applicable. Determination of HBV viral genotype (A-H) will be performed at baseline for all subjects. Subjects will maintain a Subject Dosing Diary Card to monitor subjects' compliance to study treatment at Weeks 4, 24, and 56. Subjects will maintain a diary for 10 days prior to the next visit. In addition, for subjects participating in the PK substudy, a diary card will be maintained for at least 10 days prior to the intensive PK visit. Resistance surveillance will be conducted at Baseline for all subjects, and attempted for all viremic subjects (HBV DNA ≥ 400 copies/mL [69 IU/mL]) at Weeks 48, 96, 144, and 192 (and at Early Discontinuation, if applicable). Plasma and serum for storage will be collected at every visit starting at Baseline for possible pharmacokinetic and/or virological analyses (including resistance surveillance, HBsAg and HBeAg quantification and adherence assessment). For subjects in whom a separate consent is provided, a blood sample for biomarker (including pharmacogenomic analysis) will be collected for the exploration of appropriate markers that may be predictive of virologic response and/or the tolerability of HBV therapies.
Test Product, Dose, and Mode of Administration:	Tenofovir disoproxil fumarate oral tablet • subjects ≥ 17 kg: one tablet once daily (150, 200, 250 or 300 mg tablets based on body weight) Tenofovir disoproxil fumarate oral powder • subjects < 17 kg and subjects ≥ 17 kg who are unable to swallow a tablet: once daily in a dose of 8 mg/kg up to a maximum daily dose of 300 mg

Reference Therapy, Dose, and Mode of Administration:	Matching placebo oral tablet • subjects ≥ 17 kg: one tablet once daily (matching of physical appearance as the corresponding active tablet) Matching oral placebo powder • subjects < 17 kg and subjects ≥ 17 kg who are unable to swallow a tablet: once daily with matching dose

Criteria for Evaluation:

Efficacy:

The primary efficacy endpoint is the proportion of patients with serum HBV DNA < 400 copies/mL (69 IU/mL) at Week 48 in each arm.

The key secondary endpoint is the proportion of patients with HBeAg seroconversion (in subjects with baseline HBeAg sero-positivity only) at Week 48 in each arm.

At Week 48, the secondary endpoints are:

- proportion of subjects with normal ALT and normalization of ALT
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL (69 IU/mL) and normalized ALT
- proportions of subjects with HBV DNA < 169 copies/mL (29 IU/mL)
- proportions of subjects with HBsAg loss and seroconversion
- proportions of subjects with HBeAg loss (in patients with baseline HBeAg positive patients only)
- sequence changes from baseline within the HBV polymerase for subjects who were viremic (HBV DNA ≥ 400 copies/mL [69 IU/mL]) at Weeks 48, 96, 144, 192 or Early Discontinuation; including subjects with confirmed virologic breakthrough

Similar secondary efficacy endpoints will also be explored at Weeks 144 and 192.

For the primary endpoint, key secondary endpoint, and categorical secondary efficacy endpoints, missing data will be handled using a missing = failure approach.

Safety:	Adverse events and clinical laboratory tests will be collected and characterized at every visit throughout the study. At Week 48, the safety endpoints are: • cumulative incidence of at least a 4% decrease from baseline in bone mineral density of lumbar spine • percent change from baseline in bone mineral density of lumbar spine
PK:	To evaluate the PK of different formulations of tenofovir DF in a pediatric population, two intensive PK substudies (oral powder and tablet cohorts) will be performed on a subset of subjects. The intensive PK sampling will be performed over 8 hours during one day between Week 2 and Week 12. A target of 12 randomized subjects at each dosage level (150, 200, 250, 300 mg) will be enrolled in the tablet cohort, and up to a total of 30 randomized subjects will be enrolled in the oral powder cohort. Subjects are allowed to participate in up to two intensive PK substudies with the second intensive PK substudy occurring after Week 12, but prior to the end of the double-blind phase. Subjects who are eligible to take the oral powder (either tenofovir DF or matching placebo) based on body weight and/or preference for this dosage form, and subjects who qualify for the tablet formulation but are willing to initiate treatment with the oral powder for purposes of accruing additional PK data, will be offered the opportunity to participate in the oral powder cohort. Those subjects who opt to initiate treatement with oral powder and subsequently switch to tablets following completion of the powder intensive PK visit,or those subjects who are required to change dose strengths of the tablet based on the weight-based dosing requirements (see protocol Section 5.3) will also have the option to participate in a second tablet intensive PK visit. Irrespective of choice of formulation (tablet versus oral powder), if a subject is switching from tablets to powder, the subject must be taking powder for at least 2 weeks prior to the powder intensive PK visit. Similarly, subjects must be taking tablets for at least 2 weeks prior to the tablet intensive PK visit.

Statistical Methods:

The primary efficacy analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 48. The analysis will evaluate the difference in the proportion of subjects achieving the primary efficacy endpoint between the tenofovir DF and placebo treatment groups using a two-sided Fisher's exact test with missing data handled using a missing = failure approach. Efficacy analyses of all subjects continuing on open-label tenofovir DF will be performed after the last randomized subject reaches Weeks 144 and 192.

Subgroup analyses of efficacy endpoints may include analyses by randomization stratification group (age [2 to < 6 and 6 to < 12] and geographical location of study site [North America/Europe and Asia]).

The proportion of subjects in each treatment arm with an adverse event or an abnormal laboratory test will be summarized at Week 48 and also at Weeks 144 and 192.

All continuous endpoints will be summarized using n, mean, standard deviation, median, Q1, Q3, minimum and maximum by treatment group. All categorical secondary endpoints will be summarized by number and percentage of subjects who meet the endpoint.

A sample size of 100 subjects (67 tenofovir DF, 33 placebo) would provide at least 85% power to detect a 20% treatment difference between tenofovir DF and placebo in the primary efficacy endpoint. This calculation is based on a two-sided Fisher's exact test with a significance level of 0.05, assuming a response rate of 1% in the placebo group. A similar placebo-response rate was observed in study GS-US-174-0115.

The following plasma pharmacokinetic parameters will be calculated for the PK substudy populations: CL/F,C_{max}, T_{max}, C_{last}, T_{last}, C_{tau}, λ_z, $T_{½}$, $AUC_{0\text{-}last}$, AUC_{tau}.

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

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

°C	degrees Celsius
°F	degrees Fahrenheit
ACTG	AIDS Clinical Trials Group
ADV	adefovir dipivoxil, Hepsera®
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BMD	bone mineral density
BUN	blood urea nitrogen
CBC	complete blood count
CHB	Chronic hepatitis B
CC_{50}	median concentration curve
Cl/F	apparent oral clearance following administration of the drug
CPK	creatine phosphokinase
CPMP	Committee for Proprietary Medicinal Products
CRF	Case Report Form(s)
CRO	Contract Research Organization
d4T	stavudine, Zerit®
dATP	deoxyadenosine triphosphate
$DAVG_{48}$	time-weighted average change from baseline through Week 48
DEXA	dual-energy x-ray absorptiometry
DHBV	duck hepatitis B virus
dL	Deciliter
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DSPH	Drug Safety and Public Health
EC_{50}	median effective concentration
ECG	Electrocardiogram
EEG	Electroencephalogram
EFV	Efavirenz
EMEA	European Medicines Evaluation Agency
ETV	Entecavir
EU	European Union
EudraCT	European clinical trials database
FDA	(U.S.) Food and Drug Administration
FTC	emtricitabine, Emtriva™

FAS	full analysis set
g	gram(s)
GCP	Good Clinical Practice (Guidelines)
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
HAART	highly-active antiretroviral therapy
HAV	hepatitis A virus
HBeAb	hepatitis B e antibody
HBeAg	hepatitis B e antigen
HBsAb	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDPE	high-density polyethylene
HDV	hepatitis D virus
HLGT	high level group term
HLT	high level term
HIV	human immunodeficiency virus
IC_{50}	median inhibitory concentration
ICH	International Conference on Harmonization
ID	Identification
IEC	Independent Ethics Committee
IL-2	interleukin-2
IND	Investigational New Drug (Application)
INR	international normalized ratio
IRB	Institutional Review Board
IV	Intravenous
kg	kilogram(s)
K_i	inhibition constant
L	liter
LAM	lamivudine, Epivir®, Zeffix®, 3TC
LAM^R	lamivudine resistant
LDH	lactate dehydrogenase
LFT	liver function test
LLN	lower limit of normal
LLOQ	lower limit of quantification
LLT	lower level term
LOD	limit of detection
m^2	square meter(s)

MedDRA	Medical Dictionary for Regulatory Activities
mEq	milliequivalent(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm^3	cubic millimeter(s)
mmol	millimole(s)
ng	nanogram(s)
NRTI	nucleoside reverse transcriptase inhibitor
OBR	optimized background (antiretroviral) regimen
OLT	orthotopic liver transplant
PA	Protocol Amendment
PBMC	peripheral blood mononuclear cell(s)
PCR	polymerase chain reaction
PK	Pharmacokinetic
PO	by mouth/oral (per os)
PT	prothrombin time
PTH	parathyroid hormone
RAT	randomized and treated
RBC	red blood cell
RDA	recommended daily allowance
RNA	ribonucleic acid
SAE	serious adverse event
SCr	serum creatinine
SOC	system organ class
TDF	tenofovir DF, tenofovir disoproxil fumarate, Viread®
TFV-DP	tenofovir diphosphate
µg	Microgram
µM	Micromolar
ULN	upper limit of the normal range
WBC	white blood cell
WHV	woodchuck hepatitis virus

1. INTRODUCTION

1.1. Background

Chronic hepatitis B is a serious global health care problem and a major cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC). Worldwide, approximately 350 to 400 million people have developed chronic hepatitis B, including approximately 1.2 million in the United States {Wright 2006}. Globally, approximately 1 million people die annually of complications of chronic hepatitis B {World Health Organization (WHO) 2000}, {Wright 2006} In the United States, an estimated 5,000 people die each year of complications of HBV infection {Poterucha et al 1997}.

Despite the availability of hepatitis B virus (HBV) vaccine programs in many countries, new hepatitis B infections are still common, even in areas of low endemnicity. For example, approximately 70,000 people in the United States (US) become acutely infected each year, according to estimates from the US Centers for Disease Control and Prevention {Centers for Disease Control and Prevention (CDC) 2007}. Following acute hepatitis B infection, approximately 5% of adults and up to 90% of children fail to produce an immune response adequate to clear the infection; these individuals become chronic carriers of the virus {Zuckerman 1996}, {Lok et al 2007}.

Chronic hepatitis B has a broad clinical spectrum, ranging from asymptomatic, slowly progressive illness to severe, more rapidly progressive liver disease. Chronic hepatitis B may remain quiescent for many years. However, 15% to 40% of patients with chronic hepatitis B will ultimately develop serious liver disease and complications, such as cirrhosis, hepatic decompensation, and HCC {Wright 2006}. In patients with chronic hepatitis B, the annual probability of developing cirrhosis varies from 0.1% to 1.0%, depending on the duration of active HBV replication, the severity of disease, and the presence of concomitant risk factors {Merican et al 2000}. Patients with chronic viral replication are at increased risk of complications and poor outcomes {Chen et al 2006}. Effective suppression of viral replication alters the course of the disease and decreases morbidity. However, the clinical benefit is lost if viral replication resumes as a result of emerging resistance mutations.

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 hepatitis B surface antigen (HBsAg) and hepatitis B early antigen (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 shown 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 chronic hepatitis B in children and adolescents is still evolving {Jonas et al 2010}. Current consensus is that no treatment is indicated in the immune tolerant (stage 1) or the inactive carrier state (stage 3). For children and adolescents in stages 2 or 4, however, treatment may be warranted to in order to suppress viral replication and to prevent the emergence of complications, such as cirrhosis, decompensated liver disease, and HCC. 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 hepatitis B early antigen (HBeAg)-positive patients, treatment can lead to HBeAg loss and, more rarely, anti-HBe seroconversion, with the goal of subsequent hepatitis HBsAg loss and seroconversion to anti-HBs. Achieving durable viral suppression in chronic HBV infection usually requires long-term therapy, preferably utilizing potent regimens that limit the development of resistance.

The optimal treatment for children and adolescents with chronic HBV infection is not well established. Over the past decade, interferon-alfa has been shown to be relatively effective in this patient population, resulting in viral suppression or HBeAg seroconversion in 20% to 58% of treated patients, compared with 8% to 17% of untreated controls {Sokal 2002},{Jonas 2006}. Interferon-alfa is administered for a treatment duration of 24 weeks in patients ≥ 2 years of age, and higher response rates have been reported in western countries. Some experts consider interferon to be the treatment of choice, and suggest that alternative, oral agents be limited to clinical trials. This is in large part due to concerns over the development of drug resistance {Jonas et al 2010}. Unfortunately, interferon-alfa treatment has significant limitations, including undesirable side effects, such as growth impairment, flu-like symptoms, neutropenia, and mood disturbances; inconvenient injectable dosage form; and a low rate of efficacy in patients with higher serum HBV DNA levels or only minimally elevated ALT levels {Sokal 2002},{Bortolotti 2003}.

The oral nucleoside analog lamivudine is approved for the treatment of pediatric chronic HBV infection (ages 2 to 17 years of age) in the United States, Switzerland, and Australia. Lamivudine has been shown to induce a virologic response, defined by the clearance of HBeAg and serum HBV DNA, in 23% of treated pediatric patients after 52 weeks (compared to 13% of controls, $p = 0.04$) {Jonas et al 2002}, with maintained HBeAg seroconversion {Jonas 2006}. Lamivudine therapy is usually administered for 1 year and continued for 6 months after seroconversion. In contrast to interferon alfa, lamuvidine is available in a patient-friendly oral dosage form, and is well tolerated, with no growth development changes observed after 3 years of treatment {Jonas 2006}. However, the long-term efficacy of lamivudine is limited by the development of viral resistance. As in adults, the development of the rtL180M and/or rtM204V/I mutations in the HBV DNA polymerase have been detected in children and adolescents treated with lamivudine for chronic HBV infection. In the lamivudine HBV pediatric pivotal trial, resistance mutations were detected in 19%, 49% and 64% of subjects after 1, 2, and 3 years of treatment, respectively {Jonas et al 2002},{Sokal et al 2006}. Hepatitis B viral resistance may be associated with a recurrence or acute exacerbation of hepatitis.

The oral nucleotide analog adefovir dipivoxil is approved for the treatment of chronic HBV infection in adults and children ≥ 12 years of age in the United States. Adefovir has activity against both wild-type and lamivudine-resistant HBV {Gilead Sciences Inc 2009}, {Hadziyannis et al 2003}, {Hadziyannis et al 2005}. Adefovir dipivoxil was evaluated in a large (n = 173 subjects) placebo-controlled, international trial in pediatric patients (ages 2 to 17) (Study GS-US-103-0518) {Jonas et al 2008}. After 48 weeks of treatment, adefovir dipivoxil showed modest benefits over placebo in older children and adolescents. At 48 weeks, more adefovir dipivoxil–treated subjects in the 12 to 17 years age group achieved the primary efficacy endpoint (serum HBV DNA < 1000 copies/mL and normal ALT at the end of blinded treatment), compared with placebo-treated subjects (23% vs. 0%, p = 0.007). In children aged 2 to 11 years, the efficacy difference between adefovir-treated and placebo-treated patients at the end of blinded treatment was not significantly significant, despite achievement of plasma adefovir concentrations in younger children that were comparable to those measured in older children. Adefovir dipivoxil treatment was well tolerated, and no subjects developed the rtA181V or rtN236T mutations associated with ADV resistance by Week 48.

Entecavir and telbivudine, both oral nucleoside analogs, are approved for the treatment of chronic HBV infection in patients ≥ 16 years of age in the United States and other countries. Cross-resistance exists between entecavir and lamivudine, requiring a higher dose of entecavir in patients with lamivudine-refractory HBV infection. Safety and efficacy of entecavir and telbivudine have not been established in pediatric and adolescent patients < 16 years of age.

Tenofovir disoproxil fumarate (TDF), an oral, once-daily nucleotide analog, has antiviral activity against both HBV and HIV. TDF is approved for use in the treatment of chronic HBV infection in adults in the United States, Canada, and Europe. TDF in combination with other antiretroviral agents is also approved for the treatment of HIV infection in adults and in pediatric patients > 12 years of age in the United States and Canada. The anti-HBV activity of TDF is equipotent to that of adefovir dipivoxil in vitro; however, the safety profile of TDF allows the administration of higher doses {Lok et al 2007}. In two ongoing phase 3 international comparative trials in adults, TDF was shown to be superior to adefovir dipivoxil for the treatment of HBeAg-negative (GS-US-174-0102) and HBeAg-positive (GS-US-174-0103) patients with chronic HBV infection {Marcellin et al 2007}, {Heathcote et al 2007}.

Tenofovir DF is currently being evaluated in study GS-US-174-0115, a large (n = 106 subjects) placebo-controlled, international trial in adolescent patients (12 to 17 years). After 72 weeks of treatment, tenofovir showed significant virologic response benefits over placebo (Data on file, Gilead Sciences, 2011). At 7 weeks, more tenofovir DF–treated subjects achieved the primary efficacy endpoint (serum HBV DNA < 400 copies/mL [69 IU/mL] at the end of blinded treatment), compared with placebo-treated subjects (88.5% vs. 0%, p < 0.001). Tenofovir DF treatment was well tolerated and no patients met the primary safety endpoint of a 6% decrease in lumbar spine bone mineral density (BMD). As expected for an adolescent population, both arms experienced an overall increase in mean lumbar spine BMD. Both populations also experienced an overall increase in mean whole body BMD; however, the percent increase in whole body BMD in tenofovir-treated subjects was less than the percent increase in whole body BMD attained by placebo-treated subjects (2.8% [TDF] vs. 5.4% [Placebo], p = 0.013).

With the possibility that antiviral and biochemical response in adolescents might be attainable in pediatric chronic HBV infected patients, a study in pediatric patients between 2 to11 years is proposed. Because the spontaneous clearance rate is significant but somewhat variable in this age group and serious complications of chronic HBV often require years to develop, and with no consensus regarding optimal timing of treatment in pediatric patients, a placebo-controlled study allows clearer conclusions regarding efficacy without putting patients at undue risk. In order to better define the safety profile of TDF, a more thorough analysis of the bone effects of TDF on adolescent subjects (12 to 17 years) with chronic HBV will be completed before proceeding in patients with chronic HBV age 2 to < 12.

1.2. Tenofovir Disoproxil Fumarate (Tenofovir DF)

1.2.1. General Information

Tenofovir disoproxil fumarate (TDF; 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 combination with other antiretroviral agents in the treatment of HIV-1 infection in adults.

Tenofovir DF (300 mg once daily) was approved by the US FDA in October 2001, the European Commission in February 2002, and other markets worldwide for use in combination with other antiretroviral agents for the treatment of HIV-1 infection. Cumulative patient exposure to tenofovir DF since first marketing approval in the US on 26 October 2001 to 31 July 2007 is estimated to be more than 3 million patient-years of treatment.

Tenofovir DF is approved for the treatment of CHB in adults in the United States, Canada, the EU, Turkey, Australia, New Zealand, Brazil, Argentina, Uruguay, South Korea, Hong Kong, Taiwan, and Singapore; marketing applications are also under review in other regions.

Further information is available in the Investigator's Brochure for tenofovir DF and the Package Insert for Viread® {Gilead Sciences Inc 2010}.

