



## CLINICAL STUDY PROTOCOL

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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

**Sponsor:** Gilead Sciences, Inc.  
333 Lakeside Drive  
Foster City, CA 94404 USA

**IND Number:** 71,576  
**EudraCT Number:** 2007-003704-35

**Indication:** Chronic Hepatitis B

**Protocol ID:** GS-US-174-0144

**Gilead Sciences Medical Monitor:** Name: John Flaherty, PharmD  
Telephone: PPD  
Fax: PPD  
Mobile: PPD  
E-mail: PPD

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**PROTOCOL SYNOPSIS**  
**Gilead Sciences, Inc.**  
**333 Lakeside Drive**  
**Foster City, CA 94404 USA**

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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

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**IND Number:** 71,576

**EudraCT Number:** 2007-003704-35

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**Study Centers Planned:** Approximately 35 centers in North America, Europe, and Asia

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**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

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

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**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-naïve 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 either ALT  $\geq 2 \times$  ULN at screening OR any history of ALT  $\geq 2 \times$  ULN over the past  $\leq 24$  months will be randomized in a 1:1 ratio to treatment arm A or B:

- Treatment A (n = 50): tenofovir DF PO once daily for 72 weeks
- Treatment B (n = 50): matching placebo PO once daily for 72 weeks

After 72 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for an additional 120 weeks.

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

**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  $\geq 17$  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  $\geq 16$  weeks prior to screening. Subjects must have discontinued interferon-alfa  $\geq 6$  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  $\geq 16$  weeks (i.e., failure to resolve ALT to grade  $\leq 3$  or baseline), the serial HBV DNA values

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<b>Study Design: (cont.)</b>	<p>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 study drug discontinuation.</p> <p>All subjects will take a multivitamin containing vitamin D 600 IU (to be provided by the study) once daily during the study.</p> <p>The primary analysis will be conducted at the completion of double-blinded treatment, after the last randomized subject reaches Week 72. Subsequently, ongoing 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.</p> <p>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.</p> <p>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. At each meeting, the DMC will review routine safety and DEXA data and will make recommendations regarding modification of study treatment.</p>
Number of Subjects Planned:	One hundred (100) subjects, randomized in a 1:1 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 72 weeks (1.5 years), followed by an additional 120 weeks (2.5 years) of open-label tenofovir DF therapy. The duration for a given subject is at least 4 years (each subject will receive a total of 192 weeks of study drug treatment).

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**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 AND either ALT  $\geq 2 \times$  ULN at screening OR any history of ALT  $\geq 2 \times$  ULN over the past  $\leq 24$  months, and creatinine clearance  $\geq 80$  mL/min/1.73 m<sup>2</sup> 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  $\geq 16$  weeks prior to screening. Subjects must have discontinued interferon-alfa  $\geq 6$  months prior to screening. Subjects must be without serological evidence of co-infection with HIV, HCV or HDV. Subjects with a history of significant renal disease, bone disease, decompensated liver disease, evidence of hepatocellular carcinoma (i.e.,  $\alpha$ -fetoprotein > 50 ng/mL) will not be eligible for the study.

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**Study Procedures/Frequency:** Plasma HBV DNA levels, laboratory analyses (serum chemistry, liver tests, hematology, and urinalysis), pregnancy test (post-menarchal females 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).

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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  $\geq 400$  copies/mL) at Weeks 72, 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 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.

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**Test Product, Dose, and Mode of Administration:**

Tenofovir disoproxil fumarate oral tablet

- subjects  $\geq 17$  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  $\geq 17$  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  $\geq 17$  kg: one tablet once daily (matching of physical appearance as the corresponding active tablet)

Matching oral placebo powder

- subjects  $< 17$  kg and subjects  $\geq 17$  kg who are unable to swallow a tablet: once daily with matching dose
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**Criteria for  
Evaluation:**

**Efficacy:** The primary efficacy endpoint is the proportion of patients with serum HBV DNA < 400 copies/mL at Week 72 in each arm.

At Week 72, 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 and normal ALT
- proportion of subjects with HBV DNA < 169 copies/mL
- proportions of subjects with HBsAg loss and seroconversion
- proportions of subjects with HBeAg loss and seroconversion (HBeAg positive patients only)
- genotypic changes from baseline within the HBV polymerase for subjects who were viremic (HBV DNA  $\geq$  400 copies/mL) at Weeks 72, 144, 192 or Early Discontinuation; with confirmed virologic breakthrough

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

For the primary endpoint and categorical secondary efficacy endpoints, missing data will be handled using a long-term evaluation of efficacy intention-to-treat algorithm.

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**Safety:** Adverse events and clinical laboratory tests will be collected and characterized at every visit throughout the study.

At Week 72, 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:** Following 4 weeks of dosing with tenofovir DF a PK substudy will be performed on a subset of subjects to evaluate the PK of tenofovir DF.

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**Statistical Methods:** The primary efficacy analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 72. 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 long-term evaluation of efficacy intention-to-treat algorithm. 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]), and also by presence or absence of prior oral anti-HBV treatment.

The proportion of subjects in each treatment arm with an adverse event or an abnormal laboratory test will be summarized at Week 72 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 (50 tenofovir DF, 50 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 substudy population:  $C_{\max}$ ,  $T_{\max}$ ,  $C_{\text{last}}$ ,  $T_{\text{last}}$ ,  $C_{\text{tau}}$ ,  $\lambda_z$ ,  $T_{1/2}$ ,  $AUC_{0-\text{last}}$ ,  $AUC_{\text{tau}}$ .

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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 <sup>®</sup>
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 <sub>50</sub>	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 <sup>®</sup>
dATP	deoxyadenosine triphosphate
DAVG <sub>48</sub>	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 <sub>50</sub>	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 <sup>™</sup>

## GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

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 early antibody
HBeAg	hepatitis B early 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 <sub>50</sub>	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 <sub>i</sub>	inhibition constant
L	liter
LAM	lamivudine, Epivir <sup>®</sup> , Zeffix <sup>®</sup> , 3TC
LAM <sup>R</sup>	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

## GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

m <sup>2</sup>	square meter(s)
MedDRA	Medical Dictionary for Regulatory Activities
mEq	milliequivalent(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm <sup>3</sup>	cubic millimeter(s)
mmol	millimole(s)
ng	nanogram(s)
NRTI	nucleoside reverse transcriptase inhibitor
OBR	optimized background (antiretroviral) regimen
OLT	orthotopic liver transplant
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 <sup>®</sup>
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 {10952}. Globally, approximately 1 million people die annually of complications of chronic hepatitis B {2331},{10952}. In the United States, an estimated 5,000 people die each year of complications of HBV infection {3092}.

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 endemicity. 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 {10690}. 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 {3273},{10665}.

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 {10952}. 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 {2820}. Patients with chronic viral replication are at increased risk of complications and poor outcomes {10684}. 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 show no signs or symptoms of disease. The third phase, the inactive HBsAg carrier stage, is characterized by undetectable or low levels of HBV DNA and the presence of anti-HBe antibodies. This third stage can evolve into a fourth stage, reactivation, in which ALT levels are abnormal and HBV DNA levels are increased.

The management of chronic hepatitis B in children and adolescents is still evolving {17786}. 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 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 {3689}, {10644}. Interferon-alfa is administered for a treatment duration of 24 weeks in patients  $\geq 2$  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 {17786}. 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 {3689}, {10678}.

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,  $p = 0.04$ ) {3653}, with maintained HBeAg seroconversion {10644}. Lamivudine therapy is usually administered for 1 year and continued for 6 months after seroconversion. In contrast to interferon alfa, lamivudine is available in a patient-friendly oral dosage form, and is well tolerated, with no growth development changes observed after 3 years of treatment {10644}. 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 {3653}, {10954}. 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  $\geq 12$  years of age in the United States. Adefovir has activity



against both wild-type and lamivudine-resistant HBV {14352}, {4093}, {7980}. 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) {12336}. 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  $\geq 16$  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. DF 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 {10665}. 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 {11363} {11362}.

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 72 weeks, more tenofovir DF-treated subjects achieved the primary efficacy endpoint (serum HBV DNA < 400 copies/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 to 11 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]phosphinyl]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, 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<sup>®</sup>. {16282}

### **1.2.2. Pre-Clinical Pharmacology and Toxicology**

#### **1.2.2.1. Mechanism of Action**

Tenofovir disoproxil fumarate (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 {1574}. Tenofovir is efficiently anabolized to TFV-DP in human hepatic cells, and has a half-life of 95 hours in primary human hepatocytes {9266}. TFV-DP inhibits recombinant HBV polymerase with a  $K_i$  of 0.18  $\mu\text{M}$  {9266}. 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 {1131}. TFV-DP is a very weak inhibitor of mammalian DNA polymerases  $\alpha$ ,  $\beta$ , and mitochondrial DNA polymerase  $\gamma$  {2516}, {1131}.

#### 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<sub>50</sub> values (50% effective concentration) for tenofovir were in the range of 0.14 to 1.5  $\mu$ M, with CC<sub>50</sub> (50% cytotoxicity concentration) values > 100  $\mu$ M. 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<sub>50</sub> of 0.11  $\mu$ M {10}. 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 {9266}.

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<sub>50</sub> 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<sub>50</sub> 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) {6870}, 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) {6870}. 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) {8176} 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<sub>10</sub> ( $p < 0.05$ ) from the pretreatment levels {8176}. Oral administration of tenofovir DF at 15 mg/kg for 48 weeks resulted in a mean serum viral load reduction of 2.9 log<sub>10</sub> 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<sub>10</sub>, 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 {8380}. 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<sub>50</sub>). The rtA194T mutation in combination with the rtL180M+rtM204V mutations led to a 2.4-fold increase in tenofovir EC<sub>50</sub>, 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) {9266}. 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 {6868}. In cell-based assays, HBV laboratory strains {6868}, {8381}, {8016}, {9266}, as well as those identified in clinical isolates {10916}, {10426}, {7060} 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 {10926}, {9266}, {8016}, {7788}. 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 {10926}. 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) {8683}, {10466}, {10804}. In vitro phenotypic analysis showed that the tested entecavir resistance mutations resulted in increased EC<sub>50</sub> values for tenofovir ranging from 0.6- to 6.9-fold (Report No. PC-174-2003), {10946}, {10916}.

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 {10425}, {10916}. 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<sub>50</sub> in vitro {8016}.

### 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<sub>10</sub> 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<sub>10</sub> copies/mL, and a mean ALT of 147 U/L. The studies are currently ongoing with all remaining subjects in their 5<sup>th</sup> 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 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) 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%) {13280}.



At the end of the Week 48 double-blind periods, 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  $\geq 400$  copies/mL were eligible to receive emtricitabine plus TDF combination therapy. The following describes year-4 (Week 192) data from this ongoing trial {16441}, {16437}.

In Study 102, 315 HBeAg- subjects completed W192 (84% retention). In an ITT analysis (ITT), 86% of subjects had HBV DNA  $<400$  copies/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 at W192. Four subjects (3 on TDF monotherapy) had HBV DNA  $\geq 400$  copies/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 {6909}, {1709}, and {6852}. 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  $\geq 400$  copies/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 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 (74% TDF-TDF; 84% ADV-TDF). In an on-treatment analysis 96% (TDF-TDF) and 99% (ADV-TDF) had HBV DNA  $<400$  copies/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 at W192. Overall 7 subjects (2 on TDF monotherapy and 5 on FTC/TDF) had HBV DNA  $\geq 400$  copies/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. 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. 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<sub>10</sub> 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 in 79% of all Asians (74% HBeAg+, 84% HBeAg-) and 83% of all non-Asians (79% HBeAg+, 86% HBeAg-) (ITT analysis) (p≥0.2 for all comparisons). 97% of Asians and 99% of non-Asians had HBV DNA <400 copies/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. 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 {16432}.

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 fumarate, the fixed-dose combination emtricitabine/tenofovir DF, and entecavir in the treatment of CHB with decompensated liver disease in the prevention of HB recurrence post-transplantation. The study was fully enrolled (N = 112) by Jan2008. 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 database lock is in progress. Safety and efficacy analysis will be performed in September 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 17 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 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; once all subjects complete the 96 week study visit a 96-week analysis will be performed in the first quarter of 2012.

- 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 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. The study is currently ongoing.

- 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, database lock is in progress. Safety and efficacy analysis will be performed in October 2011.

#### 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<sup>2</sup> (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<sub>10</sub> 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 completely resolved by week 96. One additional subject developed a > 6% decrease from baseline in bone mineral density by Week 96. None of the six subjects experienced a bone fracture {8548}, {9505}.

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

The primary efficacy endpoint was virologic response, measured as a change from baseline in  $\log_{10}$  HIV-1 RNA. Limited data available from these patients showed that, overall, treatment with tenofovir DF resulted in a decline in mean plasma HIV-1 RNA of  $-0.95 \log_{10}$  copies/mL from baseline to Week 72, and a decline in median plasma HIV-1 RNA of  $-0.39 \log_{10}$  copies/mL from baseline at Week 108. These overall declines were seen despite high prior exposure and virological failure/intolerance to multiple antiretrovirals in all patients. Steady state PK parameters of tenofovir DF in children and adolescents treated with tenofovir DF-containing regimens were similar to those seen in HIV-infected adults treated with 300 mg once daily. Tenofovir DF was safe and well tolerated when given for a mean duration of 100 weeks in combination with other antiretrovirals. A renal tubular disorder and increase in urinary beta-2 microglobulin were the only events to occur that were considered possibly/probably related to tenofovir DF. These events, in a subject who had a history of probable tubulopathy while receiving tenofovir DF before the study, resolved after withdrawal of tenofovir DF. The limited available bone mineral density data in this study do not suggest a decline in BMD with increased exposure to tenofovir DF. No bone fractures occurred during the study.

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

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 8mg/kg daily in HIV-1 infected children (2 to < 12 years of age) is similar to that achieved following administration of tenofovir DF 300mg 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 phosphatase) 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. With the completion of clinical studies of tenofovir DF in adolescents with chronic HBV

The optimal treatment for pediatric patients with chronic HBV infection is currently unknown. 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 72 weeks as an appropriate comparator. After 72 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  $\mu\text{M}$ ) is similar to the  $K_i$  against HBV polymerase (0.18  $\mu\text{M}$ ); (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 ( $\geq 35$  kg). All pediatric subjects in this study who weigh  $\geq 17$  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  $\geq 17$  kg who are unable to swallow a

tablet, will be given tenofovir DF oral powder (or matching placebo). Tenofovir pharmacokinetics 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) {8548}, {9505}. 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 statistically 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 and 72 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 (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

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



### 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 either ALT  $\geq 2 \times$  ULN at screening OR any history of ALT  $\geq 2 \times$  ULN over the past  $\leq 24$  months will be randomized in a 1:1 ratio to treatment arm A or B:

- Treatment A (n = 50): tenofovir DF PO once daily for 72 weeks
- Treatment B (n = 50): matching placebo PO once daily for 72 weeks

After 72 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for additional 120 weeks (see study schema at end of Section 3.).

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  $\geq 16$  weeks (i.e., failure to resolve ALT to grade  $\leq 3$  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 recoded as an adverse event leading to study drug discontinuation.

Subjects will take a daily multivitamin containing vitamin D 600 IU (to be provided by the study).

The primary efficacy and safety analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 72. Subsequently, efficacy and safety analyses of all subjects continuing on open-label tenofovir DF will be performed after the last randomized subject reaches Weeks 96, 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. 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 AND either ALT  $\geq 2 \times$  ULN at screening

OR any history of ALT  $\geq 2 \times$  ULN over the past  $\leq 24$  months, and creatinine clearance  $\geq 80$  L/min/1.73 m<sup>2</sup>. Subjects must be without serological evidence of co-infection with HCV, HIV, or HDV. Subjects with decompensated liver disease, significant renal disease, significant bone disease, evidence of hepatocellular carcinoma (i.e.,  $\alpha$ -fetoprotein  $> 50$  ng/mL), 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  $\geq 6$  months prior to the Screening Visit. Subjects experienced on other oral anti-HBV nucleoside/nucleotide therapy must have discontinued therapy  $\geq 16$  weeks prior to screening to avoid flare if randomized to the placebo arm.