1.2.2. Pre-Clinical Pharmacology and Toxicology

1.2.2.1. Mechanism of Action

Tenofovir disoproxil fumarate (tenofovir DF) is an oral prodrug of tenofovir (PMPA), an acyclic nucleotide (nucleoside monophosphate) analogue. Tenofovir DF is converted to tenofovir by serum esterases. Intracellularly, tenofovir is then converted through two phosphorylation reactions to its active phosphorylated anabolite, tenofovir diphosphate (TFV-DP), an obligate chain terminator {Robbins et al 1998}. Tenofovir is efficiently anabolized to TFV-DP in human hepatic cells, and has a half-life of 95 hours in primary human hepatocytes {Delaney et al 2006}. TFV-DP inhibits recombinant HBV polymerase with a K_i of 0.18 μM {Delaney et al 2006}. TFV-DP inhibits viral polymerases by direct binding competition with the natural

deoxyribonucleotide substrate (deoxyadenosine triphosphate - dATP) and, after incorporation into DNA, by DNA chain termination {Cherrington et al 1995}. TFV-DP is a very weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ {Kramata et al 1996},{Cherrington et al 1995}.

1.2.2.2. Anti-Hepatitis B Virus Activity

Tenofovir is a potent and selective inhibitor of HBV in vitro. The in vitro antiviral activity of tenofovir against an HBV laboratory strain was assessed in the HepG2 2.2.15 cell line. The EC_{50} values (50% effective concentration) for tenofovir were in the range of 0.14 to 1.5 μM, with CC_{50} (50% cytotoxicity concentration) values > 100 μM. In a parallel analysis, tenofovir inhibited various wild-type HBV clinical isolates with a comparable activity (Report No. PC-174-2005). It also inhibited replication of duck HBV (DHBV) in primary duck hepatocytes with an EC_{50} of 0.11 μM {Heijtink et al 1994}. As observed with anti-HIV-1 activity, the prodrug tenofovir DF had a 55-fold increased in vitro potency against HBV in comparison with tenofovir {Delaney et al 2006}.

Tenofovir demonstrated anti-HBV activity similar to adefovir in the same cell lines. TFV-DP and adefovir diphosphate also showed comparable inhibition constants for HBV polymerase in enzyme assays. The EC_{50} values of lamivudine for HBV are approximately 10-fold lower than that of tenofovir in same cell lines. However, the inhibition constants of TFV-DP and lamivudine triphosphate for HBV polymerase were similar, suggesting that the intrinsic anti-HBV potency of tenofovir is similar to that of lamivudine. The higher EC_{50} values of tenofovir versus lamivudine in cell culture may be attributed to the lower cell permeability of the negatively-charged molecule of tenofovir.

Using HBV-expressing cell lines (Report Nos. PC-174-2006, PC-164-2004) {Delaney et al 2004a}, tenofovir demonstrated additive to slightly synergistic anti-HBV activity when tested in combination with nucleoside anti-HBV reverse transcriptase inhibitors. Specifically, tenofovir in combination with lamivudine, telbivudine, entecavir and adefovir, each produced additive anti-HBV activity (Report Nos. PC-174-2006, PC-164-2004) {Delaney et al 2004a}. The combination of tenofovir and emtricitabine demonstrated additive to slightly synergistic anti-HBV activity (Report No. PC-174-2006).

The anti-viral activity of tenofovir was also evaluated in woodchucks chronically infected with WHV in short-term (4 weeks) {Menne et al 2005} and long-term (48 weeks) antiviral efficacy studies (Report No. PC-174-2004). Oral administration of tenofovir DF at 0.5, 1.5, and 5.0 mg/kg of body weight/day for 4 weeks reduced serum viral load significantly, at 0.2 ($p < 0.01$), 1.1 ($p < 0.01$), and 1.5 $\log_{10}$ ($p < 0.05$) from the pretreatment levels {Menne et al 2005}. Oral administration of tenofovir DF at 15 mg/kg for 48 weeks resulted in a mean serum viral load reduction of 2.9 $\log_{10}$ copies/mL of WHV DNA. Administration of tenofovir DF in combination with lamivudine or emtricitabine for 48 weeks resulted in a mean serum viral load reduction of 5.8 and 6.1 $\log_{10}$, respectively. Over the 48-week dosing period, there was no evidence of toxicity in woodchucks treated with tenofovir DF, either alone or in combination (Report No. PC-174-2004).

1.2.2.3. Resistance

Hepatitis B virus resistance to tenofovir is yet to be identified. An rtA194T mutation was reported to emerge in two patients receiving antiretroviral treatments including tenofovir DF, and an in vitro susceptibility assay indicated reduced susceptibility to tenofovir {Sheldon et al 2005}. The patients developed this mutation in the background of the rtL180M+rtM204V lamivudine-associated mutations, and its development was not clearly associated with HBV viral load rebound. To further characterize the rtA194T mutation, viruses containing the rtA194T mutation alone or in combination with the rtL180M+rtM204V lamivudine resistance mutations were created and their in vitro tenofovir susceptibilities were tested in parallel with the parent wild-type and lamivudine-resistant strains. The data showed that rtA194T mutation alone had no significant effect on tenofovir susceptibility (1.5-fold increase in tenofovir EC_{50}). The rtA194T mutation in combination with the rtL180M+rtM204V mutations led to a 2.4-fold increase in tenofovir EC_{50}, which was not significantly different than the 2.1-fold increase observed against virus containing the rtL180M+rtM204V mutations alone (Report No. PC-104-2012) {Delaney et al 2006}. Various clinical studies have shown potent inhibition of lamivudine-resistant HBV by tenofovir DF, suggesting that a 2- to 3- fold change in in vitro susceptibility to tenofovir is not clinically relevant. Other mutations have not been reported to date.

Four major patterns of lamivudine resistance mutations, rtL180M/rtM204V, rtV173L/rtL180M/rtM204V, rtL180M/rtM204I, and rtM204I have been identified in patients who failed lamivudine therapy {Delaney et al 2004b}. In cell-based assays, HBV laboratory strains {Delaney et al 2004b}, {Yang et al 2005}, {Brunelle et al 2005}, {Delaney et al 2006}, as well as those identified in clinical isolates {Brunelle et al 2007}, {Lacombe et al 2006}, {Lada et al 2004} expressing the rtV173L, rtL180M, and rtM204I/V mutations, showed a susceptibility to tenofovir ranging from 0.7 to 3.4-fold that of wild-type virus.

Resistance to adefovir dipivoxil is associated with development of the rtA181V/T and/or rtN236T mutations in the HBV reverse transcriptase. These mutations resulted in a 2.9- to 4.5-fold, respectively, reduced sensitivity to tenofovir in vitro {Qi et al 2007}, {Delaney et al 2006}, {Brunelle et al 2005}, {Qi et al 2005}. The effects on susceptibility to tenofovir by other adefovir resistance associated mutations or combinations of mutations (rtA181T, rtA181V/rtN236T and rtA181T/rtN236T) were also evaluated using stably transfected cell lines. The results demonstrated a reduction in susceptibility to tenofovir of 1.5-, 10.0- and 3.0-fold each, respectively {Qi et al 2007}. The clinical significance of these in vitro results is currently unknown.

The mutations conferring resistance to entecavir have been identified as changes at rtI169T, rtT184G, rtS202I/G and/or rtM250V in combination with the pre-existing lamivudine resistance mutations (rtL180M +/- rtM204V/I) {Tenney et al 2004}, {Colonno et al 2006}, {Tenney et al 2007}. In vitro phenotypic analysis showed that the tested entecavir resistance mutations resulted in increased EC_{50} values for tenofovir ranging from 0.6- to 6.9-fold (Report No. PC-174-2003), {Villet et al 2007}, {Brunelle et al 2007}.

Virus harboring combinations of mutations resulting in resistance to both lamivudine and adefovir (rtV173L/rtL180M/rtA181V/rtN236T) were observed to emerge in a patient receiving combination therapy with these drugs. Virus obtained from this patient remained susceptible to inhibition by tenofovir in vitro {Villet et al 2006}, {Brunelle et al 2007}. Virus containing the rtL180M+rtM204V+rtN236T mutations created in a laboratory strain of HBV had greatly impaired replication capacity. These mutations resulted in a 4.4-fold increase in the tenofovir EC_{50} in vitro {Brunelle et al 2005}.

1.2.3. Clinical Trials of Tenofovir DF

1.2.3.1. Gilead Sciences Sponsored Clinical Trials of Tenofovir DF in HBV Mono-infected Subjects

There are 6 completed and 3 ongoing Gilead Sciences sponsored clinical trials that are designed to study the safety and antiviral activity of tenofovir DF as monotherapy or in combination with emtricitabine in subjects mono-infected with HBV.

1.2.3.1.1. Study GS-US-174-0102: A Randomized, Double-Blind, Controlled Evaluation of Tenofovir DF versus Adefovir Dipivoxil for the Treatment of Presumed Pre-core Mutant Chronic Hepatitis B

Study GS-US-174-0103: A Randomized, Double-Blind, Controlled Evaluation of Tenofovir DF versus Adefovir Dipivoxil for the Treatment of HBeAg Positive Chronic Hepatitis B

Approval of TDF for treatment of CHB was based primarily on results from these two Phase 3 studies. In the initial 48 weeks of the trials, the studies evaluated safety and antiviral activity of TDF versus adefovir dipivoxil (ADV) in 641 HBeAg- (375) and HBeAg+ (266) patients with compensated liver function. In Study 102 at Baseline, patients had a mean Knodell necroinflammatory score of 7.8, a mean HBV DNA of 6.9 $\log_{10}$ copies/mL, and a mean ALT of 140 U/L. In Study 103 at Baseline, patients had a mean Knodell necroinflammatory score of 8.4, a mean HBV DNA of 8.7 $\log_{10}$ copies/mL, and a mean ALT of 147 U/L. The studies are currently ongoing with all remaining subjects in their 5th year in the study treatment.

In both studies at 48 weeks, TDF was superior than ADV by the comparison of subjects achieving complete response (HBV DNA < 400 copies/mL [69 IU/mL] and histologic improvement characterized by ≥ 2 point reduction in the Knodell necroinflammatory score without increase in fibrosis) in the 2 treatment arms (102: 71% vs. 49%; 103: 66% vs. 12%). TDF treatment also produced superior viral suppression (HBV DNA < 400 copies/mL [69 IU/mL]) compared to the ADV treatment (102: 93% vs. 63%; 103: 76% vs. 13%). In Studies 102 and 103, both drugs produced similar results with regard to histologic improvement at Week 48. Normalization of ALT at the end of blinded treatment was similar in both arms of Study 102; however, in Study 103, the TDF treated group had a greater proportion of subjects with normalized ALT at Week 48. In Study 103, a significantly greater proportion of TDF-treated subjects than adefovir dipivoxil-treated subjects had achieved HBsAg loss at Week 48 (3.2% vs. 0%) {Marcellin et al 2008}.

At the end of the Week 48 double-blind period, all eligible subjects from both ADV and TDF arms in Studies 102 and 103 were switched to open-label TDF (ADV-TDF and TDF-TDF, respectively). The studies have been extended to 8 years. Beginning at Week 72, subjects with a confirmed HBV DNA ≥ 400 copies/mL (69 IU/mL) were eligible to receive emtricitabine plus TDF combination therapy. The following describes year-4 (Week 192) data from this ongoing trial {Marcellin et al 2010}, {Heathcote et al 2010}.

In Study 102, 315 HBeAg- subjects completed W192 (84% retention). In an ITT analysis (ITT), 86% of subjects had HBV DNA <400 copies/mL (69 IU/mL) (85% TDF-TDF; 87% ADV-TDF). In an on-treatment analysis 99% of subjects treated with TDF for 4 years had HBV DNA < 400 copies/mL at W192. 100% of ADV treated subjects (regardless of their prior response to ADV treatment, i.e., viremic or suppressed on ADV therapy at W48) responded favorably to TDF treatment with HBV DNA <400 copies/mL (69 IU/mL) at W192. Four subjects (3 on TDF monotherapy) had HBV DNA ≥400 copies/mL (69 IU/mL) at W192 or time of discontinuation. Three of the four subjects from Study 102 that qualified for resistance testing during Year 4 were confirmed to be nonadherent to study medication {International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Steering Committee 2004}, {Arimilli et al 1998}, and {Piketty et al 2004}. Resistance analyses showed no amino acid substitutions that could be associated with TDF resistance in subjects with detectable HBV DNA at W192, at discontinuation, or when FTC was added. Of the 12 subjects who were eligible to add FTC between W72 and W192 for confirmed HBV DNA ≥400 copies/mL (69 IU/mL), 4 subjects added FTC and 8 subjects remained on TDF monotherapy. Of the subjects who remained on TDF monotherapy, 6 had HBV DNA <400 copies/mL (69 IU/mL) at W192. Overall mean ALT at W192 was 33 U/L or less than the upper limit of the normal laboratory range. TDF was well tolerated during both treatment periods with a safety profile consistent with the known safety profile of TDF. Of the 13 subjects who discontinued treatment during year 4, two had an AE that led to discontinuation (considered unrelated to TDF). Cumulatively, 4 cases (1%) of hepatocellular carcinoma were reported during open-label TDF treatment. Mean creatinine values showed minimal change through year 4. Two subjects experienced a confirmed 0.5 mg/dL increase in creatinine, one associated with advanced hepatocellular carcinoma and the other improved with QOD dosing. In summary, TDF was well tolerated and produced potent, continuous viral suppression through 4 years of TDF treatment with no detectable resistance mutations found to arise in association with TDF in this HBeAg- patient population. No subject in either treatment group had confirmed HBsAg loss or seroconverted to anti-HBs by Week 192. One subject in the ADV−TDF group was HBsAg− at Week 156; however, this subject was HBsAg+ at subsequent visits.

In Study 103, 198 HBeAg+ subjects completed W192 (74% retention). In an ITT analysis 77% of subjects had HBV DNA <400 copies/mL (69 IU/mL) (74% TDF-TDF; 84% ADV-TDF). In an on-treatment analysis 96% (TDF-TDF) and 99% (ADV-TDF) had HBV DNA <400 copies/mL (69 IU/mL) at W192. 100% of patients virally suppressed on ADV and 98% of suboptimal responders on ADV, responded favorably to a switch to TDF with HBV DNA <400 copies/mL (69 IU/mL) at W192. Overall 7 subjects (2 on TDF monotherapy and 5 on FTC/TDF) had HBV DNA ≥400 copies/mL (69 IU/mL) at W192 or after discontinuing between W144 and W192. Resistance analyses showed no amino acid substitutions that could be

associated with TDF resistance in subjects with detectable HBV DNA at W192, at discontinuation or when FTC was added. 34 subjects added FTC to TDF W192, 83% achieved HBV DNA <400 copies/mL (69 IU/mL). 9 subjects were eligible to add FTC, but did not, and of the 6 subjects on treatment at W192, 100% had HBV DNA <400 copies/mL (69 IU/mL). At W192 the mean ALT was 35.2 U/L and HBeAg loss/seroconversion were observed in 41%/31% of subjects (on-treatment analysis). Cumulatively, 10% of subjects lost HBsAg and 7.5% seroconverted to anti-HBs (Kaplan-Meier Estimate). TDF was well tolerated during both treatment periods with a safety profile consistent with the known safety profile of TDF. Mean creatinine levels remained stable through Year 4. One subject who remains on study experienced a confirmed 0.5 mg/dL increase in serum creatinine during Year 4. In summary, TDF was well tolerated and produced potent, continuous viral suppression with increasing HBeAg and HBsAg loss, with no detectable resistance mutations found to arise in association with TDF in this HBeAg+ patient population.

Among 641 total subjects enrolled in both studies, 189 (29%) were Asian (94 HBeAg- CHB; 95 HBeAg+ CHB). Mean age at entry for Asians was 40 years and 68% were male. Mean Baseline HBV DNA levels were 7.66 $\log_{10}$ copies/mL for both Asians and non-Asians. As expected, most Asian subjects had HBV genotype B or C (n=166). 163 Asians entered the open-label phase and 145 completed W192. Overall at W192, HBV DNA was <400 copies/mL (69 IU/mL) in 79% of all Asians (74% HBeAg+, 84% HBeAg-) and 83% of all non-Asians (79% HBeAg+, 86% HBeAg-) (ITT analysis) ($p \geq 0.2$ for all comparisons). 97% of Asians and 99% of non-Asians had HBV DNA <400 copies/mL (69 IU/mL) by on-treatment analysis. 7 Asian subjects (3.7%) started FTC+TDF (none in year 4), 1 discontinued, and 4 of 6 remaining on study had HBV DNA <400 copies/mL (69 IU/mL). Among Asian subjects on treatment 86% had ALT within normal range: mean value of 31 U/L (vs. 79% of non-Asian subjects, p=0.100). Of 65 HBeAg+ Asian subjects with W192 serology results 35%/26% had HBeAg loss/HBeAg seroconversion (none had HBsAg loss). During open-label TDF treatment the safety profile was similar for both groups: serious adverse events (AEs) developed in 6%/14%, Grade 3/4 AEs in 10%/12%, and Grade 3/4 laboratory abnormalities in 15%/16% of Asian/non-Asian subjects, respectively. There was 1 Asian subject with confirmed phosphorus <2 mg/dL (normal at W192), and 1 had a confirmed creatinine 0.5 mg/dL greater than Baseline and remains on study; none had creatinine clearance <50 mL/min. Antiviral efficacy and safety of TDF were similar in Asians and non-Asians over 192 weeks of treatment with good tolerability congruent with the results of the overall studies.

1.2.3.1.2. Study GS-US-174-0106: A Phase 2, Randomized, Double-Blind Study Exploring the Efficacy, Safety and Tolerability of Tenofovir Disoproxil Fumarate (DF) Monotherapy Versus Emtricitabine plus Tenofovir DF Fixed-Dose Combination Therapy in Subjects Currently Being Treated with Adefovir Dipivoxil for Chronic Hepatitis B and having Persistent Viral Replication

Study GS-US-174-0106 was fully enrolled and has been completed. This 168-week study was conducted to compare treatments of TDF versus emtricitabine (FTC) plus TDF Fixed-Dose Combination for CHB patients who had an incomplete response to ADV. The 48-week data show that TDF monotherapy and the combination of FTC and TDF had similar efficacy in the

patients with suboptimal viral suppression after therapy with ADV. Response to the treatment was not influenced by the presence of baseline LAM- or ADV-associated mutations. Initial monotherapy followed by combination therapy was as effective as early combination therapy {Berg et al 2010}.

1.2.3.1.3. Study GS-US-174-0108: A Phase 2, Double-Blind, Multi-center, Randomized Study Comparing Tenofovir Disoproxil Fumarate, Emtricitabine Plus Tenofovir Disoproxil Fumarate, and Entecavir in the Treatment of Chronic Hepatitis B Subjects with Decompensated Liver Disease and in the Prevention of Hepatitis B Recurrence Post-Transplantation

Study GS-US-174-0108 is a Phase 2, double-blind, randomized study to evaluate the safety and efficacy of tenofovir disoproxil fumerate, the fixed-dose combination emtricitabine/tenofovir DF, and entecavir in the treatment of CHB with decompenstated liver disease in the prevention of HB recurrence post-transplantation. The study was fully enrolled (N = 112) by Jan 2008. 81 subjects completed the study and 31 were early terminated. Reasons for early termination (n = 31) were investigators discretion (8 patients), patient withdrew consent (8 patients), lost to follow up (1 patient), and 14 patient deaths occurred due to various reasons. Week 48 data for this study was published in Hepatology {Liaw et al 2011}.This study is now complete, and the database has been locked. Safety and efficacy analyses were performed in the fourth quarter of 2011 and the final Clinical Study Report was completed in the first quarter of 2012.

1.2.3.1.4. Study GS-US-174-0115: A Randomized, Double-Blind Evaluation of the Antiviral Efficacy, Safety, and Tolerability of TDF versus Placebo in Adolescents with CHB Infection

Study GS-US-174-0115 was a large (n = 106 subjects) placebo-controlled, international trial in adolescent patients (12 to < 18 years). Preliminary analysis of the data suggest the following: after 72 weeks of treatment, tenofovir showed significant virologic response benefits over placebo. At 72 weeks, more tenofovir DF–treated subjects achieved the primary efficacy endpoint (serum HBV DNA < 400 copies/mL (69 IU/mL) at the end of blinded treatment), compared with placebo-treated subjects (88.5% vs. 0%, $p < 0.001$). Tenofovir DF treatment was well tolerated and no patients met the primary safety endpoint of a 6% decrease in lumbar spine bone mineral density (BMD). As expected for an adolescent population, both arms experienced an overall increase in mean lumbar spine BMD. Both populations also experienced an overall increase in mean whole body BMD; however, the percent increase in whole body BMD in tenofovir-treated subjects was less than the percent increase in whole body BMD attained by placebo-treated subjects (2.8% [TDF] vs. 5.4% [Placebo], $p = 0.013$).

1.2.3.1.5. Study GS-US-174-0121: A Randomized, Double-Blind, Double-Dummy Study Evaluating the Antiviral Efficacy, Safety, and Tolerability of TDF Monotherapy versus FTC plus TDF Fixed-Dose Combination Therapy in Subjects with CHB who are Resistant to Lamivudine

Study GS-US-174-0121 is fully enrolled (N = 280). As of January, 2011, 266 (95%) enrolled patients remain on study. Reasons for early termination (n = 14) are investigator's discretion (3 patients), protocol violation (3 patients), safety or tolerability reasons (3 patients), lost to follow-up (2 patients), withdrew consent (2 patients), and failure to meet entrance criteria (1 patient). This is a 5-year study; after all subjects complete the 96 week study visit a 96-week analysis was performed in the first quarter of 2012 for an interim Clinical Study Report.

1.2.3.1.6. Study GS-US-174-0123: A Phase 4, open-label, single-arm 48-week study evaluating the antiviral efficacy, safety, and tolerability of TDF in HBV mono-infected Asian-American adults

Study GS-US-174-0123 was fully enrolled and has been completed. Safety, efficacy and tolerability of TDF were evaluated in Asian-American adult patients with CHB. Ninety patients (HBeAg- or HBeAg+) treated by community physicians were enrolled and treated with once daily open-label 300 mg TDF for 48 week. As previously demonstrated in wider populations of HBV-infected patients, this study confirms that TDF is safe and potent in Asian-American CHB patients with greater than 80% achieving HBV-DNA < 400 copies/mL (69 IU/mL) by Week 48 and improved FibroTest fibrosis scores.

1.2.3.1.7. Study GS-US-203-0101: A Randomized, Double-Blind Study Evaluating Tenofovir Disoproxil Fumarate (DF) Monotherapy Versus the Combination of Emtricitabine and Tenofovir DF for the Treatment of Chronic Hepatitis B

Study GS-US-203-0101 is a double-blind, multi-center, randomized study to compare the safety and antiviral activity of tenofovir DF versus emtricitabine/tenofovir DF in the treatment of chronic HBV in subjects with high viral load (HBV DNA $\geq 10^8$ copies/mL) and normal ALT values. One hundred and twenty-six subjects were randomized 1:1 to tenofovir DF or emtricitabine/tenofovir DF. Key eligibility criteria for study entry include HBV DNA $\geq 10^8$ copies/mL; serum ALT $\leq$ ULN; and seronegative for HIV, HCV, and HDV. The study is 192 weeks in duration and has recently been completed. Final results are pending.

1.2.3.1.8. Study GS-US-203-0107: A Phase 2, Open-Label Randomized Study to Evaluate the Efficacy and Safety of the Combination Product, Emtricitabine/Tenofovir Disoproxil Fumarate in the Presence or Absence of Hepatitis B Immunoglobulin (HBIG) in Preventing Recurrence of Chronic Hepatitis B (CHB) Post-Orthotopic Liver Transplant (OLT)

Study GS-US-203-0107 is a Phase 2, open-label, randomized study to evaluate the safety and efficacy of the fixed-dose combination emtricitabine/tenofovir DF in the presence or absence of hepatitis B immunoglobulin in preventing recurrence of CHB after orthotopic liver transplantation. The study was fully enrolled (N = 40) by May 2009. Thirty-four subjects

completed the study and 6 were early terminated. Reasons for early termination (n = 6) were investigators discretion (2 patients), patient withdrew consent (2 patients), lost to follow up (1 patient), and 1 patient expired due to stroke. Study is now complete, and the database has been locked. Safety and efficacy analyses were performed in the fourth quarter of 2011, and the final Clinical Study Report was completed in the first quarter of 2012

1.2.3.1.9. Clinical Trials of Tenofovir DF in HIV-1-Infected Pediatric and Adolescent Subjects

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 open-label 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, open-label 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 dual-energy x-ray absorptiometry (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. Single-dose pharmacokinetic (PK) studies were evaluated with the first dose during the 7-day tenofovir DF monotherapy period and again at Week 4 when tenofovir DF was administered with an optimized background regimen (OBR) based on genotypic testing. Subjects had extensive treatment experience: median (range) duration of prior antiretroviral therapy was 9.7 years (4.8–13.5). Baseline resistance testing showed a median (range) of 7 (2–8) major NRTI mutations and 8 (1–10) major protease mutations.

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 {Hazra et al 2005}, {Gafni et al 2006}.

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 {Vigano et al 2007a}. Tenofovir DF therapy was well tolerated. Through 96 weeks, no child experienced a Grade 1 or higher increase in serum creatinine or phosphate, and mean serum creatinine, phosphate and bicarbonate values were unchanged {Vigano et al 2007b}. 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 bone mineral density (BMD) via DEXA scan in 16 subjects in this study through 12 months of tenofovir DF treatment {Giacomet et al 2005}. 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.

Two clinical trials of tenofovir DF in pediatric and/or adolescent HIV-1-infected subjects are currently ongoing. Study GS-US-104-0352 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 48-week results of Study GS-US-104-0352 showed tenofovir DF did not meet the criteria for treatment noninferiority at Week 48 (missing = failure; ITT analysis set). However, it did meet treatment noninferiority from baseline through Week 24. The lack of noninferiority by this analysis was driven by the higher number of discontinuations as well as transient low-level increases in viral load at Week 48 in the tenofovir DF group. In post hoc analyses to explore the effect of subjects who had discontinued from the study early, tenofovir DF met the criteria for treatment noninferiority at Week 48 and all timepoints from baseline to Week 48 (missing = excluded; ITT analysis set).

The development of virologic resistance in these antiretroviral-experienced children was consistent with current therapy or archived mutations from previous therapies. Tenofovir pharmacokinetics following administration of the oral powder formulation of tenofovir DF at a dose of 8 mg/kg daily in HIV-1 infected children (2 to < 12 years of age) is similar to that achieved following administration of tenofovir DF 300 mg tablets in HIV-1 infected adults, confirming the appropriateness of this dose in this age range.

Tenofovir DF was generally safe and well tolerated in this study and was consistent with the known safety profile in adult. There were no clinically relevant differences in renal function between tenofovir DF and stavudine or zidovudine groups in the randomized treatment period. At the Week 96 analysis, 4 subjects discontinued due to renal AEs (3 subjects with hypophosphatemia and 1 subject with glycosuria and chronic renal failure) that were clinically consistent with proximal renal tubulopathy, a renal disorder identified in postmarketing surveillance of tenofovir DF in adults; no additional discontinuations due to renal AEs occurred in the Week 144 analysis. No clinically relevant differences were seen between the tenofovir DF and stavudine or zidovudine groups in spine BMD. Percentage increases from baseline in total body BMD were smaller in the tenofovir DF group than in the stavudine or zidovudine group and there was a modest reduction in total body BMD Z score in the tenofovir DF group compared to no change in the stavudine or zidovudine group. The long-term clinical relevance of these observations is unknown. Biochemical markers of both bone formation (serum osteocalcin and bone-specific alkaline phophatase) and bone resorption (N- and C-telopeptides) demonstrated greater and significant changes in the tenofovir DF group compared with the stavudine or zidovudine group; however, there was no evidence of imbalance between bone formation and bone resorption. The clinical relevance of these changes in bone biochemical markers appears to be minimal given the lack of clinically relevant changes in BMD and the absence of bone fractures reported in the study.

Study GS-US-104-0321 enrolled antiretroviral treatment-experienced HIV-1 infected adolescents that were treated with tenofovir DF or placebo in addition to a reconfigured and optimized background regimen (OBR) for 48 weeks. The Week 48 results of this study showed that adolescents in both treatment groups demonstrated a virologic response to treatment. There were no statistically significant differences between tenofovir DF and placebo groups for any of the efficacy endpoints for the ITT analysis set. Treatment response was demonstrated by reductions in viral load and increases in CD4 cell count in both treatment groups. Analyses of the primary efficacy endpoint using the subgroup of subjects with a maximum of 1 active drug in their OBR (ANRS scoring system) and confirmation using post hoc analyses (Stanford database scoring system; 3- and 5-point scales) provide evidence for the antiretroviral activity of tenofovir DF in HIV-1 infected adolescents.

The development of virological resistance in these antiretroviral-experienced adolescents with extensive resistance in their HIV at screening was comparable to that observed in heavily treatment-experienced adults. Mutation K65R developed in one subject in the tenofovir DF group.

Tenofovir DF was generally safe and well tolerated when given in combination with an OBR in this study. The safety profile in adolescent subjects with HIV-1 infection was consistent with the known safety profile in adults; the most marked differences between tenofovir DF and placebo groups in reported AEs were for mild to moderate gastrointestinal disorders (vomiting, nausea, and diarrhea). No deaths or SAEs considered related to study drug were reported, and only 1 subject discontinued due to an AE (vomiting). There was no evidence of compromised renal function due to tenofovir DF.

In Study 321, bone effects were similar to adult subjects. Under normal circumstances BMD increases rapidly in this age group. In this study, the mean rate of bone gain was less in the tenofovir DF-treated group compared to the placebo group. Six tenofovir DF treated subjects and one placebo treated subject had significant (> 4%) lumbar spine BMD loss in 48 weeks. Among 28 subjects receiving 96 weeks of tenofovir DF, Z-scores declined by 0.341 for lumbar spine and -0.458 for total body. Skeletal growth (height) appeared to be unaffected. Markers of bone turnover in tenofovir DF-treated pediatric subjects 12 years of age and older suggest increased bone turnover, consistent with the effects observed in adults.

1.3. Rationale for the Current Study and Dose Selection

The aim of treatment for chronic hepatitis B infection is to maintain suppression of viral replication to prevent the development of complications. Achieving this goal requires potent, durable, well-tolerated, patient-friendly antiviral regimens. Clinical data in adults and adolescents with HBeAg-positive and HBeAg-negative/anti-HBe-positive chronic HBV infection demonstrate the efficacy and safety of tenofovir DF for the treatment of chronic HBV disease. Tenofovir DF is a promising agent for use in adolescent subjects with chronic HBV because of its favorable efficacy, safety and tolerability profiles as indicated from preliminary data from Study GS-US-174-0115. The pharmacokinetic profile of tenofovir DF allows convenient, once-daily dosing, which can facilitate adherence in this patient population.

The optimal treatment for pediatric patients with chronic HBV infection is currently evolving. As summarized in Section 1.1, treatment with interferon alfa, lamivudine, and adefovir in pediatric populations has been shown to be less than optimal. Further, the safety and efficacy of entecavir and telbivudine have not been established in patients < 16 years of age. Risk-benefit evaluation supports use of a blinded study design using placebo for 48 weeks as an appropriate comparator. After 48 weeks of blinded treatment, all subjects will switch to open-label tenofovir DF, provided that no safety concerns are identified by the independent Data Monitoring Committee monitoring the study. Use of the placebo comparator allows a more robust evaluation of tenofovir DF safety and tolerability (e.g., bone metabolism, renal safety), and does not expose subjects randomized to the placebo arm to risk of development of resistance during the blinded portion of the study.

A study evaluating tenofovir DF in pediatric patients (ages 2 to < 12, at the time of enrollment) is needed to assess the safety and efficacy of this agent in the treatment of chronic hepatitis B in this patient population. In addition, the study will help to further elucidate the pharmacokinetic and resistance profiles of tenofovir DF. Through their participation, study subjects will help generate critical new information to help guide the most optimal treatment of chronic HBV infection in pediatric patients.

1.3.1. Rationale for Dose Selection

1.3.1.1. Tenofovir DF

The selection of the 300 mg dose for tenofovir DF for use in adults is based upon the following rationale: (1) The 300 mg dose has been demonstrated to be the optimal dose of tenofovir DF for the treatment of HIV-1 infection; the K_i of tenofovir against HIV-1 reverse transcriptase (0.02-1.6 μM) is similar to the K_i against HBV polymerase (0.18 μM); (2) The safety profile of tenofovir DF 300 mg once daily has been well characterized in patients with HIV infection, and tenofovir DF 300 mg once daily has been shown to be safe in that patient population; (3) Reducing the dose of tenofovir DF may lead to an increased risk of the emergence of resistance as documented with other antivirals; (4) In the dose-ranging study GS-98-902, the safety profile of tenofovir DF 300 mg once daily was not different from the safety profiles of lower doses of tenofovir DF (75 mg and 150 mg); and (5) The safety of the 300 mg dose is similar in HIV-infected patients with or without co-infection with chronic hepatitis B, as supported by study GS-99-910. Further, in the GS-US-174-0102 and GS-US-174-0103 studies, the 300 mg dose of tenofovir DF was shown to be an effective dose for treatment of chronic hepatitis B infection in adults with HBeAg-negative/ anti-HBe-positive disease and HBeAg-positive disease, respectively.

Studies in HIV-infected children (Studies GS-01-926, GS-01-927 and GS-02-983) indicate that an 8 mg/kg dose in a pediatric population will result in a tenofovir DF systemic exposure similar to that in HIV-infected adults receiving the commercially available tenofovir DF 300 mg tablet. The recommended oral dose of tenofovir DF in children is 8 mg/kg of actual body weight, to a maximum of 300 mg/day (≥ 35 kg). All pediatric subjects in this study who weigh ≥ 17 kg and are able to swallow tablets will receive tenofovir DF as a 150, 200, 250, or 300 mg tablet (or matching placebo tablets), administered once daily with or without regard to food. Subjects weighing < 17 kg and subjects ≥ 17 kg who are unable to swallow a tablet, will be given tenofovir DF oral powder (or matching placebo). Tenofovir pharmacokinetics, following administration of both the oral powder and tablet formulations will be assessed in this study in pediatric patients ages 2 to < 12 years old.

1.3.2. Rationale for Bone Mineral Density Evaluations

Osteopenia and osteoporosis have been reported in both children and adults with HIV-1 infection. The pathophysiology associated with these findings is unclear and may be related to HIV infection, antiretroviral therapy, and/or associated co-morbidities. Preclinical data showed that high doses of tenofovir DF in animals are associated with significant effects in bone metabolism. As seen in Study 926, some HIV-1-infected children receiving salvage therapy with

tenofovir DF experienced decreases in bone mineral density (BMD) {Hazra et al 2005}, {Gafni et al 2006}. Many of the children in that study had abnormal BMD results at baseline, suggesting that other factors were related to the decrease in BMD. As seen in a preliminary analysis of Study 115, in which adolescents age 12 to 17 with chronic HBV were treated with tenofovir or placebo and followed with DEXA scans at baseline, 24, 48, and 72 weeks, tenofovir was associated with a statististically significant difference in the mean percent increase in whole body BMD (2.8% [TDF] vs. 5.4% [Placebo], p = 0.013). Using whole body BMD Z-scores to account for age specific changes, and when comparing average values, it appears that this difference is unlikely to be of clinical relevance. Individual adolescent subjects who experienced the largest decreases in Z-scores were in the tenofovir arm; however, the largest changes in Z-scores in the study did not reach a change of -1.0.

The current study was designed to evaluate, in a controlled fashion, changes from baseline in BMD in a pediatric population following 48 weeks of study drug treatment. Changes from baseline in BMD will be evaluated every 24 weeks through Week 96, then annually until each subject reaches Week 192 (approximately 4 years on study), or at the Early Study Drug Discontinuation Visit (if applicable).

2. OBJECTIVES

The primary objective of this study is:

- To evaluate the antiviral efficacy of tenofovir DF versus placebo in pediatric patients (aged 2 to < 12 years, at the time of enrollment) with chronic hepatitis B infection

The key secondary objective is:

- To evaluate the proportion of subjects with HBeAg seroconversion at Week 48 (in subjects with baseline HBeAg sero-positivity).

Secondary objectives are:

- To characterize the safety and tolerability profile of tenofovir DF in pediatric patients (aged 2 to < 12 years, at the time of enrollment) with chronic hepatitis B infection
- To evaluate the biochemical and serological responses to tenofovir DF versus placebo
- To evaluate the incidence of potential resistance mutations to tenofovir DF in the hepatitis B virus polymerase/reverse transcriptase (pol/RT)
- To assess the pharmacokinetics of tenofovir in subjects receiving the tablet formulation and those receiving the oral powder formulation.

3. STUDY DESIGN

This is a Phase 3, randomized, double-blind study to evaluate the antiviral efficacy, safety and tolerability of tenofovir DF versus placebo in pediatric subjects with chronic HBV infection. One hundred (100) tenofovir DF-naïve subjects with HBV DNA $\geq 10^5$ copies/mL and ALT $\geq 1.5 \times$ ULN will be randomized in a 2:1 ratio to treatment arm A or B:.

- Treatment A (n = 67): tenofovir DF PO once daily for 48 weeks
- Treatment B (n = 33): matching placebo PO once daily for 48 weeks

After 48 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for additional 144 weeks (see study schema at end of Section 3). Subjects under Protocol Amendment 3 who are beyond Week 48 of blinded randomized treatment will switch to open-label tenofovir DF at the Week 72 visit and will continue on open-label treatment until Week 192.

Randomization will be stratified by age (2 to < 6 and 6 to < 12) and geographical location of study site (North America/Europe and Asia).

Randomization cannot occur until after the Baseline (pre-treatment) DEXA scan has been performed.

Subjects who experience Grade 4 ALT while on blinded study medication will be evaluated weekly with serum chemistry and liver function test monitoring. In the event that any subject has sustained Grade 4 ALT for ≥ 16 weeks (i.e., failure to resolve ALT to grade ≤ 3 or baseline), the serial HBV DNA values on study will be provided to the investigator, and the subject can be offered open-label tenofovir DF, after discussion with the Gilead Medical Monitor. Such events should be recorded as an adverse event leading to blinded study drug discontinuation.

All subjects will take a daily multivitamin with no additional mineral components that contain a minimum of 400 IU of vitamin D or higher per country specific regulations.

The primary efficacy and safety analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 48. Subsequently, efficacy and safety analyses of all subjects continuing on open-label tenofovir DF will be performed after the last randomized subject reaches Weeks 144 and 192.

An external independent multidisciplinary Data Monitoring Committee (DMC) will review the progress and safety of this study approximately every 24 weeks after the first subject is randomized. During the open-label duration of the study, the DMC will convene approximately every 52 weeks. At each meeting, the DMC will review routine safety and DEXA data and will make recommendations regarding modification of study treatment.