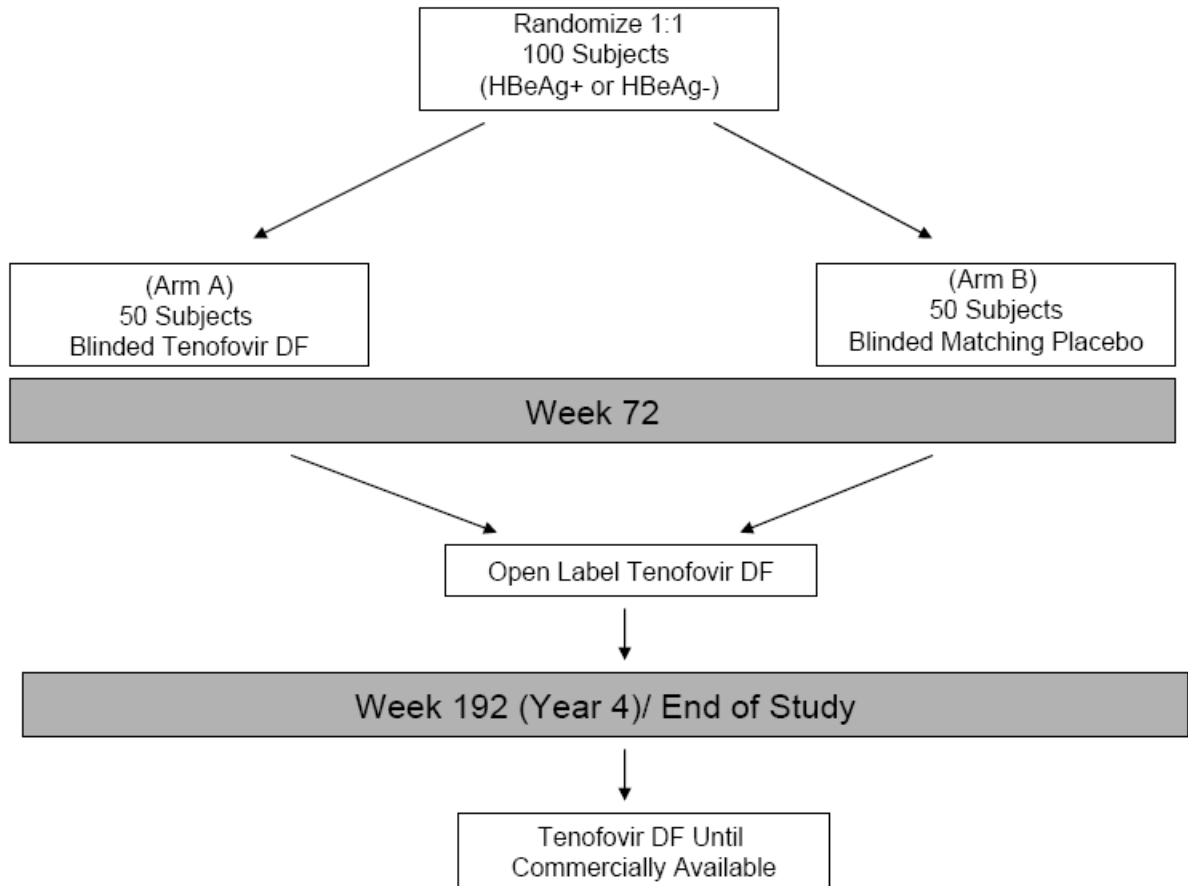
Plasma HBV DNA levels, laboratory analyses (serum chemistry, liver tests, hematology, and urinalysis), pregnancy test (post-menarchal females only), vital signs, adverse events and concomitant medications will be measured or assessed at screening, baseline, Weeks 4, 8, 16, 24, 32, 40, 48, 56, 64, 72, 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  $\geq 400$  copies/mL) at Weeks 72, 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 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 end of treatment 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.

**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 1:1 to receive blinded tenofovir DF PO once daily (Treatment A, n = 50) or blinded matching placebo PO once daily (Treatment B, n = 50).

### 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)
- Documented chronic HBV infection, defined as positive serum HBsAg  $\geq$  6 months
- HBeAg-positive or HBeAg-negative
- HBV DNA  $\geq 10^5$  copies/mL (PCR method)
- ALT  $\geq 2 \times$  ULN at screening, OR any history of ALT  $\geq 2 \times$  ULN over the past  $\leq 24$  months
- Estimated glomerular filtration rate (creatinine clearance)  $\geq 80$  mL/min/1.73m<sup>2</sup>

Estimated creatinine clearance using Schwartz Formula  
(mL/min/1.73m<sup>2</sup>) =  $k \times L / \text{Scr}$

[(k is a proportionality constant: pediatric males/females  $\geq 2$  years to < 12 years k = 0.55; for adolescent females  $\geq 12$  years old, k = 0.55, and for adolescent males  $\geq 12$  years, k = 0.70); L is height in centimeters (cm); and Scr is serum creatinine (mg/dL)]

- Adequate hematologic function (absolute neutrophil count  $\geq 1,500/\text{mm}^3$ ; hemoglobin  $\geq 10.0$  g/dL)
- Negative serum  $\beta$ -HCG pregnancy test (for post-menarchal females only)
- Female subjects of childbearing potential must agree to utilize highly effective contraception methods while on study treatment or agree to abstain from heterosexual intercourse for 30 days following the last dose of study drugs; highly effective methods

normally utilize two separate forms of contraception, one of which must be an effective barrier contraceptive method.

- Parent or 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 if they have the ability to read and write
- 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  $\geq 6$  months prior to screening; subjects experienced on other anti-HBV nucleoside/nucleotide therapy must have discontinued therapy  $\geq 16$  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 females of childbearing potential who are not willing to use an effective method of contraception during the study. (see Section 7.7. for further details).
- Decompensated liver disease defined as direct (conjugated) bilirubin  $> 1.2 \times$  ULN, PT  $> 1.2 \times$  ULN, platelets  $< 150,000/\text{mm}^3$ , 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
- $\alpha$ -fetoprotein  $> 50$  ng/mL
- Evidence of hepatocellular carcinoma (HCC)
- Co-infection with HIV, HCV, or HDV
- 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, osteochondroses, 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
  - 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 30 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 1:1 ratio to receive blinded tenofovir DF PO once daily (Treatment A, N = 50), or blinded matching placebo PO once daily (Treatment B, N = 50). 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 72 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 72 (open-label portion of the study), open-label tenofovir DF tablet or tenofovir DF oral powder will be provided to the study site in numbered bottles.

**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 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), daily multivitamin containing vitamin D 600 IU will be supplied by the study.

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 whereby an investigator finds it necessary to unblind a patient's treatment assignment, the investigator must contact the Medical Monitor at Gilead Sciences. Following discussion between the investigator and the Gilead Medical Monitor, the breaking of the blind will only be sanctioned by Gilead where knowledge of the study medication treatment would affect subject management. In order to break the blind for a subject, the investigator must call the IVRS and provide the subject number. Once the



treatment has been revealed to the investigator, the subject is automatically discontinued from the blinded phase of the study. **Consultation with the Medical Monitor is mandatory regarding any subsequent option for open-label tenofovir DF treatment.**

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. At each meeting, the DMC will review routine safety and DEXA data and will make recommendations regarding continuing or initiating study treatment.

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

### **5.2.1. Formulation**

#### 5.2.1.1. 5.2.1.1. Tenofovir DF Tablets

For pediatric patients weighing  $\geq 17$  kg ( $\geq 37$  lb) 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 are light blue, almond-shaped, plain-faced, film-coated tablets 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 Power

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, denatonium benzoate, and silicon dioxide.

#### 5.2.1.5. Multivitamin Supplement

All subjects will take a daily multivitamin containing vitamin D 600 IU. The multivitamin is to be provided by the study.

### **5.2.2. Packaging and Labeling**

The tenofovir DF tablets and 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, continuous thread, child-resistant polypropylene screw cap with an induction-sealed, aluminium-faced liner.

The tenofovir DF oral powder formulation and 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.

During Weeks 1–96, subjects will be provided with sufficient supplies for 4 to 8 weeks of dosing. Thereafter, subjects will be provided with sufficient supplies for 12 weeks of dosing.

At a minimum, each drug bottle will be labeled with a lot number, the protocol number, administration instructions, storage instructions, expiration date, Sponsor name and address. Additional information will be included according to the requirements of the protocol and local law.

### **5.2.3. Storage and Handling**

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

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).

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 (1:1) to receive one of the following treatments in a blinded fashion:

#### **Treatment A:**

- for subjects  $\geq 17$  kg - blinded tenofovir DF oral tablet (150, 200, 250, or 300 mg based on body weight) once daily for 72 weeks (oral powder may be administered to subjects if unable to swallow a tablet)
- for subjects  $< 17$  kg - 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 72 weeks

#### **Treatment B:**

- for subjects  $\geq 17$  kg – matching blinded placebo oral tablet once daily for 72 weeks (oral placebo powder may be administered to subjects if unable to swallow a tablet)
- for subjects  $< 17$  kg - matching oral placebo powder formulation of tenofovir DF PO once daily up to a maximum corresponding dose of 300 mg for 72 weeks.

After 72 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for an additional 120 weeks.

Subjects who weigh  $\geq 17$  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.

Subjects will also be instructed to take a daily multivitamin containing vitamin D 600 IU throughout the study.

### 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  $\geq 17$  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  $\geq 2$  Years of Age and Weighing  $\geq 17$  kg Using VIREAD Tablets**

Body Weight		Tablets Once Daily
Kilogram (kg)	Pound (lbs)	
17 to $<22$	37 to $<49$	150 mg
22 to $<28$	49 to $<62$	200 mg
28 to $<35$	62 to $<77$	250 mg
$\geq 35$	$\geq 77$	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  $\leq 35$  kg, in the following manner:

Spoon 2–4 ounces of applesauce or an equivalent food (defined as food that does not require chewing) into a plastic cup or bowl without grooves. 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. Note that each scoop of powder covers a 5 kg body weight increment. The appropriate number of scoops of powder should be sprinkled onto the applesauce or equivalent food (defined as food that does not require chewing). Fold over the applesauce or food with a spoon several times until thoroughly mixed. The patient should consume the entire content of the food. Pour 120 mL of water (4 fluid ounces) into the food cup or bowl and swirl to suspend all residual food and powder. Instruct patients to drink the water-food mixture followed by additional 120 mL of water (total of 240 mL or 8 fluid ounces).

**Table 5-2. Dosing Recommendations for Pediatric Patients  $\geq 2$  Years of Age Using VIREAD Oral Powder**

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

a 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, cisplatin, 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
- 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 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 study candidate must sign an Assent Form if they have the ability to read and write, and each candidate's parent or legal guardian must sign an Informed Consent Form prior to the conduct of any screening procedures, in accordance with regulatory and local Ethics Committee 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  $\geq 30$  days after the initial screen if there is a reasonable expectation that the candidate will be eligible after repeat screening.

#### 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 applicable (see above)
- Medical history, including hepatitis B history and treatment 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:
  - 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



- >  $1.5 \times \text{ULN}$ ), 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  $\geq 1.5 \times \text{ULN}$ )
- 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)
- HIV, HCV and HDV serology
- Serum  $\beta$ -HCG pregnancy test (post-menarchal females only)
- $\alpha$ -fetoprotein (AFP)
- Serum and plasma storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg quantification and adherence assessment)
- 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 30 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 30 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 within 24 hours of 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 on any changes since Screening Visit
- Complete physical examination including Tanner Staging (excluding breast, anorectal, and urogenital exams), 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  $> 1.5 \times \text{ULN}$ ), 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  $\geq 1.5 \times \text{ULN}$ )
  - 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  $\beta$ -HCG pregnancy test (post-menarchal females only)
  - Serum for HBV viral genotyping (A–H) and resistance surveillance (sequence analysis of the HBV polymerase for resistance mutations)

- Plasma for determination of tenofovir DF concentration
- Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including HBsAg 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, 56, 64 Assessments**

The following evaluations will be performed at Weeks 4, 8, 16, 24, 32, 40, 56 and 64, unless otherwise specified. Study visits are to be completed  $\pm 3$  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, excluding breast, anorectal and urogenital exams, on 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  $> 1.5 \times \text{ULN}$ ), 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  $\geq 1.5 \times \text{ULN}$ )
- At Week 24 only: bone biochemical markers (refer to Section 6.5. for details)
- Hepatitis B serology (HBeAg and HBsAg; reflex HBeAb and HBsAb if Ag negative; Weeks 16, 32 and 64 only)
- Plasma HBV DNA (PCR method)
- Plasma for determination of tenofovir DF concentration
- Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg quantification and compliance assessment)
- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - Urine pregnancy test (for post-menarchal females 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 (Week 24 only; DEXA scan must be performed  $\pm 1$  week from Week 24 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

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  $\geq 16$  weeks (i.e., failure to resolve ALT to grade  $\leq 3$  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

Study Visit for Week 48 should be completed  $\pm$  3 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 (excluding breast, anorectal, urogenital exams), 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  $> 1.5 \times$  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  $\geq 1.5 \times$  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  $\beta$ -HCG pregnancy test (for post-menarchal females only)
  - Plasma for determination of tenofovir DF concentration
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg 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  $\pm$  1 week 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

### **6.2.3. Week 72 Assessments – End of Blinded Randomized Treatment**

Study Visit for Week 72 should be completed  $\pm$  3 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 (excluding breast, anorectal and urogenital exams), 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  $> 1.5 \times$  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  $\geq 1.5 \times$  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  $\beta$ -HCG pregnancy test (for post-menarchal females only)
  - Resistance surveillance
  - Plasma for determination of tenofovir DF concentration
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg 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  $\pm$  1 week from the Week 72 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 72 weeks of blinded randomized treatment, each subject will switch to open-label tenofovir DF treatment for 2.5 additional years. An external independent multidisciplinary Data Monitoring Committee will review the progress and safety of this study every 24 weeks after the first subject is randomized. At each meeting, the DMC will review routine safety and DEXA data and will make recommendations regarding continuing or initiating study treatment.

Subjects who discontinue the study prior to the Week 72 Visit will complete all Week 72 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.

#### **6.2.4. Assessments for Weeks 80, 88, 96, 108, 120, 132, 144, 156, 168, 180**

After Week 72 through the end of the study (each subject completes Week 192), subjects will return for visits every 12 weeks (Weeks 80, 88, 96, 108, 120, 132, 144, 156, 168, 180). Each study visit will occur  $\pm$  3 days from the protocol-specified visit date.

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, excluding breast, anorectal, and urogenital exams, at Weeks 96, 120, 144, 168)
- 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  $> 1.5 \times$  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  $\geq 1.5 \times$  ULN)
- At Weeks 96 and 144: 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 80, 96, and then every 12 weeks until the end of the study)
- Plasma HBV DNA (PCR method)
- Plasma for determination of tenofovir DF concentration
- Resistance surveillance (Week 144 only)
- Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg quantification and compliance assessment)
- Urine samples for:
  - Urinalysis (protein, glucose, blood)
  - Urine pregnancy test (for post-menarchal females only; positive urine pregnancy test will be immediately confirmed with a serum pregnancy test)
  - At Weeks 96 and 144: Urine bone biochemical markers, including urine bicarbonate, urine n-telopeptide, and spot urine creatinine and phosphate
- Weeks 96 and 144 only: DEXA scan of spine and whole body (DEXA scan must be performed  $\pm 1$  week 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



### 6.2.5. Week 192 Assessments

Study Visit for Week 192 should be completed  $\pm$  3 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 (excluding breast, anorectal, and urogenital exams), 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  $> 1.5 \times$  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  $\geq 1.5 \times$  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  $\beta$ -HCG pregnancy test (for post-menarchal females only)
  - Resistance surveillance
  - Plasma for determination of tenofovir DF concentration
  - Serum and plasma for storage (for potential pharmacokinetic and/or virologic assays, including resistance surveillance, HBsAg 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  $\pm$  1 week 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 a roll-over protocol until it becomes commercially available for the indication and age group studied in this protocol.

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

#### **6.3.1. Six-Month Post-Treatment 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.

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):

- 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  $> 1.5 \times$  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  $\geq 1.5 \times \text{ULN}$ )
- 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 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 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 Week 192 assessments and procedures (see Section 6.2.5 above). DEXA scans (spine and whole body) and bone biochemical markers will not be required at the Early Discontinuation Visit if the last measurement was performed  $\leq 12$  weeks earlier. 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, and then every 48 weeks through the end of study, and the Early Study Drug Discontinuation 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.** In addition, a DEXA scan will be performed at the time of switch in subjects switching from placebo to

tenofovir DF. DEXA scan will not be required at the Early Discontinuation Visit or at the time of switch if the last DEXA scan was performed  $\leq 12$  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 and then every 48 weeks through the end of study, and the Early Study Drug Discontinuation 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. Bone biochemical marker measurements will not be required at the Early Discontinuation Visit or at the time of switch if the last measurement was performed  $\leq 12$  weeks earlier.

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 renal excretion of calcium, phosphorous, and magnesium through calculation 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 ( $\pm 2$  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**

An intensive PK substudy will be performed on a subset of subjects following 4 weeks of dosing to evaluate the PK of tenofovir DF. PK sampling will occur over a period of 12 hours. Specimens will be drawn at 0, 1, 2, 4, 8, and 12 hours after tenofovir DF has been dosed. For the purpose of scheduling, the PK visit does not have to occur on the same day as the Week 4 Visit, but should occur within 2 weeks of the scheduled visit date. All specimens will be sent to an external lab for analysis. Details of the substudy procedures and specimen management will be documented in the PK manual.

### **6.7. Serum and Plasma for Storage**

Additional blood (plasma and serum) samples will be collected at each visit for long-term storage and possible future testing for potential pharmacokinetic and/or virologic assays (including resistance surveillance, HBsAg 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.

### **6.8. 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.

Genotypic sequence analysis of the HBV polymerase for resistance mutations will be conducted at Baseline for all subjects, and attempted for all viremic subjects (HBV DNA  $\geq$  400 copies/mL) at Weeks 72, 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 6.7).