Subjects with the following characteristics will be eligible for enrollment: 2 to < 12 years of age with chronic HBeAg-positive or HBeAg-negative HBV infection (HBsAg-positive for at least 6 months; HBV DNA levels $\geq 10^5$ copies/mL, ALT $\geq 1.5 \times$ ULN, and creatinine clearance ≥ 80 mL/min/1.73 m^2 at screening). Subjects must be without serological evidence of co-infection with HCV, HIV, acute HAV, or HDV. Subjects with decompensated liver disease, significant renal disease, significant bone disease, evidence of hepatocellular carcinoma (i.e., α-fetoprotein > 50 ng/mL), or any chronic liver disease not related to HBV-infection, as well as pregnant or breast-feeding females, will be excluded from the study. Subjects must be naïve to tenofovir DF, but could have received prior treatment with other anti-HBV nucleoside/nucleotide therapy or interferon. Previous treatment with interferon must have ended ≥ 6 months prior to the Screening Visit. Subjects experienced on other oral anti-HBV nucleoside/nucleotide therapy must have discontinued therapy ≥ 16 weeks prior to screening to avoid flare if randomized to the placebo arm.

Plasma HBV DNA levels, laboratory analyses (serum chemistry, liver tests, hematology, and urinalysis), pregnancy test (females of childbearing potential only), vital signs, adverse events and concomitant medications will be measured or assessed at screening, baseline, Weeks 4, 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, then every 12 weeks thereafter until the end of the study (and at Early Discontinuation, if applicable). HBV serology (HBsAg, HBeAg, and reflex HBeAb and HBsAb if Ag negative) will be conducted at Screening, Baseline, Weeks 16, 32, 48, 64, 72, 80, and 96, then every 12 weeks through the end of study (and at Early Discontinuation, if applicable). DEXA scans of the spine and whole body will be performed at Baseline, and Weeks 24, 48, 72, and 96, then annually until completion of the study (and at Early Discontinuation if applicable). Bone biochemical markers will be measured at Screening, Baseline, every 24 weeks through Week 96, then annually until the end of study (and at Early Discontinuation, if applicable). DEXA and bone biochemical markers will also be required at time of switching from placebo to tenofovir DF, if the last measurement was performed > 12 weeks prior to switch. Complete physical examinations (including Tanner Staging starting at Baseline) will be performed at Screening, Baseline, Week 24 and then every 24 weeks through the end of study (and at Early Discontinuation, if applicable). Determination of HBV viral genotype (A-H) will be performed at baseline for all subjects. Resistance surveillance will be conducted at Baseline for all subjects, and attempted for all viremic subjects (HBV DNA ≥ 400 copies/mL [69 IU/mL]) at Weeks 48, 96, 144, and 192 (and at Early Discontinuation, if applicable).

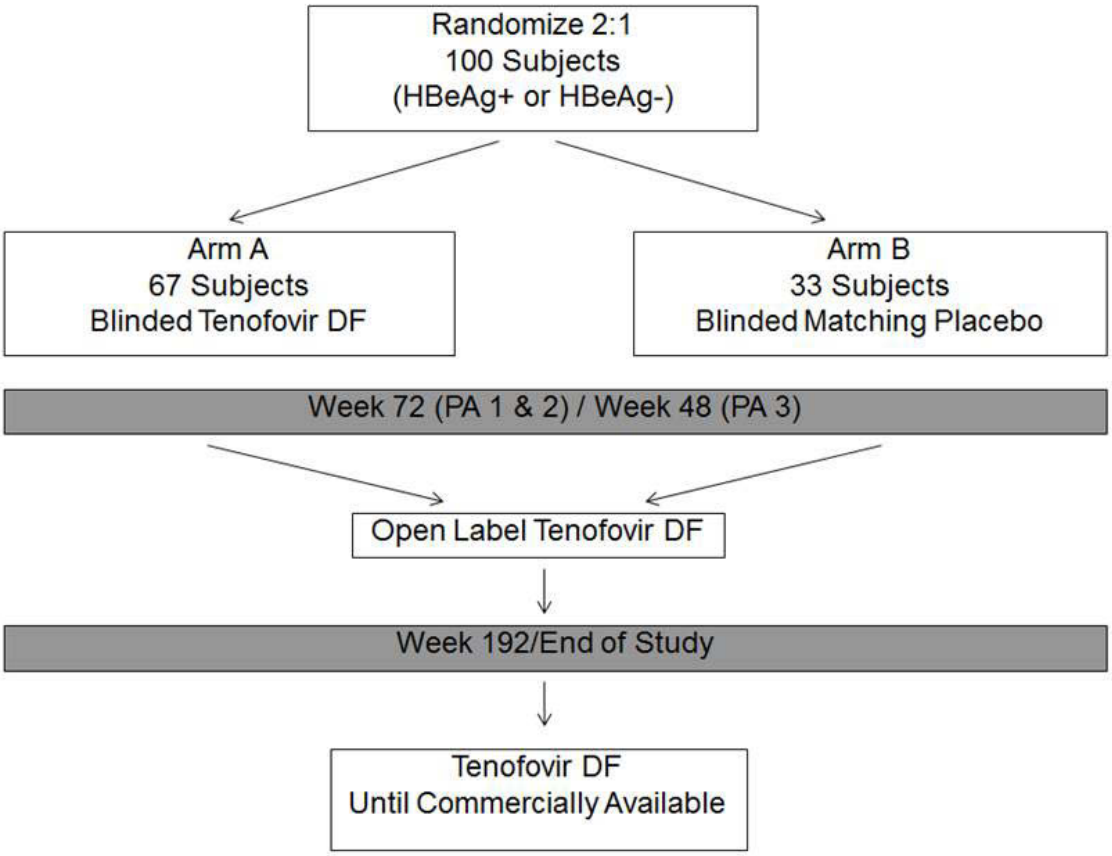
Plasma and serum for storage will be collected at every visit for possible pharmacokinetic and/or virological analyses (including resistance surveillance, HBsAg and HBeAg quantification and adherence assessment).

For subjects for whom a separate consent is provided, an appropriate blood sample for biomarker (including pharmacogenomic) analysis will be collected for the exploration of markers that may be predictive of virologic response and/or the tolerability of HBV therapies.

Subjects who permanently discontinue study drug will be asked to return for an Early Study Drug Discontinuation visit within 72 hours of the last dose of study drug. Subjects who permanently discontinue study drug will be followed for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first. For subjects off treatment, post-treatment follow-up evaluations, i.e., serum chemistry, liver function tests and plasma HBV DNA, will be performed every 4 weeks for 24 weeks. The treatment-free follow-up period could occur for early discontinuation subjects or at Week 192 study completion.

After the completion of the study at Week 192, all subjects who have completed the study will be offered open label tenofovir DF under the Extension Phase of the protocol until it is commercially available in that country for treatment of chronic HBV in patients of their age and weight. During the Extension Phase, subjects will return for study visits every 12 weeks to assess efficacy and safety, to conduct study drug accountability, and dispense study drug.

Figure 3-1. Study Schema



4. SUBJECT POPULATION

4.1. Number of Subjects and Subject Selection

A total of 100 tenofovir DF-naïve pediatric subjects with chronic HBV will be randomized 2:1 to receive blinded tenofovir DF PO once daily (Treatment A, n = 67) or blinded matching placebo PO once daily (Treatment B, n = 33).

4.2. Inclusion Criteria

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

- Male or female
- 2 years to < 12 years of age (consent of parent or legal guardian required)
- Body weight ≥ 10kg
- Documented chronic HBV infection, defined as positive serum HBsAg ≥ 6 months
- HBeAg-positive or HBeAg-negative
- HBV DNA ≥ 10^5 copies/mL (PCR method)
- ALT ≥ 1.5 × ULN at screening
- 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 proportionality constant: pediatric males/females ≥ 2 years to < 12 years k = 0.55; 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)]

- Adequate hematologic function (absolute neutrophil count ≥ 1,500/mm^3; hemoglobin ≥ 10.0 g/dL)
- Negative serum β-HCG pregnancy test (for females of childbearing potential only)
- Male and female subjects of childbearing potential (defined in Section 7.7.1) identified as choosing to become sexually active must agree to utilize highly effective contraception methods or agree to abstain from heterosexual intercourse while on study treatment and for 30 days following the last dose of study drugs (refer to Section 7.7.2).

- Parent or legal guardian able to provide written informed consent prior to any screening evaluations and willing to comply with study requirements
- Subject able to provide written assent as determined by IRB/IEC/local requirements and Investigator's discretion.
- No prior tenofovir DF therapy (subjects may have received prior interferon-alfa and/or other oral anti-HBV nucleoside/nucleotide therapy; subjects must have discontinued interferon-alfa therapy ≥ 6 months prior to screening; subjects experienced on other anti-HBV nucleoside/nucleotide therapy must have discontinued therapy ≥ 16 weeks prior to screening to avoid flare if randomized to the placebo arm)

4.3. Exclusion Criteria

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

- Pregnant or lactating subjects.
- Sexually-active males or females of childbearing potential who are not willing to use a highly effective method of contraception during the study. (see Section 7.7.2 for further details).
- Decompensated liver disease defined as PT > 1.2 × ULN, platelets < 150,000/mm^3, serum albumin < 3.5 g/dL, or prior history of clinical hepatic decompensation (e.g., ascites, jaundice, encephalopathy, variceal hemorrhage).
- Receipt of interferon (pegylated or not) therapy within 6 months of the Screening Visit
- Receipt of anti-HBV nucleoside/nucleotide therapy within 16 weeks of the Screening Visit
- α-fetoprotein > 50 ng/mL
- Evidence of hepatocellular carcinoma (HCC)
- Co-infection with HIV, acute HAV, HCV, or HDV
- Chronic liver disease of non-HBV etiology (e.g., hemochromatosis, alpha-1 antitrypsin deficiency, cholangitis)
- History of significant renal disease (e.g., nephrotic syndrome, renal dysgenesis, polycystic kidney disease, congenital nephrosis, acute tubular necrosis, other renal disease)
- History of significant bone disease (e.g., osteomalacia, chronic osteomyelitis, osteogenesis imperfecta, osteochrondroses, multiple bone fractures)
- Significant cardiovascular, pulmonary or neurological disease
- Evidence of a gastrointestinal malabsorption syndrome that may interfere with absorption of orally administered medications

- History of solid organ or bone marrow transplantation
- Ongoing therapy with any of the following:
  - Nephrotoxic agents
  - Parenteral aminoglycoside antibiotics (e.g., gentamicin, tobramycin, amikacin)
  - Cidofovir
  - Cisplatin
  - Foscarnet
  - IV amphotericin B
  - IV pentamidine
  - Oral or IV ganciclovir
  - Cyclosporine
  - Tacrolimus
  - IV vancomycin
- Chronic daily non-steroidal anti-inflammatory drug therapy
  - Competitors of renal excretion (e.g., probenecid)
  - Systemic chemotherapeutic agents
  - Systemic corticosteroids (pulmonary administration via MDI/nebulizer and oral steroids administered for less that 5 days are permitted)
  - Interleukin-2 (IL-2) and other immunomodulating agents
  - Investigational agents (except with the expressed approval of the Sponsor)
  - Administration of any of the above medications must be discontinued at least 45 days prior to the Baseline Visit and for the duration of the study period.
- Known hypersensitivity to the study drugs, the metabolites or formulation excipients
- Any other 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 dosing requirements

5. STUDY DRUGS

5.1. Randomization, Blinding and Unblinding

Approximately 100 tenofovir DF-naïve subjects will be randomized in a 2:1 ratio to receive blinded tenofovir DF PO once daily (Treatment A, N = 67), or blinded matching placebo PO once daily (Treatment B, N = 33). Randomization will be stratified by age (2 to < 6, 6 to < 12) and geographical location of study site (North America/Europe and Asia). Subjects will be assigned a screening number at the time of consent/assent. Once eligibility has been confirmed, subjects will be assigned a subject number and treatment arm at the time of randomization. A centralized randomization procedure will be used, whereby numbered bottles of tenofovir DF or placebo are assigned to subjects via an interactive voice response system (IVRS) according to the randomization code. For the first 48 weeks of the study (blinded phase), study drugs will be dispensed to the subject in a blinded fashion in numbered bottles from supplies stored at the study site. After Week 48 (open-label portion of the study), open-label tenofovir DF tablet or tenofovir DF oral powder will be provided to the study site.

Randomization cannot occur until after the Baseline (pre-treatment) DEXA scan has been performed. All Baseline tests and procedures must be completed prior to the administration of the first dose of study drugs. Initiation of treatment with study drugs should take place on-site within 24 hours of the Baseline Visit.

For the double-blind treatment period, tenofovir DF tablet or tenofovir DF oral powder and matching placebo (tablet or powder) will be supplied by Gilead. For the open-label treatment period, tenofovir DF tablet or tenofovir DF oral powder will be supplied by Gilead. Throughout the entire study period (blinded and open-label), all subjects will take a daily multivitamin with no additional mineral components that contains a minimum of 400 IU of vitamin D or higher per country specific regulations.

During the blinded portion of the study, HBV DNA results will not be distributed to investigators, subjects, or clinical research personnel involved in the clinical conduct of the study. The only exception will be in the event where Grade 4 ALT is maintained for 16 weeks and in the case of ALT flare, both considered situations of medical need, when serial HBV DNA values from Screening through time of event will be made available to the investigator. Additionally, Gilead Drug Safety and Public Health will be provided with unblinding information upon request for expedited reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs).

In the event of a medical emergency where unblinding is required for treating the subject, the investigator may unblind via the IXRS. Gilead recommends but does not require that the investigator contact the Gilead Sciences medical monitor before unblinding in such instances. Treatment assignment should remain blinded unless that knowledge is necessary to determine emergency medical care for the subject. The rationale for unblinding must be clearly explained in source documentation and on the electronic case report form (eCRF), along with the date on which the code was broken. Additionally, the investigator is requested to inform the Gilead medical monitor within 24 hours of obtaining the treatment code.

Blinding of study treatment is critical to the integrity of this clinical trial and therefore, if a subject's treatment assignment is disclosed to the investigator, the subject will have study treatment discontinued. The subject can be offered open-label tenofovir DF after discussion with the Gilead Medical Monitor. All subjects will be followed until study completion unless consent to do so is specifically withdrawn by the subject.

Gilead Drug Safety and Public Health (DSPH) may independently unblind cases for expedited reporting of suspected unexpected serious adverse reactions (SUSARs).

5.2. Description and Handling of Study Drugs – Tenofovir DF Tablet/Oral Powder and Matching Placebos

5.2.1. Formulation

5.2.1.1. Tenofovir DF Tablets

For pediatric patients weighing ≥17 kg who can swallow an intact tablet, one Tenofovir DF tablet, 150, 200, 250 or 300 mg based on body weight, can be taken orally once daily.

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

Tenofovir DF tablets 250 mg contain 250 mg of tenofovir disoproxil fumarate, which is equivalent to 204 mg of tenofovir disoproxil. The tablets are capsule-shaped, white, debossed and film-coated.

Tenofovir DF tablets 200 mg contain 200 mg of tenofovir disoproxil fumarate, which is equivalent to 163 mg of tenofovir disoproxil. The tablets are round-shaped, white, debossed, and film-coated.

Tenofovir DF tablets 150 mg contain 150 mg of tenofovir disoproxil fumarate, which is equivalent to 123 mg of tenofovir disoproxil. The tablets are triangle-shaped, white, debossed, and film-coated.

In addition to the active ingredient, the tenofovir DF tablets 150, 200, and 250 mg contain the following inactive ingredients: microcrystalline cellulose, lactose monohydrate, pregelatinized starch, croscarmellose sodium, and magnesium stearate. The film-coating contains: hypromellose (hydroxypropyl methylcellulose), lactose monohydrate, titanium dioxide, and glycerol triacetate.

5.2.1.2. Tenofovir DF Powder

Tenofovir DF oral powder formulation consists of polymer coated particles containing 40 mg of tenofovir disoproxil fumarate per 1 gram (1 scoop) of powder. In addition to the active ingredient, the oral powder contains the following inactive ingredients: hydroxypropyl cellulose, mannitol, ethylcellulose, and silicon dioxide.

5.2.1.3. Placebo Tablets

Placebo tablets to match tenofovir DF 300 mg are light blue, almond shaped, plain faced, film-coated tablets. Each tablet contains the following inactive ingredients: denatonium benzoate, lactose monohydrate, pregelatinized starch, croscarmellose sodium, and magnesium stearate. The film coating contains the following inactive ingredients: lactose monohydrate, hypromellose (hydroxypropyl methylcellulose), glycerol triacetate, titanium dioxide, and indigo carmine aluminum lake.

Placebo tablets to match tenofovir DF 150 mg, 200 mg, and 250 mg are white and of identical dimensions as the corresponding active tablet. Each tablet contains the following inactive ingredients: denatonium benzoate, lactose monohydrate, pregelatinized starch, croscarmellose sodium, and magnesium stearate. The film coating contains the following inactive ingredients: lactose monohydrate, hypromellose (hydroxypropyl methylcellulose), glycerol triacetate, and titanium dioxide.

5.2.1.4. Placebo Powder

Placebo powder to match tenofovir DF oral powder formulation is a white to off white powder. The placebo to match tenofovir DF oral powder contains the following inactive ingredients: hydroxypropyl cellulose, mannitol, ethylcellulose, and silicon dioxide.

5.2.1.5. Multivitamin Supplement

All subjects will take a daily multivitamin with no additional mineral components that contain a minimum of 400 IU of vitamin D or higher per country specific regulations.

The multivitamin is to be provided by the study site.

5.2.2. Packaging and Labeling

Thirty (30) tenofovir DF tablets or matching placebo tablets are packaged in white, high-density polyethylene (HDPE) bottles with one gram of desiccant and fiber packing present in each bottle. Each bottle is enclosed with a white, continous thread, child-reststant polypropylene screw cap with an induction-sealed, aluminium-faced liner.

Sixty (60) grams of tenofovir DF oral powder formulation or matching placebo powder formulation are packaged in white, high density polyethylene (HDPE) bottles. Each bottle is capped with a white, continuous thread, child-resistant polypropylene screw cap with an induction-sealed, aluminum-faced liner. One or more polypropylene dosing scoops will be provided with each bottle.

Study drug to be distributed to centers in the US and other participating countries shall be labeled to meet applicable requirements of the United States Food and Drug Administration (FDA), EU Guideline to Good Manufacturing Practice - Annex 13 (Investigational Medicinal Products), and/or other local regulations.

5.2.3. Storage and Handling

Tenofovir DF tablets and matching placebo should be stored at 25°C (77°F); excursions are permitted to 15°–30°C (59°–86°F). Storage conditions are specified on the label.

Tenofovir DF oral powder formulation and matching placebo powder formulation should be stored at 25°C (77°F); excursions permitted to 15°C–30°C (59°F–86°F). Storage conditions are specified on the label.

Until dispensed to the subjects, all bottles of study drugs should be stored in a securely locked area, accessible only to authorized site personnel.

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

5.3. Dosage and Administration of Study Drugs

Subjects will be randomly assigned (2:1) to receive one of the following treatments in a blinded fashion:

Treatment A:

- for subjects ≥ 17 kg - blinded tenofovir DF oral tablet (150, 200, 250, or 300 mg based on body weight) once daily for 48 weeks (oral powder may be administered to subjects if unable to swallow a tablet)

- for subjects < 17 kg or ≥ 17 kg who are unable to swallow a tablet - blinded tenofovir DF oral powder administered in a dose of 8 mg/kg PO once daily up to a maximum dose of 300 mg (ie, subjects weighing more than 35 kg will continue to receive only 300 mg) for 48 weeks

Treatment B:

- for subjects ≥ 17 kg – matching blinded placebo oral tablet once daily for 48 weeks (oral placebo powder may be administered to subjects if unable to swallow a tablet)

- for subjects < 17 kg or ≥ 17 kg who are unable to swallow a tablet - matching oral placebo powder formulation of tenofovir DF PO once daily up to a maximum corresponding dose of 300 mg for 48 weeks.

After 48 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for an additional 144 weeks. Subjects under Protocol Amendment 3 who are beyond Week 48 of blinded randomized treatment will switch to open-label tenofovir DF at the Week 72 visit and will continue on open-label treatment until Week 192.

Subjects who weigh ≥ 17 kg may be switched from the oral power formulation to tenofovir DF oral tablet once daily. For subjects < 17 kg, tenofovir DF oral powder will be administered through 192 weeks or switch to tenofovir DF tablets when subjects weigh more than 17 kg.

All subjects will take a daily multivitamin with no additional mineral components that contain a minimum of 400 IU of vitamin D or higher per country specific regulations.

5.3.1. Dosing Recommendation – Tenofovir DF or Matching Placebo Tablet

One tenofovir DF tablet or its matching placebo (as the comparator treatment) will be administered once daily to subjects who weigh ≥ 17 kg. Administration of study drug will be followed by 240 mL (8 fluid ounces) of water and will be dosed with or without food.

Table 5-1. Dosing Recommendations for Pediatric Patients ≥2 Years of Age and Weighing ≥17 kg Using VIREAD Tablets

Body Weight		
Kilogram (kg)	**Pound (lbs)**	**Tablets Once Daily**
17 to <22	37 to <49	150 mg
22 to <28	49 to <62	200 mg
28 to <35	62 to <77	250 mg
≥35	≥77	300 mg

5.3.2. Dosing Recommendation - Oral Powder

The tenofovir DF oral powder containing 4% weight/weight tenofovir DF can be administered at a dose of 8 mg/kg once daily to subjects who are ≤ 35 kg, in the following manner:

Spoon 2-4 ounces (1/4-1/2 cup) of soft food that can be swallowed without chewing (Examples of soft foods you can use are: applesauce, baby food, or yogurt) into a plastic cup or bowl without grooves. Do not mix powder with liquid (powder may float to the top even after stirring). Determine the appropriate number of level scoops (see below) of tenofovir DF oral powder that should be removed from the labeled high density polyethylene (HDPE) bottle. Use the flat edge of clean knife to make the powder even with the top of the scoop. Note that each scoop of powder covers a 5 kg body weight increment. The scoop provided contains a line marking for half scoops. The appropriate number of scoops of powder should be sprinkled on the soft food. Stir with a spoon until well mixed and give the entire dose to patient to consume right away after mixing to avoid a bad taste.

Table 5-2. Dosing Recommendations for Pediatric Patients ≥2 Years of Age Using VIREAD Oral Powder

Body Weight		Oral Powder Once Daily
Kilogram (kg)	**Pound (lbs)**	**Scoops of Powder**
10 to <12	22 to <26	2.0
12 to <14	26 to <31	2.5
14 to <17	31 to <37	3.0
17 to <19	37 to <42	3.5
19 to <22	42 to <49	4.0
22 to <24	49 to <53	4.5
24 to <27	53 to <60	5.0
27 to <29	60 to <64	5.5
29 to <32	64 to <71	6.0
32 to <34	71 to <75	6.5
34 to <35	75 to <77	7.0
≥35a	≥77	7.5

Subjects > 35 kg who are unable to swallow tablet may dose with oral powder at 7.5 level scoops once daily.

5.4. Prior and Concomitant Medications

5.4.1. Prior to Study Entry

Refer to Exclusion Criteria in Section 4.3. Subjects must not have received therapy with interferon within six months prior to screening for this study. Subjects must not have received therapy with anti-HBV nucleotide/nucleoside within 16 weeks prior to screening for this study.

5.4.2. During the Study

Use of the following medications is prohibited while subjects are on study drug:

- Antiviral agents with anti-HBV activity, including lamivudine, emtricitabine, entecavir, adefovir, telbivudine, clevudine, or others
- Interferon-alfa and pegylated interferon-alfa
- Nephrotoxic agents such as aminoglycoside antibiotics, cidofovir, cisplain, foscarnet, IV amphotericin B, IV pentamidine, ganciclovir, cyclosporine, tacrolimus, chronic daily non-steroidal anti-inflammatory drugs, or other agents with significant nephrotoxic potential

- Hepatotoxic agents such as anabolic steroids, isoniazid, itraconazole, ketoconazole, lovastatin, rifabutin, rifampin, simvastatin, and other agents with significant hepatotoxic potential
- Competitors of renal excretion, such as probenecid
- Systemic chemotherapeutic agents
- Interleukin-2 [IL-2]) and other immunomodulating agents
- Systemic corticosteroids (pulmonary administration via MDI/nebulizer and oral steroids administered for less that 5 days are permitted)
- Investigational agents, except with written approval of the Sponsor

Should subjects need to start treatment with any excluded concomitant medication, the Sponsor must be consulted prior to initiation of the new medication. In instances where an excluded medication is initiated prior to discussion with the Sponsor, the Investigator must notify the Sponsor as soon as s/he is aware of the use of the excluded medication.

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. Study Drug Accountability

The investigator or designee (e.g., pharmacist) is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgement of receipt of each shipment of study drug (quantity and condition). All used and unused study drug dispensed to subjects must be returned to the site.

Investigational Drug Accountability records will be provided to each study site to:

- Record the date received and quantity of study drug.
- Record the date, subject number, subject initials, the study drug dispensed.
- Record the date, quantity of used and unused study drug returned. Dispensing records will include the initials of the person dispensing the study drug or supplies.

5.6. Study Drug Return or Disposal

At the start of the study, the study monitor will evaluate each study center's study drug disposal procedures and provide appropriate instruction for return or destruction of unused study drug supplies. If the site has an appropriate Standard Operating Procedure (SOP) for drug destruction, the site may destroy used and unused study drug supplies performed in accordance with the site's (hospital/pharmacy) SOP. If the site does not have acceptable procedures in place for drug

destruction, arrangements will be made between the site and Gilead Sciences (or Gilead Sciences' representative) for return of unused study drug supplies. A copy of the site's SOP will be obtained for central files. Where possible, study drug will be destroyed at the site. Upon study completion, a copy of the drug accountability records must be filed at the site. Another copy will be returned to Gilead Sciences. If drug is destroyed on site, the investigator must maintain accurate records for all study drug destroyed. Records must show the identification and quantity of each unit destroyed, the method of destruction, and person who disposed of the drug. All study drug records must be maintained at the site and copies must be submitted to Gilead Sciences at the end of the study.

6. STUDY PROCEDURES

Study procedures to be conducted for each subject enrolled in the study are presented in tabular form in Appendix 2 and in the text that follows. Additional information on the study procedures will be provided in written materials by the Contract Research Organization (CRO).

Any deviation from protocol procedures should be noted in the Case Report Forms (CRFs) and the sponsor or Contract Research Organization (CRO) should be notified.

All protocol-specified laboratory tests on blood and urine samples must be performed at the selected central laboratory. Refer to the appropriate central laboratory instruction manuals for information on sample collection and shipment of all required study samples.

6.1. Subject Enrollment and Treatment Assignment

Each candidate's parent or legal guardian must sign an Informed Consent Form prior to the conduct of any screening procedures. Each study candidate must sign an Assent Form, as required by IRB/IEC/local requirements. Screening evaluations will be used to determine the eligibility of each candidate for study enrollment. Candidates who fail to meet eligibility criteria by screening evaluations may be re-screened once ≥ 45 days after the initial screen if there is a reasonable expectation that the candidate will be eligible after repeat screening.

Retesting of exclusionary laboratory values during the Screening period is permitted only if, in the Principal Investigator's opinion, there is reason to believe the retest value will be within accepted parameters; if the initial value was deemed to be inaccurate, inconsistent with the subject's previous result(s); in error (e.g. mishandled sample); or due to an extenuating circumstance.

6.1.1. Screening Visit

The following assessments and procedures will be performed and recorded on CRFs at the initial Screening Visit:

- Written informed consent from parent or legal guardian and assent from subject, if/when applicable (see above)
- Medical history, including hepatitis B history, hepatitis B treatment history, and family history
- Complete physical examination (excluding breast, anorectal, and urogenital exams), vital signs (temperature, blood pressure, pulse, respiratory rate)
- Body weight and height

- Blood samples for the following will be collected. If all the blood samples cannot be collected in one screening visit, subject may come in at a different visit prior to Baseline to have the remainder of the blood samples collected.

  — Hematology (complete blood count [CBC] with differential and platelet count)

  — Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, total iron, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)

  — Prothrombin time (PT), international normalized ratio (INR)

  — Alpha-1 antitrypsin and protease inhibitor (PI) typing

  — Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative)

  — Plasma HBV DNA (PCR method)

  — HIV, HAV, HCV and HDV serology

  — Serum β-HCG test (females of childbearing potential only)

  — α-fetoprotein (AFP)

  — Serum bone biochemical markers, including serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, vitamin D levels (25-hydroxy), 1,25 (dihydroxyvitamin) D levels, and fasting creatinine and fasting phosphate

- Urine samples for:

  — Urinalysis (protein, glucose, blood)

  — Urine bone biochemical markers, including urine bicarbonate, urine n-telopeptide, and spot urine creatinine and phosphate

- Review of AEs and concomitant medications

- Review of all inclusion and exclusion criteria

- Dual energy x-ray absorptiometry (DEXA) scan of spine and whole body (DEXA scan may be performed between Screening and Baseline Visits, but must be performed no later than the Baseline Visit, and prior to receipt of study drugs).

Subjects meeting all of the inclusion criteria and none of the exclusion criteria will return to the clinic within 45 days of the initial Screening Visit for entry into the study. All candidates who meet screening requirements will be randomized and enrolled into the study until the planned enrollment of 100 subjects has been met.

Randomization cannot occur until after the Baseline (pre-treatment) DEXA scan has been performed.

6.1.2. Baseline Assessments

The Baseline Visit should occur within 45 days of the initial Screening Visit. Once eligibility has been confirmed, subjects will be assigned a subject number and treatment arm at the time of randomization. All baseline tests and procedures must be completed prior to the receipt of the first dose of study drugs. Subjects will be dispensed study medication at the Baseline Visit. Study medication should be initiated on-site at the Baseline Visit.

The following assessments and procedures will be performed and recorded on CRFs at the Baseline Visit:

- Review of medical history, hepatitis B history and hepatitis B treatment history and any changes since Screening Visit
- Complete physical examination including Tanner Staging and vital signs
- Body weight and height
- Blood samples for:
  - Hematology (CBC with differential and platelet count)
  - Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)
  - Prothrombin time (PT), international normalized ratio (INR)
  - Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative)
  - Plasma HBV DNA (PCR method)
  - Serum bone biochemical markers, including serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, vitamin D levels (25-hydroxy), 1,25 (dihydroxyvitamin) D levels, and fasting creatinine and fasting phosphate

- Serum β-HCG pregnancy test (females of childbearing potential only)
- Serum for HBV viral genotyping (A–H) and resistance surveillance (sequence analysis of the HBV pol/RT for resistance mutations)
- Plasma for determination of tenofovir concentration
- Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including HBsAg and HBeAg quantification and compliance assessment)
- For subjects for whom a separate consent is provided, an appropriate blood sample for biomarker (including pharmacogenomic) analysis will be collected at Baseline (or at the subsequent visits if necessary)

- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - Urine bone biochemical markers, including urine bicarbonate, urine n-telopeptide, and spot urine creatinine and phosphate

- DEXA scan of spine and whole body (DEXA scan may be performed between Screening and Baseline Visits, but must be performed no later than the Baseline Visit), and prior to receipt of study drugs.
- Review of AEs and concomitant medications
- Study drug dispensing (including multivitamin) and instructions on appropriate dosing and administration

6.2. Treatment Assessments

6.2.1. Week 4, 8, 16, 24, 32, 40 Assessments

The following evaluations will be performed at Weeks 4, 8, 16, 24, 32, and 40, unless otherwise specified. Study visits are to be completed ± 4 days of the protocol-specified visit date, based on the Baseline Visit. Week 48 assessments are outlined in the following section (Section 6.2.2).

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

- Symptom-directed physical examination, including vital signs (complete physical examination including Tanner Staging at Week 24 only)
- Body weight and height

- Blood samples for:
  - Hematology (CBC with differential and platelet count)
  - Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)
  - Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative; Weeks 16 and 32 only)
  - Plasma HBV DNA (PCR method)
  - Plasma for determination of tenofovir concentration
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg and HBeAg quantification and compliance assessment)
- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - At Week 24 only: urine biochemical markers (refer to Section 6.5. for details)
  - Urine pregnancy test (for females of childbearing potential only; positive urine pregnancy test will be immediately confirmed with a serum pregnancy test)
- At Week 24 only: dual energy x-ray absorptiometry (DEXA) scan of spine and whole body (DEXA scan must be performed ± 10 days from visit)
- Review of AEs and changes in concomitant medications
- Retrieval of study medication and assessment of medication adherence
- Study drug dispensing (including multivitamin) and instructions on appropriate dosing and administration
- At Weeks 4 and 24 only: Provide all subjects with a Subject Dosing Diary Card to complete the diary daily for 10 days prior to the next visit
- At Weeks 8 and 32 only: Collect the Subject Dosing Diary Card completed by the subject daily for 10 days prior to these visits

Subjects who experience Grade 4 ALT while on blinded study medication will be evaluated weekly with serum chemistry and liver function test monitoring. In the event that any subject has sustained Grade 4 ALT for ≥ 16 weeks (i.e., failure to resolve ALT to grade ≤ 3 or baseline), the serial HBV DNA values on study will be provided to the investigator, and the subject can be offered open-label tenofovir DF, after discussion with the Gilead Medical Monitor.

6.2.2. Week 48 Assessments – End of Blinded Randomized Treatment

Study Visit for Week 48 should be completed ± 4 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 examination including Tanner Staging and vital signs
- Body weight and height
- Blood samples for:
  - Hematology (CBC with differential and platelet count)
  - Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)
  - Serum bone biochemical markers, including serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, vitamin D levels (25-hydroxy), 1,25 (dihydroxyvitamin) D levels, and fasting creatinine and fasting phosphate
  - Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative)
  - Plasma HBV DNA (PCR method)
  - Serum β-HCG pregnancy test (females of childbearing potential only)
  - Serum for resistance surveillance
  - Plasma for determination of tenofovir concentration
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg and HBeAg quantification and compliance assessment)

- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - Urine bone biochemical markers, including urine bicarbonate, urine n-telopeptide, and spot urine creatinine and phosphate
- DEXA scan of spine and whole body (DEXA scans may be performed ± 10 days from the Week 48 visit).
- Review of AEs and concomitant medications
- Retrieval of study medication and assessment of medication adherence
- Study drug dispensing (including multivitamin) and instructions on appropriate dosing and administration

After 48 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for an additional 144 weeks. Subjects under Protocol Amendment 3 who are beyond Week 48 of blinded randomized treatment will switch to open-label tenofovir DF at the Week 72 visit and will continue on open-label treatment until Week 192.

Subjects who discontinue the study prior to the Week 48 Visit will complete an Early Study Drug Discontinuation Visit as detailed in Section 6.3. 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 study drug and permanently discontinue study drug will be followed for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first.

6.2.3. Assessments for Weeks 56, 64, 72, 80, 88, 96, 108, 120, 132, 144, 156, 168, 180, and Extension Phase

After Week 48 through the end of the study treatment (Week 192), subjects will return for visits at Weeks 56, 64, 72, 80, 88, 96, 108, 120, 132, 144, 156, 168, and 180. Each study visit will occur ± 6 days from the protocol-specified visit date, based on the Baseline Visit.

Subjects in this study who complete all study visits and are on tenofovir DF at the Week 192 visit will be eligible to continue in the Extension Phase of the study, where they will receive open-label tenofovir DF (dose adjusted for weight) until Viread is commercially available in that country for treatment of Chronic HBV in patients of their age and weight. During the Extension Phase, subjects will return for visits every 12 weeks to assess efficacy and safety, to conduct study drug accountability, and dispense study drug. Study Visits for the Extension Phase should be completed within ± 14 days of the protocol-specified visit date, based on the Baseline Visit. When commercial Viread becomes available, subjects will complete the End of Treatment Visit assessments at the next scheduled visit (see Section 6.2.4), and the subject's participation in the study will end.

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

- Symptom-directed physical examination, including vital signs (complete physical examination including Tanner Staging at Weeks 72, 96, 120, 144, 168, and every 48 weeks during Extension Phase)
- Body weight and height
- Blood samples for:
  - Hematology (CBC with differential and platelet count)
  - Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)
  - At Weeks 96, 144, and every 48 weeks during the Extension Phase: Serum bone biochemical markers, including serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, vitamin D levels (25-hydroxy), 1,25 (dihydroxyvitamin) D levels, and fasting creatinine and fasting phosphate
  - Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative) (Weeks 64, 72, 80, 96, and then every 12 weeks until the end of the study including the Extension Phase)
  - Plasma HBV DNA (PCR method)
  - Plasma for determination of tenofovir concentration
  - At Weeks 96 and 144: Serum for resistance surveillance
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg and HBeAg quantification and compliance assessment)
- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - Urine pregnancy test (for females of childbearing potential only; positive urine pregnancy test will be immediately confirmed with a serum pregnancy test)
  - At Weeks 72, 96, 144, and every 48 weeks during the Extension Phase: Urine bone biomarkers, including urine bicarbonate, urine n-telopeptide, and spot urine creatinine and phosphate

- Weeks 72, 96, 144, and every 48 weeks during the Extension Phase: DEXA scan of spine and whole body (DEXA scan must be performed ± 10 days from scheduled study visit)
- Review of AEs and concomitant medications
- Retrieval of study medication and assessment of medication adherence
- Study drug dispensing (including multivitamin) and instructions on appropriate dosing and administration
- At Week 56 only: Provide all subjects with a Subject Dosing Diary Card to complete the diary for 10 days prior to the next visit.
- At Week 64 only: Collect the Subject Dosing Diary Card completed by the subject daily for 10 days prior to these visits.

6.2.4. Week 192 Assessments/Early Discontinuation/End of Treatment

Study Visit for Week 192 should be completed ± 6 days of the protocol-specified visit date, based on the Baseline Visit. If the Early Discontinuation visit is performed prior to Week 96, DEXA and bone biochemical markers are required if the previous measurements were > 12 weeks prior. If the Visit is performed after Week 96 or after the Extension Phase, DEXA and biochemical markers are required if the previous measurements were > 24 weeks prior.