### **6.9. 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

## 7. ADVERSE EVENTS AND TOXICITY MANAGEMENT

### 7.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and 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)

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., *Overdose*).
- 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 (subject is at **immediate** risk of death)

- 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  $> 2 \times$  baseline and  $> 10 \times$  ULN, with or without associated symptoms.
- Confirmed ALT elevation (defined as 1-grade shift or  $2 \times$  previous value) associated with confirmed changes outside of the normal range in other laboratory parameters suggestive of worsening hepatic function (total bilirubin  $\geq 2$  mg/dL above baseline, abnormal PT  $\geq 2$  seconds or INR  $\geq 0.5$  over baseline, abnormal serum albumin  $\geq 1$  g/dL below baseline or elevated serum lactate levels (if available), defined as  $2 \times$  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 of an AE, and not an adverse event in itself.
- 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” means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity.



- 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. Overdose**

A dose known to have been taken (accidentally or intentionally) exceeding the dose as prescribed by the protocol, misuse or abuse of blinded study medication or Gilead study drug, as well as any Gilead product taken as a concomitant medication, whether suspected or confirmed, and whether or not associated with an adverse experience, must be reported using the Gilead Drug Safety & Public Health (DSPH) Clinical Overdose form. The completed form must be forwarded within 24 hours to the Gilead Drug Safety & Public Health, or if applicable, the CRO.

Overdose will be considered an SAE only if any of the seriousness criteria are met. Any clinical sequelae in association with the overdose should be reported as an AE (as outlined in Section 7.1.) or SAE (as outlined in Section 7.3.). Details of signs or symptoms, clinical management and outcome should be reported, if available.

#### **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 to the attention of the Drug Safety and Public Health (DSPH) Department within 24 hours of the investigator’s knowledge of the event. Contact information is listed below.

Gilead Sciences DSPH  
Representative:

PPD  
Fax: PPD  
Email: Safety\_FC@gilead.com

- For fatal or life-threatening events, also fax copies of hospital case reports, autopsy report, and other documents when requested and applicable.
- 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 probably or possibly related to the use of study drugs, he/she should promptly document and report the event to Gilead Sciences.

### **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.

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.

All clinical and clinically significant laboratory toxicities will be managed according to uniform guidelines detailed in [Appendix 4](#).

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, the study drug should be restarted at full dose or modified dose dependent upon discussion with the Gilead Sciences Medical Monitor.

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  $\leq$  Grade 2.

If a laboratory abnormality recurs to  $\geq$  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:

$$\text{Schwartz Formula (mL/min/1.73 m}^2\text{)} = k \times L/\text{Scr}$$

[(k is a proportionality constant: pediatric males/females  $\geq 2$  years to < 12 years  $k = 0.55$ ; for adolescent females  $\geq 12$  years old,  $k = 0.55$ , and for adolescent males  $\geq 12$  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<sup>2</sup> (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<sup>2</sup> and serum creatinine increased  $\geq 0.5$  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<sup>2</sup> and serum creatinine increased  $\geq 0.5$  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  $\geq 70$  mL/min/1.73/m<sup>2</sup>, study medication may be resumed, with monitoring as described below. If the estimated estimated creatinine clearance remains  $< 70$  mL/min/1.73 m<sup>2</sup>, 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<sup>2</sup> (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  $\geq 16$  weeks (i.e., failure to resolve ALT to grade  $\leq 3$  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  $> 2 \times$  baseline and  $> 10 \times$  ULN, with or without associated symptoms OR
- Confirmed ALT elevation (defined as 1-grade shift or  $2 \times$  previous value) associated with confirmed changes outside of the normal range in other laboratory parameters suggestive of worsening hepatic function (total bilirubin  $\geq 2$  mg/dL above baseline, abnormal PT  $\geq 2$  seconds or INR  $\geq 0.5$  over baseline, abnormal serum albumin  $\geq 1$  g/dL below baseline or elevated serum lactate levels (if available), defined as  $2 \times$  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  $> 2 \times$  baseline and  $> 10 \times$  ULN OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (abnormal bilirubin  $\geq 2$ mg/dL above baseline, abnormal PT  $\geq 2$  sec above baseline, INR  $\geq 0.5$  above baseline, abnormal albumin  $\geq 1$  g/dL decrease from baseline or elevated serum lactate levels  $> 2 \times$  ULN along with any ALT elevation (i.e., grade shift or  $2 \times$  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.,  $> 2 \times$  baseline and  $> 10 \times$  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.,  $> 2 \times$  baseline and  $> 10 \times$  ULN) or have worsened, with bilirubin  $\leq 2.5 \times$  ULN, PT  $\leq 1.5 \times$  ULN, or abnormal albumin or lactate levels, the investigator should consult with the Gilead Medical Monitor.
- If ALT remains  $> 2 \times$  baseline and  $> 10 \times$  ULN and the bilirubin or PT values are confirmed at  $> 2.5 \times$  ULN or  $> 1.5 \times$  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 ( $> \text{Grade } 2$ ), Symptomatic Elevated Lactate ( $> 2 \times$  ULN) or Asymptomatic Elevated Lactate ( $> 4 \times$  ULN)**

If ALT/AST values are elevated (i.e.,  $> 2 \times$  baseline and  $> 10 \times$  ULN) and bilirubin or PT values are confirmed at  $> 2.5 \times$  ULN or  $> 1.5 \times$  ULN, respectively, or lactate levels are increased (symptomatic and  $> 2 \times$  ULN or asymptomatic and  $> 4 \times$  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  $> 2 \times$  baseline and  $> 10 \times$  ULN OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (bilirubin  $\geq 2$  mg/dL above baseline, abnormal PT  $\geq 2$  secs above baseline, abnormal albumin  $\geq 1$  g/dL below baseline or elevated lactate levels  $> 2 \times$  ULN) along with any ALT elevation (i.e., 1 grade shift or  $2 \times$  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  $> 2 \times$  baseline and  $> 10 \times$  ULN) OR (2) abnormal laboratory parameters suggestive of worsening hepatic function (abnormal bilirubin  $\geq 2$  mg/dL above baseline, abnormal PT  $\geq 2$  secs above baseline, abnormal albumin  $\geq 1$  g/dL below baseline, or elevated lactate levels  $> 2 \times$  ULN) along with any ALT elevation (i.e., 1 grade shift or  $2 \times$  previous value), request the clinical laboratory to conduct reflex testing for serum HBV DNA, HBV serology (HBeAg, HBeAb, and HBsAg), HDV, HAV IgM and HCV. If serum HBV DNA is increasing, the investigator should consider immediate initiation of approved therapy.
- The subject should be followed until the abnormal ALT/AST values, bilirubin, PT/INR, albumin or lactate laboratory parameters return to normal or baseline up to a maximum of 6 months after the initial occurrence of the event. Refer to [Appendix 6](#) for specific guidelines for the management of symptomatic and asymptomatic hyperlactatemia.



## **7.7. 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. Tenofovir DF is known to be excreted in human breast milk, so lactating females must discontinue nursing prior to study drug administration. 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 woman who has not had a hysterectomy, bilateral oophorectomy, or medically documented ovarian failure.

### **7.7.2. Contraceptive Requirements**

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 should be counseled appropriately on the protocol-recommended method(s) for avoiding pregnancy in case the subject chooses to engage in heterosexual intercourse.

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 counsel subjects 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 must discontinue nursing before investigational medicinal product administration.

**Table 7-1. Protocol-Recommended Contraceptive Methods**

Single Methods	Combination Methods
<p><b>Intra-uterine devices (IUDs)</b></p> <ul style="list-style-type: none"> <li>• Copper T 380A IUD</li> <li>• LNG 20 IUD</li> </ul> <p><b>Progesterone</b></p> <ul style="list-style-type: none"> <li>• Implant</li> </ul> <p><b>Tubal sterilization</b>            Vasectomy</p>	<p><b>Estrogen and Progesterone</b></p> <ul style="list-style-type: none"> <li>• Oral contraceptives plus barrier</li> <li>• Transdermal patch plus barrier</li> <li>• Vaginal ring plus barrier</li> </ul> <p><b>Progesterone</b></p> <ul style="list-style-type: none"> <li>• Injection plus barrier</li> </ul> <p><b>Two Barrier Methods</b></p> <ul style="list-style-type: none"> <li>• Female barrier/spermicide plus condom</li> </ul>

Female subjects who utilize hormonal contraceptives as one of their birth control methods must have used the same method for at least 3 months before study dosing.

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 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](mailto: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

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

### **8.2. Primary Endpoint**

The primary efficacy endpoint is the proportion of subjects with serum HBV DNA < 400 copies/mL at Week 72. A long-term evaluation of efficacy intention-to-treat algorithm will be employed for handling missing data; the details of this algorithm will be included in a separate statistical analysis plan.