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

- Complete physical examination including Tanner Staging and vital signs
- Body weight and height
- Blood samples for:
  - Hematology (CBC with differential and platelet count)
  - Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)
  - Serum bone biochemical markers, including serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, vitamin D levels (25-hydroxy), 1,25 (dihydroxyvitamin) D levels, and fasting creatinine and fasting phosphate
  - Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative)

- Plasma HBV DNA (PCR method)
- Serum β-HCG pregnancy test (females of childbearing potential only)
- Serum for resistance surveillance
- Plasma for determination of tenofovir concentration
- Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg and HBeAg quantification and compliance assessment)

- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - Urine bone biochemical markers, including urine bicarbonate, urine n-telopeptide, and spot urine creatinine and phosphate

- DEXA scan of spine and whole body (DEXA scans may be performed ± 10 days from the Week 192 visit). Review of AEs and concomitant medications

- Retrieval of study medication and assessment of medication adherence

Subjects who discontinue the study prior to the Week 192 Visit will complete all Week 192 assessments and procedures at an Early Study Drug Discontinuation Visit, to be completed within 72 hours of last dose of study drug. 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 study drug and permanently discontinue study drug will be followed for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first.

The study treatment will end after the each subject reaches Week 192. At Week 192, all active subjects will be offered tenofovir DF through the Extension Phase of the protocol until it becomes commercially available in that country for treatment of chronic HBV in patients of their age and weight. If the subject is off treatment following Week 192, the treatment-free follow-up period is required (Section 6.3.1).

6.3. Post-Treatment/Treatment-Free Follow-up Assessments

6.3.1. Follow-Up Assessments

For subjects with known bridging fibrosis or cirrhosis, study drug discontinuation with treatment-free follow-up is contraindicated due to the potential risk of exacerbation of hepatitis in the setting of low hepatic reserve which could lead to decompensation. Subjects with bridging fibrosis or cirrhosis should be placed on commercially available HBV therapy following study drug discontinuation.

Subjects who have received at least one dose of study drug and permanently discontinue study drug will be followed for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first. The treatment-free follow-up period could occur for early discontinuation subjects or at Week 192 study completion.

For those subjects who remain off treatment, the following evaluations are to be completed every 4 weeks through the Week 24 Follow-Up Visit (Follow-Up Weeks 4, 8, 12, 16, 20 and 24). Each follow up visit should be conducted within ± 3 days from the protocol-specified visit date, calculated from the date of last dose.

- Symptom-directed physical examination, including vital signs
- Body weight and height
- Blood samples for:
  - Serum chemistry and liver function tests, including albumin, alkaline phosphatase, AST, ALT, total bilirubin (reflex to direct [conjugated] bilirubin if total bilirubin > 1.5 × ULN), bicarbonate, BUN, calcium, chloride, CPK, creatinine (and calculated creatinine clearance), glucose, LDH, magnesium, phosphorus, potassium, sodium, uric acid, and amylase (reflex lipase testing if total amylase is ≥ 1.5 × ULN)
  - Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative) (Follow-Up Week 24 only)
  - Plasma HBV DNA (PCR method)
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg and HBeAg quantification and compliance assessment)
  - Prothrombin time (PT), international normalized ratio (INR) (reflex test in case of post-treatment exacerbation of hepatitis)
- Review of AEs and concomitant medications

Subjects experiencing post-treatment exacerbation of hepatitis during the 24-week post-treatment period should be followed weekly until their ALT levels return to Grade 2 or baseline, as described in the Toxicity Management section of the protocol (Section 7.6.)

6.3.2. Assessments for Premature Discontinuation from the Study (Early Study Drug Discontinuation Visit)

Subjects who permanently discontinue study drug prior to the end of the study (Week 192) will be asked to return to the clinic within 72 hours of stopping study drug for an Early Study Drug Discontinuation Visit. Subjects who discontinue the study prior to the Week 192 Visit will complete all Early Discontinuation assessments and procedures (see Section 6.3.1 above). Subjects who have received at least one dose of study drug and permanently discontinue study drug will be followed with visits every 4 weeks for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first. Subsequent off-study therapy, if any, is at the discretion of the subject/physician and will not be provided by Gilead Sciences.

Subjects experiencing post-treatment exacerbation of hepatitis during the 24-week post-treatment period should be followed weekly until their ALT levels return to Grade 2 or baseline, as described in the Toxicity Management section of the protocol (Section 7.6).

6.4. Bone Mineral Density

Dual energy x-ray absorptiometry (DEXA) scans will be performed between the Screening and the Baseline Visits, at Weeks 24, 48, 72, 96, 144, 192, every 48 weeks during the Extension Phase, and the Early Study Drug Discontinuation/End of Treatment Visit, if applicable, using pediatric software approved by the DEXA vendor selected by Gilead Sciences. **Randomization may not occur until after the Baseline (pre-treatment) DEXA scan has been performed.** Subsequent DEXA scans should be performed within 10 days of the scheduled visit. In addition, a DEXA scan will be performed at the time of switch in subjects switching from placebo to tenofovir DF, unless the previous scan was ≤ 12 weeks earlier. Scans will be made of the spine and whole body to measure changes in bone mineral density and bone mineral content. All DEXA scan results (spine bone mineral density, whole body bone mineral density) will be provided to the study sites.

A complete description of the procedures to be performed for the DEXA scans will be provided by the DEXA vendor in a DEXA procedure manual.

6.5. Bone Biochemical Markers

Laboratory samples will be taken at screening, baseline and at Weeks 24, 48, 72, 96, 144, 192, every 48 weeks during the Extension Phase, and the Early Study Drug Discontinuation/End of Treatment Visit, if applicable, for measuring bone biochemical markers. In addition, bone biochemical markers will be measured at the time of switch in subjects switching from placebo to tenofovir DF, unless the previous measurements were ≤ 12 weeks prior.

Analyses will include measurements of urine bicarbonate and n-telopeptide; measurements of serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, and vitamin D levels (25-hydroxy) and 1,25 (dihydroxyvitamin) D levels; and fasting serum creatinine/phosphate and urine creatinine (spot)/phosphate for measurement of the renal phosphate threshold (TmP/GFR).

The mean of the Screening and Baseline Visit values will constitute the study baseline value to which all subsequent values will be compared. Bone biochemical markers can be affected by the time of day (diurnal variation) and food. Therefore, subjects will be asked to report for each of these visits in the morning in a fasted state or, if fasting is not feasible, at the same time (± 2 hours from the Baseline Visit draw time) at each subsequent visit. All samples will be sent to a central laboratory in accordance with the laboratory procedure manual. Bone biochemical marker results will not be provided to the study sites.

6.6. Pharmacokinetic Assessment

Two intensive PK substudies will be performed on a subset of subjects in two separate cohorts; an oral powder and a tablet cohort. Each cohort will have an intensive PK substudy performed over one day between Week 2 and Week 12 to evaluate the PK of different tenofovir DF formulations in a pediatric population.

Powder Cohort

Subjects who are eligible to take the oral powder (either tenofovir DF or matching placebo) based on body weight and/or preference for this dosage form, and subjects who qualify for the tablet formulation but are willing to initiate treatment with the oral powder for purposes of accruing additional PK data, will be offered the opportunity to participate in the oral powder cohort. Up to 30 randomized subjects will be able to participate in the powder cohort. In order to participate in the oral powder cohort, the subject must be taking the oral powder for at least 2 weeks prior to the intensive PK visit.

Tablet Cohort

Randomized subjects who are assigned to take tablet (either tenofovir DF or matching placebo) will be offered the opportunity to participate in the tablet cohort. A target of 12 subjects will be enrolled in the tablet cohort at each dosage level (150, 200, 250, 300 mg). Subjects may participate in both the powder and the tablet cohorts intensive PK substudies, with the second intensive PK substudy occurring after the Week 12, but prior to the end of the double-blind phase. For those subjects who opt to initiate treatment with oral powder and subsequently switch to tablets following completion of the powder intensive PK visit, or those subjects who are required to change dose strengths of the tablet based on the weight-based dosing requirement (see protocol Section 5.3) will also have the option to participate in a second tablet intensive PK visit. Subjects initially treated with tablets or subjects switching from oral powder to tablet, must be receiving tablets for at least two weeks prior to the tablet intensive PK visit.

Intensive and Sparse PK Sampling

Both cohorts (oral powder and tablets) will have the intense PK sampling occurring over a single day for a period of 8 hours. Specimens will be drawn at 0 hours (pre-dose), and at 1, 2, 4, and 8 hours after study drug dosing (tenofovir DF or placebo).

Additionally, sparse PK sampling will be performed on all subjects at every study visit to monitor for adherence and potentially for other PK analyses (e.g. population pharmacokinetics). At the time each blood sample is collected (both intensive and sparse sampling), the following information will be recorded:

- The date and time of last dose taken
- Whether last dose was taken with or without food
- The date and time of blood draw

All specimens will be sent to an external lab for analysis. Details of the substudy procedures and specimen management will be described in the PK manual.

6.7. Drug Administration and Fasting Criteria for the Pharmacokinectic (PK) Substudy

In order to allow for ease of PK sampling over an 8-hour period, subjects participating in the PK substudy should be taking their tenofovir DF dose in the AM on the day of the PK visit. Therefore, subjects who have been routinely dosing in the PM will need to switch to an AM dosing regimen at least 10 days prior to the intensive PK visit. The AM dose should be taken on each day with a meal (breakfast). In addition, subjects should fast overnight (a minimum of 8 hours) prior to the scheduled intensive PK visit.

6.8. Serum and Plasma for Storage

Additional blood (plasma and serum) samples will be collected at each visit (including during the Extension Phase) starting at Baseline for long-term storage and possible future testing for potential pharmacokinetic and/or virologic assays (including resistance surveillance, HBsAg and HBeAg quantification and compliance assessment). No human genetic testing will be performed. At the conclusion of this study, these samples may be retained in storage for a period of up to 15 years.

6.9. Subject Dosing Diary Card

As an added measure to assess adherence to treatment, all subjects will be provided with a Subject Dosing Diary Card at Weeks 4, 24, and 56. Subjects will complete the card daily for 10 days prior to the next visit. Subjects will return their completed card at Week 8, 32 and 64. The Investigator or designee will review the diary card to monitor subject compliance with study drug regimen.

In addition to the above, all subjects participating in the intensive PK substudy will be required to complete the Subject Dosing Diary Card for at least 10 days prior to the intensive PK visit.

6.10. Resistance Surveillance

The objectives of the resistance surveillance are: (1) to identify mutations in the HBV polymerase gene from HBV subject isolates that are potentially associated with virological resistance to tenofovir DF, (2) to determine the correlation of the effects of these mutations to the

clinical response to tenofovir DF therapy, (3) to determine whether these mutations alter antiviral susceptibility to tenofovir DF using in vitro HBV replication assays, and (4) to evaluate the cross resistance profile of these mutations.

Sequence analysis of the HBV polymerase/reverse transcriptase for resistance mutations will be conducted at Baseline for all subjects, and attempted for all viremic subjects (HBV DNA ≥ 400 copies/mL [69 IU/mL]) at Weeks 48, 96, 144, and 192, as well as at the Early Discontinuation Visit, if applicable. Serum for storage will be collected at every visit for possible virologic analyses (see Section 6.8).

6.11. Criteria for Discontinuation of Study Treatment

Study 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
- Subject or legal guardian requests to discontinue for any reason
- Subject noncompliance
- Pregnancy during the study
- Discontinuation of the study at the request of Gilead Sciences, regulatory agency or an IRB/IEC.

If a subject discontinues study medication dosing, every attempt should be made to keep the subject in the study and continue to perform the required study-related follow-up and procedures. If this is not possible or acceptable to the subject or investigator, the subject may be withdrawn from the study.

7. ADVERSE EVENTS AND TOXICITY MANAGEMENT

7.1. 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 also include the following:

- Pre-or post-treatment complications that occur as a result of protocol mandated procedures (e.g., invasive procedures such as venipuncture, biopsy) during or after screening (before the administration of study drug).

- Any pre-existing condition that increases in severity, or changes in nature during or as a consequence of the study drug phase of a human clinical trial, will also be considered an AE.

- Complications and terminations of pregnancy (see Section 7.7 for additional information)

- Reports of adverse reactions in infants following exposure from breastfeeding

All AEs that occur after the subject consents to participate in the study and throughout the duration of the study, including the follow-up off-study medication period should be recorded as an AE.

An AE does not include the following:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion) performed; the condition that leads to the procedure is an adverse event

- Pre-existing diseases or 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 (see Section 7.3.1., *Special Situations Report).*

- 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 eCRF.

- Uncomplicated pregnancy

- An induced elective abortion to terminate a pregnancy without medical reason

7.2. Assessment of Adverse Events

All AEs will be assessed by the investigator and recorded on the AE CRF page. The AE entry should indicate whether or not the AE was serious, the start date (AE onset), the stop date (date of AE resolution), whether or not the AE was related to study drug or to a study procedure, the action taken with study drug due to the AE, and the severity of the AE.

The relationship to study drug therapy should be assessed using clinical judgment and the following definitions:

- **No**: Evidence exists that the adverse event has an etiology other than the study drug. For SAEs, an alternative causality must be provided (e.g., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

- **Yes**: A temporal relationship exists between the AE onset and administration of the study drug that cannot be readily explained by the subject's clinical state or concomitant therapies. Furthermore, the AE appears with some degree of certainty to be related, based on the known therapeutic and pharmacologic actions or adverse event profile of the study drug. In case of cessation or reduction of the dose, the AE abates or resolves and reappears upon rechallenge.

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 definitions:

- **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-mandated procedures such as venipuncture, biopsy or diagnostic tests.

7.3. Serious Adverse Events

A **serious adverse event** (SAE) is defined as follows:

Any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death

- Life-threatening situation. Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred.

- In-patient hospitalization or prolongation of existing hospitalization (excluding those for study therapy or placement of an indwelling catheter, unless associated with other SAEs)

- Persistent or significant disability/incapacity

- Congenital anomaly/birth defect in the offspring of a subject who received study drug

- Other: medically significant events that may not be immediately life-threatening or result in death or hospitalization, but based upon appropriate medical and scientific judgment, may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above

- Examples of such events are as follows:

  — Intensive treatment in an emergency room or at home for allergic bronchospasm

  — Blood dyscrasias or convulsions that do not result in hospitalization

  — Development of drug dependency or drug abuse

For the purposes of this study, in addition to the above criteria, the following must be reported as SAE's:

- Serum ALT > 2 × baseline and > 10 × ULN, with or without associated symptoms.

- 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 (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).

- Any clinical manifestations of hepatic decompensation (variceal bleeding, hepatic encephalopathy, or worsening of ascites requiring diuretics or paracentesis).

Clarification of Serious Adverse Events

- Death is an outcome; therefore, not an AE in itself; the SAE is the condition(s) that leads to death.

- The subject may not have been on study drug at the occurrence of the event. Dosing may have been given as treatment cycles or interrupted temporarily before the onset of the SAE, but may have contributed to the event.

- "Life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

- Complications that occur during hospitalizations are AEs. If a complication prolongs the hospitalization, it is a SAE.

- "In-patient hospitalization" means the subject has been formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. It does not include presentation and care within an emergency department.

- The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

- A distinction should be drawn between seriousness and severity of AEs. An AE that is assessed as Grade 4 (potentially life-threatening) should not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 4. An event is defined as "serious" when it meets one of the predefined outcomes described above in Section 7.3.

7.3.1. Special Situations Report

Defintions of Special Situations

Special situation reports include all reports of medication error, abuse, misuse, overdose, reports of adverse events associated with product complaints, occupational exposure and pregnancy reports.

A pregnancy report is used to report pregnancies occurring in subjects during the study whether or not exposure to the product occurred.

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.

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

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).

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

Instructions for Reporting Special Situations: Pregnancies

The Investigator should report all pregnancies that are identified after the subject first consents to participate in the study (ie, signing the informed consent) and throughout the study, including the post-study drug follow-up period, to the CRO using the Pregnancy Report form within 24 hours of becoming aware of the pregnancy. Subjects who become pregnant or who suspect that they are pregnant during the study must report the information to the Investigator and discontinue study drug immediately.

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 (e.g., 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 adverse event term.

A spontaneous abortion is always considered to be an SAE and will be reported as described in the Adverse and Serious Adverse Events section. Furthermore, any SAE occurring as an adverse pregnancy outcome post-study must be reported to the CRO.

The subject should receive appropriate monitoring and care until the conclusion of the pregnancy. The outcome should be reported. Please refer to Sections 7.4.1 All Serious Adverse Events and 7.4.3 Post-Study Reporting Requirements for contact information for reporting.

Instructions for Reporting Other Special Situations

Please refer to Sections 7.4.1 All Serious Adverse Events and 7.4.3 Post-Study Reporting Requirements for contact information for reporting.

These reports must consist of situations that involve study investigational medicinal products (IMPs), but do not apply to concomitant medications (i.e., except for situations that result in adverse events, special situations involving concomitant medications will not be reported). Any inappropriate use of non-protocol approved medications should not be reported as “misuse” but may be more appropriately documented as a protocol deviation if applicable.

7.4. Serious Adverse Event Reporting Requirements

7.4.1. All Serious Adverse Events

Gilead is required to expedite to worldwide regulatory authorities reports of Serious Adverse Events, Serious Adverse Drug Reactions or Suspected Unexpected Serious Adverse Reactions (SUSARs) in line with relevant legislation, including the European Commission Clinical Trials

Directive (2001/20/EC); therefore, Gilead (or the CRO on the behalf of Gilead) must be notified immediately regarding the occurrence of any SAE or SADR that occurs after the subject consents to participate in the study, including SAEs/SADRs resulting from protocol-associated procedures as defined in relevant legislation including 2001/20/EC, performed from screening onwards. The procedures for reporting all SAEs, regardless of causal relationship, are as follows:

- Record the SAE on the AE CRF and complete the "Serious Adverse Event Report" form.
- Fax or email the SAE form within 24 hours of the Investigator's knowledge of the event. Contact information listed below.

For US (PPD):	Fax:	1-888-529-3580
For EU and Asia (PPD):	Fax:	+44 1223-374-102
For India (KlinEra):	Email:	Safetyreporting@klinera.com
	Fax:	+91-22-25004588
	Tel:	+91-22-25091470, if you have questions

- For post-study reporting, SAE forms will be reported as outlined in Section 7.4.3 *Post-Study Reporting Requirements.*
- For fatal or life-threatening events, also fax copies of hospital case reports, autopsy report, and other documents when requested and applicable. Transmission of such documents should occur with Personal Subject Details de-identified, without losing the traceability of a document to the Subject Identifiers.
- Gilead Sciences may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications necessary for treatment of the SAE must be recorded onto the concomitant medication section of the subject's CRF.

Follow-up of adverse events will continue through the last day on study (including the follow-up off-study medication period of the study) and/or until the investigator and/or Gilead Sciences determine that the subject's condition is stable. Gilead Sciences may request that certain adverse events be followed until resolution.

7.4.2. Investigator and Sponsor Reporting Requirements for SAEs

An event may qualify for expedited reporting to worldwide regulatory authorities if it is a Serious Adverse Event, Serious Adverse Drug Reaction or Suspected Unexpected Serious Adverse Reaction (SUSAR) in line with relevant legislation, including the European Commission Clinical Trials Directive (2001/20/EC). Expectedness of SAEs will be determined by Gilead using the reference safety information specified in the Investigator's Brochure.

All investigators will receive a safety letter notifying them of relevant SUSAR reports. The investigator should notify the IRB or IEC as soon as is practical, of serious events in writing where this is required by local regulatory authorities, and in accordance with the local institutional policy.

In accordance with the European Commission Directive 2001/20/EC, Gilead Sciences or specified designee will notify worldwide regulatory authorities and the relevant Ethics Committees in concerned Member States of applicable SUSARs as individual notifications or through a periodic line listing.

7.4.3. Post-Study Reporting Requirements

All AEs and SAEs including deaths, regardless of cause or relationship, must be reported for subjects on study through the protocol-required post-treatment follow-up.

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

Investigators are not obligated to actively seek out SAEs beyond the follow-up period for subjects. However, if the investigator learns of an AE or SAE occurring after the completion/termination visit and the event is deemed by the investigator to be relevant to the use of study drugs, he/she should promptly document and report the event to Gilead Sciences DSPH. For all SAE reporting, the SAE form should be completed and fax or email to the attention of Gilead Sciences DSPH within 24 hours of the Investigator's knowledge of the event. Contact information is listed below.

Gilead Sciences DSPH Representative:	Fax: Email:	1-650-522-5477 Safety_FC@gilead.com
Gilead Sciences Medical Monitor/Study Director:	Name: John Flaherty, PharmD Telephone: Fax: Mobile: E-mail:	PPD PPD PPD PPD

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

Laboratory abnormalities are usually not recorded as AEs or SAEs. However, laboratory abnormalities (e.g. clinical chemistry, hematology, urinalysis) independent of the underlying medical condition that require medical or surgical intervention or lead to study drug interruption or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (e.g., 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 and 7.3. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (i.e, anemia), not the labotatory results (i.e., decreased hemoglobin).

Severity should be recorded and graded according to the GSI Grading Scale for Severity of Adverse Events and Laboratory Abnormalities (Appendix 4).

For adverse events 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.

7.6. 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 4. The Gilead Sciences Medical Monitor is available for consultation on all medical and toxicity-related issues.

Grade 3 and 4 clinically significant laboratory abnormalities should be confirmed by repeat testing within 3 calendar days of receipt of results and before study 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 study drug following resolution of the adverse event, there should be discussion with the Medical Monitor prior to restarting drug; unlike therapy for adults, the protocol does not allow for dose modification in children.

Any recurrence of the study drug-related Grade 3 or 4 clinical or clinically significant laboratory adverse event following dose interruption mandates permanent discontinuation of study drug.

Any questions regarding toxicity management should be directed to the Gilead Sciences Medical Monitor.

7.6.1. Grades 1 and 2 Laboratory Abnormality or Clinical Event

Continue study drug at the discretion of the investigator.

7.6.2. Grade 3 Laboratory Abnormality or Clinical Event

For Grade 3 clinically significant laboratory abnormality or clinical event, study drug may be continued if the event is considered to be unrelated to study drug.

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

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

7.6.3. 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 study drug, study drug 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.

Study drug 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 nonfasting or that can be medically managed) or a clinical event considered unrelated to study drug.

7.6.4. 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 2 to <12 as follows:

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

  [(k is proportionality constant: pediatric males/females ≥ 2 years to < 12 years k = 0.55; 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)]

- If creatinine clearance decreases to < 50 mL/min/1.73 m^2 (confirmed) at any time during the study, study drug 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 study drug 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 study medication 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, study medication may be resumed, with monitoring as described below. If the estimated estimated creatinine clearance remains < 70 mL/min/1.73 m^2, study drug 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 rechallenge with study drug, study drug should be permanently discontinued, followed by 24 weeks of monthly monitoring in treatment-free follow up if alternative HBV therapy is not initiated.

7.6.5. Special Considerations During Blinded Treatment – Grade 4 ALT Management

Subjects who experience Grade 4 ALT while on blinded study medication will be evaluated weekly with serum chemistry and liver function test monitoring. In the event that any subject has sustained Grade 4 ALT for ≥ 16 weeks (i.e., failure to resolve ALT to grade ≤ 3 or baseline), the serial HBV DNA values on study will be provided to the investigator, and the subject can be offered open-label tenofovir DF, after discussion with the Gilead Medical Monitor.

7.6.6. On-Treatment ALT Flare and Post-Treatment Exacerbation of Hepatitis Management

For subjects with bridging fibrosis or cirrhosis, study drug discontinuation with treatment-free follow-up is contraindicated due to the potential risk of exacerbation of hepatitis in the setting of low hepatic reserve which could lead to decompensation. Subjects with bridging fibrosis or cirrhosis should be placed on commercially available HBV therapy following study drug discontinuation.

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 (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).

7.6.6.1. Management of ALT Flare in Subjects Receiving Study Medication

If laboratory results indicate (1) elevation of ALT > 2 × baseline and > 10 × ULN OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (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/INR, 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 study medication 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 study medication 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 study medication 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, study medication 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.

7.6.6.2. Management of Exacerbation of Hepatitis in Subjects Who Have Discontinued Study Medication

If laboratory results indicate (1) an ALT elevation > 2 × baseline and > 10 × ULN OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (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 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, 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.

7.7. Risks for Women of Childbearing Potential or During Pregnancy

The risks of treatment with tenofovir DF during pregnancy have not been evaluated in pregnant women. Animal studies do not indicate direct or indirect harmful effects of TDF with respect to pregnancy. Please refer to the latest version of the Investigator's Brochure for additional information.

7.7.1. Definition of Childbearing Potential

For the purposes of this study, a female subject of childbearing potential is a pubertal female regardless of whether or not she has had a menses (premenarchal, Tanner Stage 3). This also includes a female subject who has not had any of the following conditions or procedures considered very rare in this age group: hysterectomy, bilateral oophorectomy, or medically documented ovarian failure.

7.7.2. Contraceptive Requirements

Male study subjects and female study subjects of childbearing potential who are not heterosexually active should have periodic confirmation of continued abstinence from heterosexual intercourse and regular pregnancy testing while taking tenofovir DF. Female study subjects of childbearing potential who have been identified during the study as choosing to become sexually active should be counseled appropriately on the protocol-recommended method(s) for avoiding pregnancy in case the subject chooses to continue with heterosexual intercourse.

Male and female subjects of childbearing potential who engage in heterosexual intercourse must either agree to use protocol-recommended method(s) of contraception or agree to abstain from heterosexual intercourse from the Screening/Enrollment Visit throughout the study period and for 30 days following the last dose of tenofovir DF. The investigator should identify those subjects at risk for becoming sexually active and provide counsel appropriately on the protocol-recommended method(s) for avoiding pregnancy during the trial. These methods are recommended due to the low failure rate (i.e., less than 1% per year). See Table 7-1 for the protocol-recommended methods.

Female subjects of childbearing potential must have a negative serum pregnancy test at Screening and Baseline (Day 1) prior to receiving the first dose of study drug. Lactating females will not be enrolled in this study.

Table 7-1. Protocol-Recommended Contraceptive Methods

Single Methods	Combination Methods
Intra-uterine devices (IUDs) • Copper T 380A IUD • LNg 20 IUD **Progesterone** • Implant	**Estrogen and Progesterone** • Oral contraceptives plus barrier • Transdermal patch plus barrier • Vaginal ring plus barrier **Progesterone** • Injection plus barrier **Barrier methods** • Diaphragm • Cervical cap • Male condom

Other contraceptive methods may be acceptable after discussion with the Medical Monitor.

7.7.3. Procedures to be Followed in the Event of Pregnancy

The investigator must report all pregnancies to Gilead Sciences DSPH via the CRO within 24 hours of becoming aware of the pregnancy. Any pregnancies that occur within 30 days of last investigational medicinal product dose should also be reported. The subject must be instructed to discontinue all investigational medicinal products and inform the investigator **immediately** if she becomes pregnant or suspects that she is pregnant during the study.

The investigator should counsel the subject regarding the possible effects of prior investigational medicinal product exposure on the fetus and the need to inform the study site of the outcome of the pregnancy.

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

However, an induced therapeutic abortion to terminate any pregnancy due to complications or other medical reasons will be recorded as an AE or an SAE. The underlying medical reason for this procedure should be recorded as the adverse event term.

A spontaneous abortion is always considered to be an SAE and will be reported as described in the Adverse and Serious Adverse Events section. Furthermore, any SAE occurring as an adverse pregnancy outcome post-study must be reported to Gilead Sciences DSPH or the CRO.

All pregnancies of female study subjects that occur during the study should be reported using the Pregnancy Report CRF page and the Gilead DSPH Pregnancy form and Pregnancy Outcome form. Monitoring of the subject should continue until the conclusion of the pregnancy. If the end of the pregnancy occurs after the study has been completed, the outcome should be reported directly to Gilead Sciences DSPH, fax number +1 650 522-5477 or email safety_fc@gilead.com.

8. STATISTICAL CONSIDERATIONS

8.1. Analysis Objectives

The primary objective of this study is:

- To evaluate the antiviral efficacy of tenofovir DF versus placebo in pediatric patients (aged 2 to <12 years, at the time of enrollment) with chronic hepatitis B infection

The key secondary objective is:

- To evaluate the proportion of subjects with HBeAg seroconversion at Week 48 (in subjects with baseline HBeAg sero-positivity).

Secondary objectives are:

- To characterize the safety and tolerability profile of tenofovir DF in pediatric patients (aged 2 to <12 years, at the time of enrollment) with chronic hepatitis B infection
- To evaluate the biochemical and serological responses to tenofovir DF versus placebo
- To evaluate the incidence of potential resistance mutations to tenofovir DF in the hepatitis B virus polymerase /reverse transcriptase (pol/RT)
- To assess the pharmacokinetics of tenofovir in subjects receiving the tablet formulation and those receiving the oral powder formulation.

8.2. Primary Endpoint

The primary efficacy endpoint is the proportion of subjects with serum HBV DNA < 400 copies/mL (69 IU/mL) at Week 48. A missing = failure approach will be employed for handling missing data.

8.3. Key Secondary Endpoint

The key secondary endpoint is the proportion of subjects with HBeAg seroconversion at Week 48 (in subjects with baseline HBeAg sero-positivity). A missing = failure approach will be employed for handling missing data.

8.4. Secondary Endpoints

For Week 48, secondary endpoints to be evaluated in all subjects include:

- proportion of subjects with normal ALT and normalization of ALT
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL and normal ALT

- proportion of subjects with HBV DNA < 169 copies/mL (29 IU/mL)
- proportions of subjects with HBsAg loss and seroconversion
- sequence changes from baseline within the HBV polymerase for subjects who were viremic (HBV DNA ≥ 400 copies/mL [69 IU/mL]) at Weeks 48, 96, 144, 192 or Early Discontinuation; including subjects with confirmed virologic breakthrough
- cumulative incidence of at least a 4% decrease from baseline in bone mineral density of lumbar spine
- percent change from baseline in bone mineral density of lumbar spine

8.5. Other Endpoints of Interest

For Week 48, secondary endpoints to be evaluated in HBeAg-positive subjects include:

- proportions of subjects with HBeAg loss
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL (69 IU/mL), normal ALT and HBeAg loss
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL (69 IU/mL), normal ALT, and HBeAg seroconversion.

For Week 48, secondary endpoints to be evaluated in subjects with abnormal ALT at baseline include:

- proportion of subjects with normalized ALT
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL (69 IU/mL) and normalized ALT

For Week 48, secondary endpoints to be evaluated in HBeAg-positive subjects with abnormal ALT at baseline include:

- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL (69 IU/mL), normalized ALT and HBeAg loss
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL (69 IU/mL), normalized ALT, and HBeAg seroconversion.

Secondary endpoints to be evaluated in PK substudy populations on tablet formulation and oral powder are the plasma pharmacokinetic parameters including CL/F,C_{max}, T_{max}, C_{last}, T_{last}, C_{tau}, λ_z, $T_{½}$, $AUC_{0\text{-}last}$, AUC_{tau}.

For all categorical secondary endpoints, missing data will be handled using a missing = failure approach.

All endpoints at Week 48 will also be analyzed at Weeks 144 and 192.

8.6. Methods of Analysis

All summary tables for efficacy endpoints will present data by treatment group (TDF or Placebo) and overall.

8.6.1. Analysis Sets

8.6.1.1. Efficacy

The primary analysis set for efficacy analyses will be defined as all randomized subjects who received at least one dose of study drug (referred to hereafter as the full analysis set [FAS]). Subjects who withdraw after randomization prior to receiving study drug will be excluded from the FAS. Subjects discontinuing randomized therapy prior to Week 48 will be handled using a missing = failure approach, as noted in Sections 8.2 and 8.3, for the purpose of the primary and key secondary efficacy analyses. Subjects will be analyzed according to the randomized treatment assignment.

8.6.1.2. Safety

The primary analysis set for safety analyses will include all randomized subjects who received at least one dose of study drug. All data collected during the course of the study (on treatment and during treatment-free follow up) will be included in the safety summaries. Subjects will be analyzed according to the treatment actually received.

8.6.1.3 Pharmacokinetics

The PK analysis set will include all subjects who have evaluable pharmacokinetic data.

8.6.2. 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 considered a failure, as noted in Sections 8.2 and 8.3.

Sensitivity analyses will be performed if warranted.

8.6.3. Multiplicity Adjustments

A sequential gatekeeping procedure will be employed. The key secondary endpoint will only be tested at a 0.05 level if the primary endpoint is statistically significant at a 0.05 level.

8.6.4. Interim Analysis

An external independent multidisciplinary Data Monitoring Committee (DMC) will review the progress and safety of this study approximately every 24 weeks after the first subject is randomized. During the open-label duration of the study, the DMC will convene approximately every 52 weeks. See Section 8.12 for discussion of Data Monitoring Committee interim analyses.

No efficacy analyses are planned prior to the primary efficacy analysis, which will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 48.

8.7. Demographic Data and Baseline Characteristics

Demographic and baseline measurements will be summarized using standard descriptive methods by treatment group, and overall.

Demographic summaries will include gender, ethnic origin (with Asian-Chinese, Asian-Indian, Asian-Other all combined to Asian), racial minority, age, and geographical location of study site [North America/Europe, and Asia]).

Baseline data will include a summary of body weight, height, body mass index, $\log_{10}$ (HBV DNA) level, HBV serology, ALT/AST values, previous nucleoside and interferon exposure, genotype, bone mineral density (via DEXA scan), and serum bone biochemical markers.

8.8. Efficacy Analysis

8.8.1. Primary Analysis

The primary efficacy analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 48. The analysis will evaluate the difference between treatment groups in the proportion of subjects achieving the primary endpoint, using a two-sided Fisher's exact test.

8.8.2. Key Secondary Analysis

The key secondary efficacy analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 48. The analysis will evaluate the difference between treatment groups in the proportion of subjects achieving the key secondary efficacy endpoint, using a two-sided Fisher's exact test.

8.8.3. Secondary Analyses

Continuous secondary efficacy endpoints will be summarized using conventional descriptive statistics (n, mean, standard deviation, median, Q1, Q3, minimum and maximum) by treatment group, and a Wilcoxon rank sum test may be used to compare treatment groups. Categorical secondary efficacy endpoints will be summarized by number and percentage of subjects that meet the endpoint, and a two-sided Fisher's exact test may be used to compare treatment groups. For analyses of categorical secondary efficacy endpoints, missing data will be handled using a missing = failure approach.

Subgroup analyses of efficacy endpoints may include analyses by geographical location of study site (North America/Europe, and Asia), and also by presence/absence of prior oral anti-HBV treatment.

Efficacy analyses of all subjects continuing on open-label tenofovir DF will also be performed after the last randomized subject reaches Weeks 144 and 192.

8.9. Safety Analysis

All safety data collected on or after the date that study drug was first dispensed will be summarized by randomized treatment group. Data for the pretreatment and treatment-free follow-up periods will be included in listings.

The proportion of subjects in each treatment group with an AE leading to permanent discontinuation of study drug through Week 48 (also at Weeks 144 and 192) will be summarized.

8.9.1. Extent of Exposure

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

8.9.2. Adverse Events

Clinical and laboratory adverse events 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, and Lower Level Term (LLT) will be attached to the clinical database. Severity of adverse events will be graded using the grading scale defined in Appendix 4.

Summaries (number and percentage of subjects) of treatment-emergent adverse events (by SOC, HLT and Preferred Term) will be provided by treatment group as follows:

- all treatment-emergent adverse events,
- all related treatment-emergent adverse events,
- combined Grade 2, 3, and 4 treatment-emergent adverse events,
- combined Grade 3 and 4 treatment-emergent adverse events,
- combined Grade 2, 3, and 4 related treatment-emergent adverse events,
- combined Grade 3 and 4 related treatment-emergent adverse events,
- all adverse events that caused permanent discontinuation from study drug,
- all adverse events that caused change in dose or temporary interruption of study drug,

- all serious adverse events, and
- all serious related adverse events.

Events will be summarized based on the date of onset for the event.

Treatment-emergent AEs are defined as follows:

- Any AE with onset date on or after the study drug start date and no later than the study drug stop date for those who discontinued study drug permanently, or
- Any AE with onset date on or after the study drug start date for those who haven't discontinued study drug permanently, or
- Any AE leading to study drug discontinuation.

8.9.3. Laboratory Evaluations

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. 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 treatment 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 study drug or after the last dose of study drug will be included in a data listing.

8.9.4. Analyses of Bone Mineral Density and Bone Biochemical Markers

Cumulative incidence of at least a 4% decrease from baseline in bone mineral density of lumbar spine will be summarized and compared between treatment groups using 95% confidence intervals for the difference in proportions.

The percent change from baseline in bone mineral density of lumbar spine will be summarized over time and compared between treatment groups using the Wilcoxon rank sum test.

Lumbar spine Z-scores (derived from BMD assessment obtained via DEXA scan) and lumbar spine changes in Z-scores from study baseline will be summarized (n, mean, standard deviation, median, Q1, Q3, minimum and maximum) by treatment group and study week.

BMD assessments for whole body (via DEXA scan) and serum bone biochemical markers will be summarized over time. Changes from study baseline in bone biochemical markers will be summarized by treatment group, where study baseline will be defined as the mean of the Screening and Baseline Visit values.

Correlations between renal parameters and measurements of bone minerals density will be explored including exploration of the potential relationship between renal phosphate threshold (TmP/GFR) and measurements of bone mineral density.

8.10. Pharmacokinetic Analysis

Pharmacokinetic parameters *{ CL/F, C_{max}, T_{max}, C_{last}, T_{last}, C_{tau}, λ_z, $T_{½}$, $AUC_{0\text{-}last}$ and AUC_{tau} }* will be listed and summarized for tenofovir using descriptive statistics (e.g., sample size, arithmetic mean, geometric mean, % coefficient of variation, standard deviation, median, minimum, and maximum). Plasma concentrations of the investigational medicinal product over time will be plotted in semilogarithmic and linear formats as mean ± standard deviation.

8.11. Sample Size

With respect to the primary efficacy endpoint, a sample size of 100 subjects (67 in TDF and 33 in placebo) would provide at least 85% power to detect a difference of 20% between the groups, based on a two-sided Fisher's exact test with a significance level of 0.05, assuming a placebo response rate of 1%. A placebo response rate of 0% was observed in Study GS-US-174-0115 evaluating 54 adolescent patients aged 12-17 years, treated with placebo and in Study GS-US-103-0158 evaluating 58 pediatric patients aged 2-17 years. For the purpose of this sample size calculation, we assume a placebo response rate of 1%.

8.12. Data Monitoring Committee

An external independent multidisciplinary Data Monitoring Committee (DMC) will review the progress of the study and perform interim reviews of the safety data approximately every 24 weeks after the first subject is randomized, in order to protect subject welfare and preserve study integrity. Specifically, the DMC will review bone mineral density data, in addition to other laboratory (particularly renal) safety data and adverse events. During the open-label duration of the study, the DMC will convene approximately every 52 weeks. At each meeting, the DMC will review routine safety and DEXA data and will make recommendations regarding modification of study treatment.The DMC will also provide recommendations as needed regarding study design, modification and conduct.