### **8.3. Secondary Endpoints**

For Week 72, 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
- proportions of subjects with HBsAg loss and seroconversion
- genotypic changes from baseline within the HBV polymerase for subjects who were viremic (HBV DNA  $\geq$  400 copies/mL) at Weeks 72, 144, 192 or Early Discontinuation; 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

#### **8.4. Other Endpoints of Interest**

For Week 72, secondary endpoints to be evaluated in HBeAg-positive subjects include:

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

For Week 72, 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 and normalized ALT

For Week 72, 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, normalized ALT and HBeAg loss
- composite endpoint of proportion of subjects with HBV DNA < 400 copies/mL, normalized ALT, and HBeAg seroconversion.

For all categorical secondary endpoints, missing data will be handled using a long-term evaluation of efficacy intention-to-treat algorithm, as noted in Section 8.2.

All endpoints at Week 72 will also be analyzed at Weeks 144 and 192.

#### **8.5. Methods of Analysis**

All summary tables for efficacy endpoints will present data by randomization stratification groups (age [2 to < 6 or 6 to < 12], geographical location [North America/Europe, and Asia]), treatment naïve and treatment experience and overall.

### **8.5.1. Analysis Sets**

#### 8.5.1.1. 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 72 will be handled using a long-term evaluation of efficacy intention-to-treat algorithm, as noted in Section 8.2, for the purpose of the primary efficacy analysis. Subjects will be analyzed according to the randomized treatment assignment.

#### 8.5.1.2. 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.

#### 8.5.1.3. Pharmacokinetics

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

### **8.5.2. 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 handled using a long-term evaluation of efficacy intention-to-treat algorithm, as noted in Section 8.2; the details of this algorithm will be included in a separate statistical analysis plan.

Sensitivity analyses will be performed if warranted.

### **8.5.3. 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. See Section 8.10 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 72.

## **8.6. 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]).

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.

## **8.7. Efficacy Analysis**

### **8.7.1. Primary Analysis**

The primary efficacy analysis will be conducted at the end of double-blind treatment, after the last randomized subject reaches Week 72. 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. Missing data will be handled using a long-term evaluation of efficacy intention-to-treat algorithm, as noted in Section 8.2.

### **8.7.2. 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 long-term evaluation of efficacy intention-to-treat algorithm, as noted in Section 8.2.

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.

## **8.8. 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 72 (also at Weeks 144 and 192) will be summarized.

### **8.8.1. 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.

### **8.8.2. 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 permanent discontinuation from study,
- 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. A treatment-emergent adverse event will be defined as any adverse event that begins on or after the date of first dose of study drug. Events that occur prior to the first dose of study drug or after the last dose of study drug will be included in data listings.



A Fisher's exact test comparing treatment groups may be conducted for each HLT or preferred term that occurs with at least 5% incidence in either treatment group.

### **8.8.3. 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.

### **8.8.4. 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.

Bone mineral density (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.

## **8.9. Sample Size**

With respect to the primary efficacy endpoint, a sample size of 100 subjects (50 in TDF and 50 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%.

#### **8.10. 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. 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. For studies conducted under a United States Investigational New Drug Application (IND), the investigator will ensure that 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, are adhered to.

Since this is a “covered” clinical trial, the investigator will ensure that 21 CFR, Part 54, 1998, is adhered to; a “covered” clinical trial is any “study of a drug or device in humans submitted in a marketing application or reclassification petition subject to this part that the applicant or FDA relies on to establish that the product is effective (including studies that show equivalence to an effective product) or that make a significant contribution to the demonstration of safety.” This requires that investigators and all sub-investigators must provide documentation of their financial interest or arrangements with Gilead, or proprietary interests in the drug being studied. This documentation must be provided before participation of the investigator and any subinvestigator. The investigator and sub-investigator agree to notify Gilead of any change reportable interests during the study and for one year following completion of the study. Study completion is defined as the date that the last subject has completed the protocol defined activities.

This study is also subject to and will be conducted in accordance with 21 CFR, part 320, 1993, “Retention of Bioavailability and Bioequivalence Testing Samples.”

#### **9.1.2. Institutional Review Board/Independent Ethics Committee Approval**

This protocol 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/assent) will be submitted by the investigator to an IRB (for studies conducted in the United States) or IEC (for studies conducted outside of the United States). Approval from the IRB or IEC must be obtained **before** starting the study and should be documented in a letter to the investigator specifying the protocol number, protocol version, protocol date, documents reviewed, and date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of IRB or IEC approval must also be submitted to the IRB or IEC for approval before implementation.

### **9.1.3. Informed Consent/Assent**

The investigator is responsible for obtaining written informed consent/assent 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 utilize an IRB- or IEC-approved consent/assent form for documenting written informed consent. Each informed consent/assent will be appropriately signed and dated by the subject or the subject's legally authorized representative and the person obtaining consent/assent.

### **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 and an identification code (i.e., not names) should be recorded on any form or biological sample submitted to the sponsor, IRB or IEC, or laboratory. The investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial.

The investigator agrees that all information received from Gilead, including but not limited to the Investigator's Brochure, this protocol, CRFs, the investigational new drug, 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.

Subject clinical source documents (usually defined by the project in advance to record key activity/safety parameters independent of the CRFs) would include (although not be limited to) the following: subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, electroencephalogram, X-ray, pathology and special assessment reports, consultant letters, screening and enrollment log, etc.

All clinical study documents must be retained by the investigator until at least 2 years after the last approval of a marketing application in an ICH region (i.e., United States, Europe, or Japan) and until there are no pending or contemplated 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 required by applicable regulatory requirements or 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 guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Gilead to store these in sealed containers outside of the site so that they can be returned sealed to the investigator in case of a regulatory audit. When source documents are required for the continued care of the subject, appropriate copies should be made for storage outside of the site.

Biological samples at the conclusion of this study may be retained in storage by the Sponsor for a period up to 15 years for purposes of this study.

#### **9.1.6. Case Report Forms**

For each subject enrolled, a CRF must be completed and signed by the principal investigator or subinvestigator within a reasonable time period after data collection. This also applies to records for those subjects who fail to complete the study (even during a prerandomization screening period if a CRF was initiated). If a subject withdraws from the study, the reason must be noted on the CRF. If a subject is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

#### **9.1.7. Drug Accountability**

The investigator or designee (i.e., pharmacist) is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgment of receipt of each shipment of study product (quantity and condition) and subject dispensing records and returned or destroyed study product. Dispensing records will document quantities received from Gilead and quantities dispensed to subjects, including lot number, date dispensed, subject identifier number, subject initials, and the initials of the person dispensing the medication.

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

dispose of and/or destroy all unused study drug supplies, including empty containers, according to these procedures. If the site cannot meet Gilead's requirements for disposal, arrangements will be made between the site and Gilead or its representative for destruction or return of unused study drug supplies.

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

#### **9.1.8. Inspections**

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from Gilead or its representatives, 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. All protocol modifications must be submitted to the IRB or IEC in accordance with local requirements. Approval must be obtained before changes can be implemented.

#### **9.2.2. Study Report and Publications**

A clinical study report 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.

After conclusion of the study and without prior written approval from Gilead, 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.

No such communication, presentation, or publication will include Gilead's confidential information (see Section 9.1.4).

The investigator will submit 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. 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. Access to Information for Monitoring**

In accordance with ICH Good Clinical Practice (ICH GCP) guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

The monitor is responsible for routine review of the CRFs 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 CRFs. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

#### **9.3.2. 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.

#### **9.3.3. 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.

## 10. REFERENCES

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## 11. APPENDICES

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**Appendix 1. Investigator Signature Page**

**GILEAD SCIENCES, INC.  
333 LAKESIDE DRIVE  
FOSTER CITY, CA 94404 USA**

**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, 22 July 2011**

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

**PPD**

Author

**PPD**

22 July 2011

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 <sup>a</sup>	Baseline	Study Week									
			4	8	16	24	32	40	48	56	64	72
Written Informed Consent, Subject Assent	X											
Medical History	X	X <sup>c</sup>										
Complete Physical Examination (including Tanner Staging starting at Baseline)	X	X				X			X			X
Symptom-Directed Physical Examination			X	X	X		X	X		X	X	
Vitals Signs, Height, Weight <sup>f</sup>	X	X	X	X	X	X	X	X	X	X	X	X
HIV-1, HCV, HDV, $\alpha$ -fetoprotein	X											
HBV DNA Levels (PCR-Based Assay)	X	X	X	X	X	X	X	X	X	X	X	X
HBV Serology <sup>g</sup>	X	X			X		X		X		X	X
HBV Genotyping, Resistance Surveillance <sup>h</sup>		X							X			X
Hematology Profile	X	X	X	X	X	X	X	X	X	X	X	X
Serum Chemistry and Liver Tests <sup>i</sup>	X	X	X	X	X	X	X	X	X	X	X	X
Prothrombin Time/INR <sup>j</sup>	X	X										
Urinalysis	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test <sup>k</sup>	X	X	X	X	X	X	X	X	X	X	X	X
Plasma for Tenofovir DF Concentration		X	X	X	X	X	X	X	X	X	X	X
Serum and Plasma for Storage <sup>l</sup>	X	X	X	X	X	X	X	X	X	X	X	X
DEXA Scan – Spine and Whole-Body <sup>m</sup>		X				X			X			X
Bone Biochemical Markers <sup>n</sup>	X	X				X			X			X
Blood sample storage for genomic testings <sup>o</sup>		X										
Concomitant Medications	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
Study Drug Dispensing/Return <sup>p</sup>		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

**Appendix 2. Study Procedures Table (continued)**

Study Procedures	Study Week											Early DC <sup>c</sup>	24-Wk FU <sup>d</sup>
	80	88	96 <sup>b</sup>	108	120	132	144	156	168	180	192		
Complete Physical Examination			X		X		X		X		X	X	
Symptom-Directed Physical Examination	X	X		X		X		X		X			X
Vital Signs, Height, Weight <sup>f</sup>	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
HBV Serology <sup>g</sup>	X		X	X	X	X	X	X	X	X	X	X	X <sup>q</sup>
Hematology Profile	X	X	X	X	X	X	X	X	X	X	X	X	
HBV Resistance Surveillance <sup>h</sup>			X				X				X	X	
Serum Chemistry and Liver Tests <sup>i</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X
Prothrombin Time/INR <sup>j</sup>													X <sup>j</sup>
Urinalysis	X	X	X	X	X	X	X	X	X	X	X	X	
Pregnancy Test <sup>k</sup>	X	X	X	X	X	X	X	X	X	X	X	X	
Plasma for Tenofovir DF Concentration	X	X	X	X	X	X	X	X	X	X	X	X	
Serum and Plasma for Storage <sup>l</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X
DEXA Scan – Spine and Whole-Body <sup>m</sup>			X				X				X	X <sup>r</sup>	
Bone Biochemical Markers <sup>n</sup>			X				X				X	X <sup>r</sup>	
Concomitant Medications	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
Study Drug Dispensing/Return <sup>o</sup>	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	

a Evaluations to be completed within 30 days prior to Baseline Visit.

b After Week 96, subjects will return for study visits every 12 weeks until each subject reaches Week 192.

c The Early Study Drug Discontinuation Visit should be completed within 72 hours of last dose of study drug; subjects who discontinue study drug will be followed every 4 weeks for 24 weeks off treatment or up to initiation of active treatment, whichever comes first.

d Subjects who receive at least one dose of study drug will be followed every 4 weeks for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first.

- 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  $\geq$  400 copies/mL) at Weeks 72, 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 post-menarchal females only; a serum  $\beta$ -HCG pregnancy test will be performed at Screening, Baseline, Weeks 48, 72, 192 and Early Study Drug Discontinuation Visit (if applicable). Urine pregnancy testing will be performed at all other study visits through the end of the study. A positive urine pregnancy test must be confirmed immediately with a serum pregnancy test.
- l Blood (serum and plasma) collected for longterm 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  $\pm$  1 week 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. After each subject has reached Week 192 (or at the Early Study Drug Discontinuation Visit), study medication will not be dispensed. Subjects who have received at least one dose of study drug and permanently discontinue study drug will be followed every 4 weeks for 24 weeks off treatment or up to initiation of active treatment, whichever occurs first.
- q During the 24-week follow-up period, HBV serology testing will only be performed at the Follow-Up Week 24 Visit.
- r DEXA scan is required at Early Discontinuation Visit if most recent DEXA scan was > 12 weeks prior to the date of the Early Discontinuation Visit. Bone biochemical markers are required at Early Discontinuation Visit if most recent measurement was > 12 weeks prior.

**Appendix 3. Clinical Laboratory Assessments**

<b>Hematology:</b> Erythrocytes Hemoglobin Hematocrit Platelets Total leukocytes and differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils)	<b>Liver Tests:</b> Total bilirubin (reflex direct [conjugated] bilirubin) AST ALT Alkaline phosphatase Prothrombin time, INR
<b>Chemistry:</b> Creatine phosphokinase Lactic dehydrogenase (LDH) Creatinine and calculated creatinine clearance Albumin Glucose Serum amylase Lipase (if serum amylase is $\geq 1.5 \times$ ULN) $\alpha$ -fetoprotein (Screening Visit) Blood urea nitrogen (BUN) Uric acid	<b>Urinalysis:</b> Protein Blood Glucose <b>Urine Pregnancy Test:</b> Post-menarchal females only (reflex serum $\beta$ -HCG if positive)
<b>Electrolytes:</b> Sodium Potassium Bicarbonate Phosphorus+ Calcium Magnesium Chloride	<b>HBV Serology:</b> HBsAg, HBeAg (reflex HBsAb and HBeAb).

<b>Bone Biochemical Markers:</b>	<ul style="list-style-type: none"> <li>• urine bicarbonate and n-telopeptide;</li> <li>• serum c-telopeptides, osteocalcin, bone-specific alkaline phosphatase, PTH, and vitamin D levels (25-hydroxy) and 1,25 (dihydroxyvitamin) D levels;</li> <li>• fasting serum creatinine/ phosphate and urine creatinine (spot)/phosphate for measurement of renal excretion of calcium, phosphorous and magnesium through calculation of the renal phosphate threshold (TmP/GFR)</li> </ul>	<b>Virology:</b> Plasma and serum for HBV DNA and serology HIV, HDV and HCV (Screening Visit) Viral genotyping (A–H), resistance surveillance
<b>Serum <math>\beta</math>-HCG Pregnancy Test:</b>	Post-menarchal females only; at Screening, Baseline, Weeks 48, 72, 192, or Early Study Discontinuation Visits; reflex if urine test positive at all other study visits	<b>Tenofovir DF Concentration:</b> Plasma concentration  <b>Storage:</b> Plasma and serum for storage



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

<b>HEMATOLOGY</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Hemoglobin	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
Absolute Neutrophil Count (ANC)	1000 to 1300/mm <sup>3</sup> 1.00 to 1.30 GI/L	750 to < 1000/mm <sup>3</sup> 0.75 to < 1.00 GI/L	500 to < 750/mm <sup>3</sup> 0.50 to < 0.75 GI/L	< 500/mm <sup>3</sup> < 0.50 GI/L
Platelets	100,000 to < 125,000/mm <sup>3</sup> 100.0 to < 125.0 GI/L	50,000 to < 100,000/mm <sup>3</sup> 50.0 to < 100.0 GI/L	25,000 to < 50,000/mm <sup>3</sup> 25.0 to < 50.0 GI/L	< 25,000/mm <sup>3</sup> < 25.0 GI/L
WBCs	2000/mm <sup>3</sup> to 2500/mm <sup>3</sup> 2.0 GI/L to 2.5 GI/L	1,500 to < 2,000/mm <sup>3</sup> 1.5 to < 2.0 GI/L	1000 to < 1,500/mm <sup>3</sup> 1.0 to < 1.5 GI/L	< 1000/mm <sup>3</sup> < 1.0 GI/L
Hypofibrinogenemia	100 to < LLN mg/dL 1.00 to < LLN 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

**HEMATOLOGY**

	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 microg/mL 20 to 40 mg/L	> 40 to 50 microg/mL > 40 to 50 mg/L	> 50 to 60 microg/mL > 50 to 60 mg/L	> 60 microg/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
Activated Partial Thromboplastin (APPT)	> 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%