While the Data Monitoring Committee is being asked to advise Gilead regarding future conduct of the study, including possible early study termination, Gilead retains final decision-making authority on all aspects of the study.

9. RESPONSIBILITIES

9.1. Investigator Responsibilities

9.1.1. 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 will ensure adherence to the basic principles of Good Clinical Practice, as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, part 50, 1998, and 21 CFR, part 56, 1998.

The investigator and all applicable subinvestigators will comply with 21 CFR, Part 54, 1998, providing documentation of their financial interest or arrangements with Gilead, or proprietary interests in the investigational drug under study. This documentation must be provided prior to the investigator's (and any subinvestigator's) participation in the study. The investigator and subinvestigator 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.

9.1.2. Institutional Review Board (IRB)/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 IRB/IEC. The investigator will not begin any study subject activities until approval from the IRBIEC has been documented and provided as a letter to the investigator.

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

9.1.3. Informed Consent/Assent

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. The investigator must use the most current IRB- or 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 local requirements. The consent form will inform subjects about pharmacogenomic testing and sample retention, and their right to receive clinically relevant pharmacogenomic analysis results.

9.1.4. 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, IRB [or] IEC, or laboratory. Laboratory specimens must be labeled in such as 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, the IMP, 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.

9.1.5. 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, IRB or 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, ie, 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, as required by the protocol;
- Start and end date (including dose regimen) of IMP, including dates of dispensing and return;
- Record of all adverse events 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.

9.1.6. 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. The Eligibility Criteria eCRF should be completed only after all data related to eligibility have been received. 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 9.1.5.

9.1.7. Investigational Medicinal Product Accountability and Return

Gilead recommends that used and unused IMP 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 IMP disposal procedures and provide appropriate instruction for destruction of unused IMP 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 IMP supplies in accordance with that site's approved SOP. A copy of the site's approved SOP will be obtained for central files.

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

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

9.1.8. Inspections

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

9.1.9. Protocol Compliance

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

9.2. Sponsor Responsibilities

9.2.1. Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Gilead. The investigator must submit all protocol modifications to the IRB [or] IEC in accordance with local requirements and receive documented IRB [or] IECapproval before modifications can be implemented.

9.2.2. 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 9.1.4).

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.

9.3. Joint Investigator/Sponsor Responsibilities

9.3.1. 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.

9.3.2. 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.

9.3.3. 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.

9.3.4. 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), IRBs, 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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11. APPENDICES

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Appendix 1. Investigator Signature Page

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STUDY ACKNOWLEDGEMENT

A Randomized, Double-Blind Evaluation of the Antiviral Efficacy, Safety, and Tolerability of Tenofovir Disoproxil Fumarate Versus Placebo in Pediatric Patients with Chronic Hepatitis B Infection

GS-US-174-0144 Protocol Amendment 4, 04 August 2016

This protocol has been approved by Gilead Sciences, Inc. The following signature documents this approval.

PPD

Author

PPD

8-August-2016

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, Inc. 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	Screening[a]	Baseline	Study Week						
			4	8	16	24	32	40	48
Written Informed Consent, Subject Assent	X								
Medical History	X	X[e]							
Complete Physical Examination (including Tanner Staging starting at Baseline)	X	X				X			X
Symptom-Directed Physical Examination			X	X	X		X	X	
Vitals Signs, Height, Weight[f]	X	X	X	X	X	X	X	X	X
HIV-1, HAV, HCV, HDV, α-fetoprotein	X								
HBV DNA Levels (PCR-Based Assay)	X	X	X	X	X	X	X	X	X
HBV Serology[g]	X	X			X		X		X
HBV Genotyping, Resistance Surveillance[h]		X							X
Hematology Profile	X	X	X	X	X	X	X	X	X
Serum Chemistry and Liver Tests[i]	X	X	X	X	X	X	X	X	X
Prothrombin Time/INR[j]	X	X							
Alpha-1 antitrypsin and protease inhibitor (PI) typing	X								
Urinalysis	X	X	X	X	X	X	X	X	X
Pregnancy Test[k]	X	X	X	X	X	X	X	X	X
Plasma for Tenofovir Concentration		X	X	X	X	X	X	X	X
Serum and Plasma for Storage[l]		X	X	X	X	X	X	X	X
DEXA Scan – Spine and Whole-Body[m]	X	X				X			X
Bone Biochemical Markers[n]	X	X				X			X
Blood sample storage for genomic testings[o]		X							

Study Procedures	Screening[a]	Baseline	Study Week						
			4	8	16	24	32	40	48
Concomitant Medications	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X
Subject Dosing Diary Card Dispensing			X			X			
Subject Dosing Diary Card Return				X			X		
Study Drug Dispensing/Return[p]		X	X	X	X	X	X	X	X
Drug Accountability/Adherence Assessment			X	X	X	X	X	X	X

Appendix 2. Study Procedures Table (continued)

Study Procedures	Study Week														Early DC/EOT[c]	OL Ext[t]	FU[d]
	56	64	72	80	88	96[b]	108	120	132	144	156	168	180	192			
Complete Physical Examination (including Tanner Staging starting at Baseline)			X			X		X		X		X		X	X	X[s]	
Symptom-Directed Physical Examination	X	X		X	X		X		X		X		X			X[s]	X
Vital Signs, Height, Weight[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBV DNA Levels (PCR-Based Assay)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HBV Serology[g]		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X[q]
Hematology Profile	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
HBV Genotyping, Resistance Surveillance[h]						X				X				X	X		
Serum Chemistry and Liver Tests[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prothrombin Time/INR[j]																	X[j]
Urinalysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pregnancy Test[k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Plasma for Tenofovir Concentration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Serum and Plasma for Storage[l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DEXA Scan – Spine and Whole-Body[m]			X			X				X				X	X[r]	X[s]	
Bone Biochemical Markers[n]			X			X				X				X	X[r]	X[s]	
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Study Procedures	Study Week														Early DC/EOT[c]	OL Ext[t]	FU[d]
	56	64	72	80	88	96[b]	108	120	132	144	156	168	180	192			
Subject Dosing Diary Card Dispensing	X																
Subject Dosing Diary Card Return		X															
Study Drug Dispensing/Return[p]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Drug Accountability/Adherence Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

a Evaluations to be completed within 45 days prior to Baseline Visit.
b After Week 96, subjects will return for study visits every 12 weeks until each subject reaches Week 192, as well as during the Extension Phase if applicable.
c The Early Study Drug Discontinuation Visit should be completed within 72 hours of last dose of study drug.
d Subjects who receive at least one dose of study drug will complete Follow Up visits every 4 weeks post treatment discontinuation for 24 weeks or up to initiation of active treatment, whichever occurs first. The treatment-free follow-up period could occur for early discontinuation subjects or at Week 192 study completion.
e Review of medical history and any changes since Screening Visit, including changes in concomitant medications.
f Vital signs = temperature, blood pressure, pulse, respiratory rate.
g HBeAg and HBsAg and, if negative, reflex HBeAb and HBsAb, respectively.
h Determination of HBV viral genotype (A–H) will be performed at the Baseline Visit for all subjects. Resistance surveillance will be conducted at Baseline for all subjects, and attempted for all viremic subjects (HBV DNA ≥ 400 copies/mL [69 IU/mL]) at Weeks 48, 96, 144, and 192, as well as at the Early Discontinuation Visit, if applicable.
i Including estimated creatinine clearance, by the Schwartz Formula (2 to <12 years old).
j PT/INR will be performed at the Screening and Baseline Visits, then as a reflex test in subjects experiencing exacerbation of hepatitis thereafter.
k For females of childbearing potential only; a serum β-HCG pregnancy test will be performed at Screening, Baseline, Weeks 48, 72, 192 and Early Study Drug Discontinuation/End of Treatment Visit (if applicable). Urine pregnancy testing will be performed at all other study visits through the end of the study including the Extension Phase. A positive urine pregnancy test must be confirmed immediately with a serum pregnancy test.
l Blood (serum and plasma) collected for long-term storage and possible future testing (e.g., pharmacokinetic and/or virologic analysis).
m DEXA scan will be performed between the Screening and Baseline Visits, and must be performed prior to randomization and receipt of study drugs. All other DEXA scans must be performed within ± 10 days of the scheduled study visits. DEXA scan will be performed at the time of switch for subjects switching from placebo to tenofovir DF, unless the most recent DEXA scan was performed within 12 weeks earlier.
n Specimens for bone biochemical markers must be collected in the morning in a fasted state, or, if fasting is not feasible, within two hours of the time of day that the Baseline Visit specimen was collected. For example, if the bone biochemical markers were drawn at 7:00 AM for the Baseline Visit, they must be drawn no earlier than 5:00 AM and no later than 9:00 AM for Week 24, 48, 72, 96, and subsequent specified visits. Bone biochemical markers will also be collected at the time of switch for subjects switching from placebo to tenofovir DF if not done within 12 weeks prior to switch. Bone biochemical markers will also be collected at the Screening Visit; the mean of the Screening and Baseline Visit values will constitute the study baseline, to which subsequent values will be compared.
o For subjects in whom a separate consent is provided, an appropriate blood sample will be collected for biomarker (including pharmacogenomic) analysis that may be predictive of virologic response and/or the tolerability of HBV therapies. This sample may be obtained after the Baseline visit, if necessary.
p Initiation of study drugs (including multivitamin) must occur within 24 hours after the Baseline Visit. At every study visit, subjects will return unused study medication, and new study medication will be dispensed.
q During the 24-week follow-up period, HBV serology testing will only be performed at the Follow-Up Week 24 Visit.

r If the Early Discontinuation Visit is performed prior to Week 96, DEXA and bone biochemical markers are required if the previous measurements were > 12 weeks prior. If the Visit is performed after Week 96 or after the Extension Phase, DEXA and bone biochemical markers are required if the previous measurements were > 24 weeks prior.

s Complete Physical Exam including Tanner Staging, DEXA scan and bone biochemical markers are required every 48 weeks during the Extension Phase. Symptom Directed PE will be done at all other visits.

t Open Label Extension Phase visits will be conducted following Week 192 until the visit after commercial Viread becomes available for the subject's age and weight (at Weeks 204, 216, 228, 240, 252, 264, 276, 288, 300, 312, 324, 336, 348, 360, 372, 384, 396, 408, 420, 432, 444, 456, 468, 480.) Study visits should be completed within ±14 days of the protocol-specified visit date, based on the Baseline Visit.

Appendix 3. Clinical Laboratory Assessments

Hematology:	Erythrocytes Hemoglobin Hematocrit Platelets Total leukocytes and differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)	**Liver Tests:**	Total bilirubin (reflex direct [conjugated] bilirubin) AST ALT Alkaline phosphatase Prothrombin time, INR Alpha-1 antitrypsin and protease inhibitor (PI) typing
Chemistry:	Creatine phosphokinase Lactic dehydrogenase (LDH) Creatinine and calculated creatinine clearance Albumin Glucose Serum amylase Lipase (if serum amylase is ≥ 1.5 × ULN) α-fetoprotein (Screening Visit) Blood urea nitrogen (BUN) Uric acid Total iron (Screening Visit)	**Urinalysis:** **Urine Pregnancy Test:**	Protein Blood Glucose Post-menarchal females only (reflex serum β-HCG if positive)
Electrolytes:	Sodium Potassium Bicarbonate Phosphorus+ Calcium Magnesium Chloride	**HBV Serology:**	HBsAg, HBeAg (reflex HBsAb and HBeAb).

Bone Biochemical Markers:	• urine bicarbonate and n-telopeptide; • serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, and vitamin D levels (25-hydroxy) and 1,25 (dihydroxyvitamin) D levels; • fasting serum creatinine/phosphate and urine creatinine (spot)/phosphate for measurement of the renal phosphate threshold (TmP/GFR)	**Virology:**	Plasma and serum for HBV DNA and serology HIV, HAV, HDV and HCV (Screening Visit) Viral genotyping (A–H), resistance surveillance
		Tenofovir DF Concentration:	Plasma concentration
Serum β-HCG Pregnancy Test:	Post-menarchal females only; at Screening, Baseline, Weeks 48, 72, 192, or Early Study Discontinuation/End of Treatment Visits; reflex if urine test positive at all other study visits	**Storage:**	Plasma and serum for storage

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

Antiviral Toxicity Grading Scale Version: 01 April 2015

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 Months#**	1000 to 1300/mm^3 1.00 to 1.30 GI/L	750 to < 1000/mm^3 0.75 to < 1.00 GI/L	500 to < 750/mm^3 0.50 to < 0.75 GI/L	< 500/mm^3 < 0.50 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
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

HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
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%

An overlap between the Grade 1 scale and the Lab's normal range for absolute neutrophils may result for pediatric subjects. Please follow the Gilead convention of grading any result within the LLN and ULN a 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	>ULN to 150 mEq/L >ULN 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 **Adult and Pediatric ≥ 1 Year**	3.0 to <LLN mEq/L 3.0 to <LLN 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
Infant <1 Year	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 mmolL	2.0 to < 2.5 mEq/L 2.0 t o <2.5 mmolL	< 2.0 mEq/L <2.0 mmolL
Hyperkalemia **Adult and Pediatric ≥ 1 Year**	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
Infant <1 Year	>ULN to 6.0 mEq/L >ULN 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**	55 to 64 mg/dL 3.03 to 3.58 mmol/L	40 to < 55 mg/dL 2.20 to < 3.03 mmol/L	30 to < 40 mg/dL 1.64 to < 2.20 mmol/L	< 30 mg/dL < 1.64 mmol/L
Infant, < 1 Month	50 to 54 mg/dL 2.8 to 3.0 mmol/L	40 to < 50 mg/dL 2.2 to < 2.8 mmol/L	30 to < 40 mg/dL 1.7 to < 2.2 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

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
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
Hypocalcemia (corrected for albumin if appropriate*) **Adult and Pediatric ≥2 Years**	7.8 <LLN mg/dL 1.94 to <LLN 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
Pediatric ≥7 days -2 Years	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 mmolL	6.1 to <7.0 mg/dL 1.51 to < 1.74 mmolL	< 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 <LLN mg/dL 0.96 to <LLN 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 <LLN mg/dL 1.12 to <LLN 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

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hypouricemia **Adult and Pediatric ≥ 1 year**	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
Infant < 1 Year	N/A	1.0 mg/dl to <LLN- 57 µmol to <LLN	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
Bicarbonate **Adult and Pediatric ≥ 4 Years**	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
Pediatric < 4 Years	NA	11.0 mEq/Lto <LLN 11.0 mmol/L to <LLN	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) Adult	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
LDL (Fasting) 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

CHEMISTRY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
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
** An overlap between the Grade 1 scale and the Lab's normal range for creatinine may result for Male subjects >70 yrs. Please follow the Gilead convention of grading any result within the LLN and ULN a 0.

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 **Pediatrics <16 years**	-	2.0 to < LLN g/dL 20 to < LLN g/L	< 2.0 g/dL < 20 g/L	NA
≥ 16 years	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) **Pediatric ≤ 17 Years** (with repeat testing at same visit)	140–159 mmHg systolic OR 90–99 mmHg diastolic NA	> 159–179 mmHg systolic OR > 99–109 mmHg diastolic 91st–94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	> 179 mmHg systolic OR > 109 mmHg diastolic ≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization (other than ER visit) indicated 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
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

CARDIOVASCULAR				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
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
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
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

NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
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
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)

NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
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 **Pediatric < 21 Years**	BMD t-score or z-score –2.5 to –1.0 BMD z-score –2.5 to –1.0	BMD t-score or z-score < –2.5 BMD z-score < –2.5	Pathological fracture (including loss of vertebral height) Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences 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

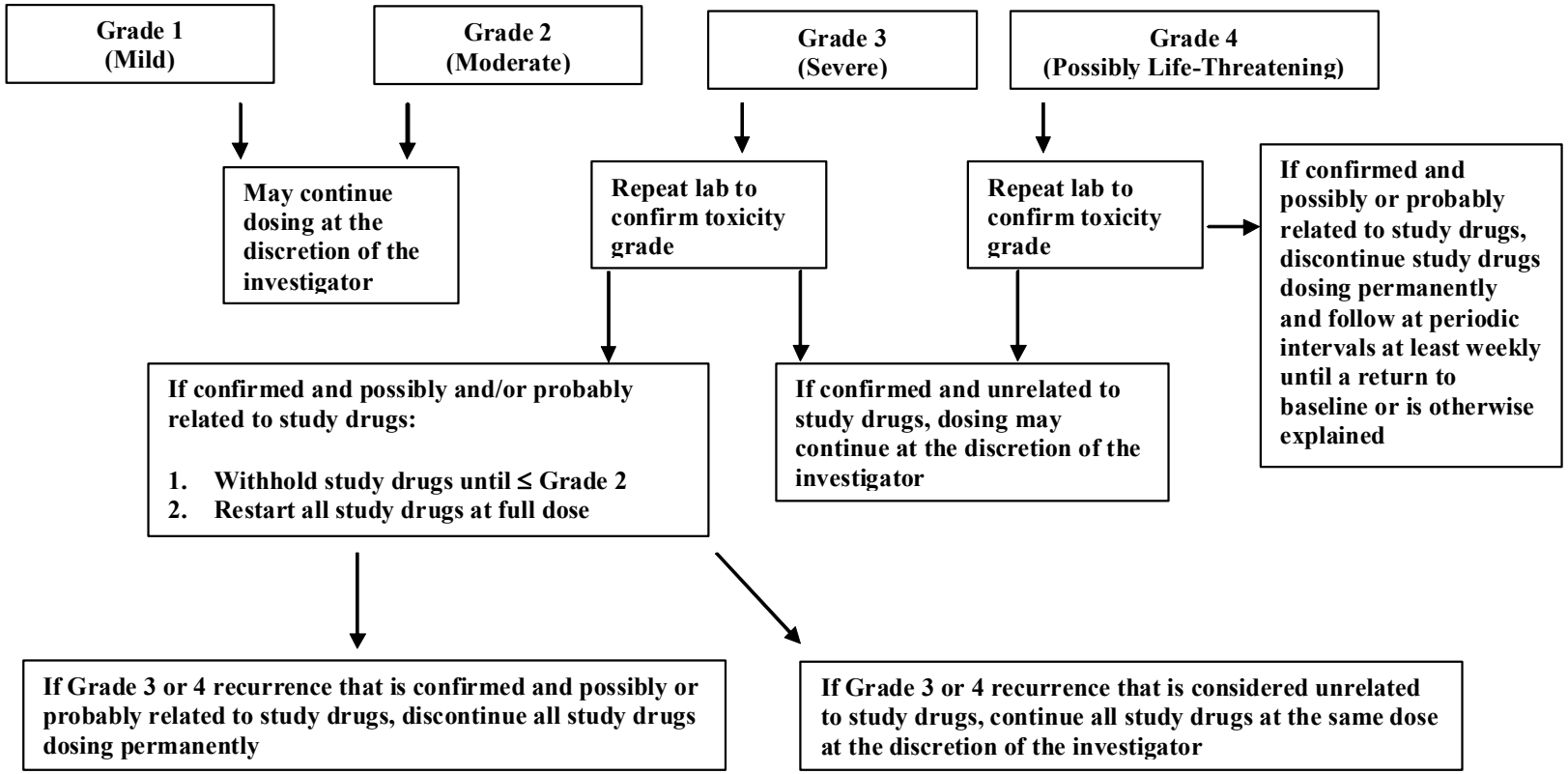
ENDOCRINE/METABOLIC				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Lipodystrophy (eg, back of neck, breasts, abdomen)	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual 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. Management of Clinical and Laboratory Adverse Events



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.

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.