<b>CHEMISTRY</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Hyponatremia	130 mEq/L to < LLN	125 to < 130 mEq/L	121 to < 125 mEq/L	< 121 mEq/L
	130 mmol/L to < LLN	125 to < 130 mmol/L	121 to < 125 mmol/L	< 121 mmol/L
Hypernatremia	> ULN to 150 mEq/L	> 150 to 154 mEq/L	> 154 to 159 mEq/L	> 159 mEq/L
	> ULN to 150 mmol/L	> 150 to 154 mmol/L	> 154 to 159 mmol/L	> 159 mmol/L
Hypokalemia	3.0 mEq/L to < LLN	2.5 to < 3.0 mEq/L	2.0 to < 2.5 mEq/L	< 2.0 mEq/
	3.0 mmol/L to < LLN	2.5 to < 3.0 mmol/L	2.0 to < 2.5 mmol/L	< 2.0 mmol/L
Hyperkalemia	5.6 to 6.0 mEq/L	> 6.0 to 6.5 mEq/L	> 6.5 to 7.0 mEq/L	> 7.0 mEq/L
	5.6 to 6.0 mmol/L	> 6.0 to 6.5 mmol/L	> 6.5 to 7.0 mmol/L	> 7.0 mmol/L
Hypoglycemia	55 to 64 mg/dL	40 to < 55 mg/dL	30 to < 40 mg/dL	< 30 mg/dL
	3.1 to 3.5 mmol/L	2.2 to < 3.1 mmol/L	1.7 to < 2.2 mmol/L	< 1.7 mmol/L
Hyperglycemia, Nonfasting and no prior diabetes	> ULN to 160 mg/dL	> 160 to 250 mg/dL	> 250 to 500 mg/dL	> 500 mg/dL
	> ULN to 8.9 mmol/L	> 8.9 to 13.9 mmol/L	> 13.9 to 27.7 mmol/L	> 27.7 mmol/L
Hypocalcemia (corrected for albumin)	7.8 mg/dL to < LLN	7.0 to < 7.8 mg/dL	6.1 to < 7.0 mg/dL	< 6.1 mg/dL
	1.94 mmol/L to < LLN	1.74 to < 1.94 mmol/L	1.51 to < 1.74 mmol/L	< 1.51 mmol/L
Hypercalcemia (corrected for albumin)	> ULN to 11.5 mg/dL	> 11.5 to 12.5 mg/dL	> 12.5 to 13.5 mg/dL	> 13.5 mg/dL
	> ULN to 2.88 mmol/L	> 2.88 to 3.13 mmol/L	> 3.13 to 3.38 mmol/L	> 3.38 mmol/L
Hypocalcemia (ionized)	3.0 mg/dL to < LLN	2.5 to < 3.0 mg/dL	2.0 to < 2.5 mg/dL	< 2.0 mg/dL
	0.74 mmol/L to < LLN	0.62 to < 0.74 mmol/L	0.49 to < 0.62 mmol/L	< 0.49 mmol/L

<b>CHEMISTRY</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Hypercalcemia (ionized)	> ULN to 6.0 mg/dL	> 6.0 to 6.5 mg/dL	> 6.5 to 7.0 mg/dL	> 7.0 mg/dL
	> ULN to 1.50 mmol/L	> 1.50 to 1.63 mmol/L	> 1.63 to 1.75 mmol/L	> 1.75 mmol/L
Hypomagnesemia	1.45 mg/dL to < LLN	1.09 to < 1.45 mg/dL	0.72 to < 1.09 mg/dL	< 0.72 mg/dL
	0.60 mmol/L to < LLN	0.45 to < 0.60 mmol/L	0.30 to < 0.45 mmol/L	< 0.30 mmol/L
Hypophosphatemia > 14 years	2.0 mg/dL to < LLN	1.5 to < 2.0 mg/dL	1.0 to < 1.5 mg/dL	< 1.0 mg/dL
	0.63 mmol/L to < LLN	0.47 to < 0.63 mmol/L	0.31 to < 0.47 mmol/L	< 0.31 mmol/L
<b>Pediatric 1 year – 14 years</b>	3.0 to 3.5 mg/dL	2.5 to < 3.0 mg/dL	1.5 to < 2.5 mg/dL	< 1.5 mg/dL
	0.96 to 1.14 mmol/L	0.80 to < 0.96 mmol/L	0.47 to < 0.80 mmol/L	< 0.47 mmol/L
Hyperbilirubinemia	> 1.0 to 1.5 × ULN	> 1.5 to 2.5 × ULN	> 2.5 to 5.0 × ULN	> 5.0 × ULN
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	> 10.0 to 12.0 mg/dL	> 12.0 to 15.0 mg/dL	> 15.0 mg/dL
	> ULN to 597 micromol/L	> 597 to 716 micromol/L	> 716 to 895 micromol/L	> 895 micromol/L
Hypouricemia	1.5 mg/dL to < LLN	1.0 to < 1.5 mg/dL	0.5 to < 1.0 mg/dL	< 0.5 mg/dL
	90 micromol/L to < LLN	60 to < 90 micromol/L	30 to < 60 micromol/L	< 30 micromol/L
Creatinine	> 1.5 to 2.0 mg/dL	> 2.0 to 3.0 mg/dL	> 3.0 to 6.0 mg/dL	> 6.0 mg/dL
	> 137 to 181 micromol/L	> 181 to 269 micromol/L	> 269 to 535 micromol/L	> 535 micromol/L
Bicarbonate	16.0 mEq/L to < LLN	11.0 to < 16.0 mEq/L	8.0 to < 11.0 mEq/L	< 8.0 mEq/L
	16.0 mmol/L to < LLN	11.0 to < 16.0 mmol/L	8.0 to < 11.0 mmol/L	< 8.0 mmol/L

<b>CHEMISTRY</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Triglycerides (Fasting)	—	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
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	—
<b>Pediatric &lt; 18 years</b>	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
Lactate	ULN to < 2.0 × ULN without acidosis	≥ 2.0 × ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences

**ENZYMES**

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

**URINALYSIS**

	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Hematuria (Dipstick)	1+	2+	3+	NA
Hematuria (Quantitative)	6–10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria (Dipstick)	1+	2–3+	4+	NA
Proteinuria, 24 hour collection	200 to 999 mg/24 h	> 999 to 1999 mg/24 h	> 1999 to 3500 mg/24 h	> 3500 mg/24 h
Glycosuria (Dipstick)	1+	2+	3+	4+

<b>CARDIOVASCULAR</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 $\leq 2$ units packed RBCs (for children $\leq 10$ cc/kg) indicated	Life-threatening hypotension OR Transfusion of $> 2$ units packed RBCs indicated (for children $\leq 10$ cc/kg) indicated
Hypertension (with repeat testing at same visit)	$> 140\text{--}159$ mmHg systolic OR $> 90\text{--}99$ mmHg diastolic	$> 159\text{--}179$ mmHg systolic OR $> 99\text{--}109$ mmHg diastolic	$> 179$ mmHg systolic OR $> 109$ mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization (other than ER visit) indicated
<b>Pediatric <math>\leq 17</math> years</b> (with repeat testing at same visit)	NA	91 <sup>st</sup> –94 <sup>th</sup> percentile adjusted for age, height, and gender (systolic and/or diastolic)	$\geq 95^{\text{th}}$ percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (e.g., 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

<b>CARDIOVASCULAR</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 (e.g., tamponade) OR Urgent intervention indicated
Prolonged PR Interval	PR interval 0.21 to 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec	Complete AV block
<b>Pediatric ≤ 16 years</b>	1 <sup>st</sup> degree AV block (PR > normal for age and rate)	Type I 2 <sup>nd</sup> degree AV block	Type II 2 <sup>nd</sup> 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, e.g., Torsade de pointes or other associated serious ventricular dysrhythmia
<b>Pediatric ≤ 16 years</b>	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, e.g., Torsade de pointes or other associated serious ventricular dysrhythmia
Thrombosis/embolism	NA	Deep vein thrombosis AND No intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Deep vein thrombosis AND Intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Embolic event (e.g., 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



<b>RESPIRATORY</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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
<b>Pediatric ≤ 14 years</b>	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

<b>OCULAR/VISUAL</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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)

<b>SKIN</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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

<b>GASTROINTESTINAL</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 [e.g., tube feeding or total parenteral nutrition]
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., 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 (e.g., sepsis or perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of $\leq 3$ stools over baseline/24 hr	Persistent episodes of unformed to watery stools OR Increase of 4–6 stools over baseline per 24hrs.	Bloody diarrhea OR Increase of $\geq 7$ stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., 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
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 (e.g., aspiration, choking)

**GASTROINTESTINAL**

	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than ER visit)	Symptomatic AND Hospitalization indicated (other than ER visit)	Life-threatening consequences (e.g., 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 (e.g., 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 (e.g., hypotensive shock)

<b>NEUROLOGICAL</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Alteration in personality-behavior or in mood (e.g., 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 (e.g., 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 – <b>Pediatric ≤ 16 years</b>	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

<b>NEUROLOGICAL</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)

<b>NEUROLOGICAL</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 (e.g., severity or focality)	Seizures of any kind that are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure – <b>Pediatric &lt; 18 years</b>	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

<b>MUSCULOSKELETAL</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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  <b>Pediatric &lt; 21 years</b>	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



<b>SYSTEMIC</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 [e.g., 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	Erythema OR Induration of 5 × 5 cm to 9x9 cm (or 25-81 cm <sup>2</sup> )	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm <sup>2</sup> )	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
<b>Pediatric ≤ 15 years</b>	<b>Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter</b>	<b>Erythema OR Induration OR Edema &gt; 2.5 cm diameter but &lt; 50% surface area of the extremity segment (e.g., upper arm/thigh)</b>	<b>Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage</b>	<b>Necrosis (involving dermis and deeper tissue)</b>
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

<b>ENDOCRINE/METABOLIC</b>				
	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Lipodystrophy (e.g., 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 (e.g., 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 (e.g., 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 (e.g., myxedema coma)
Lipoatrophy (e.g., 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**

	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
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 (e.g., 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

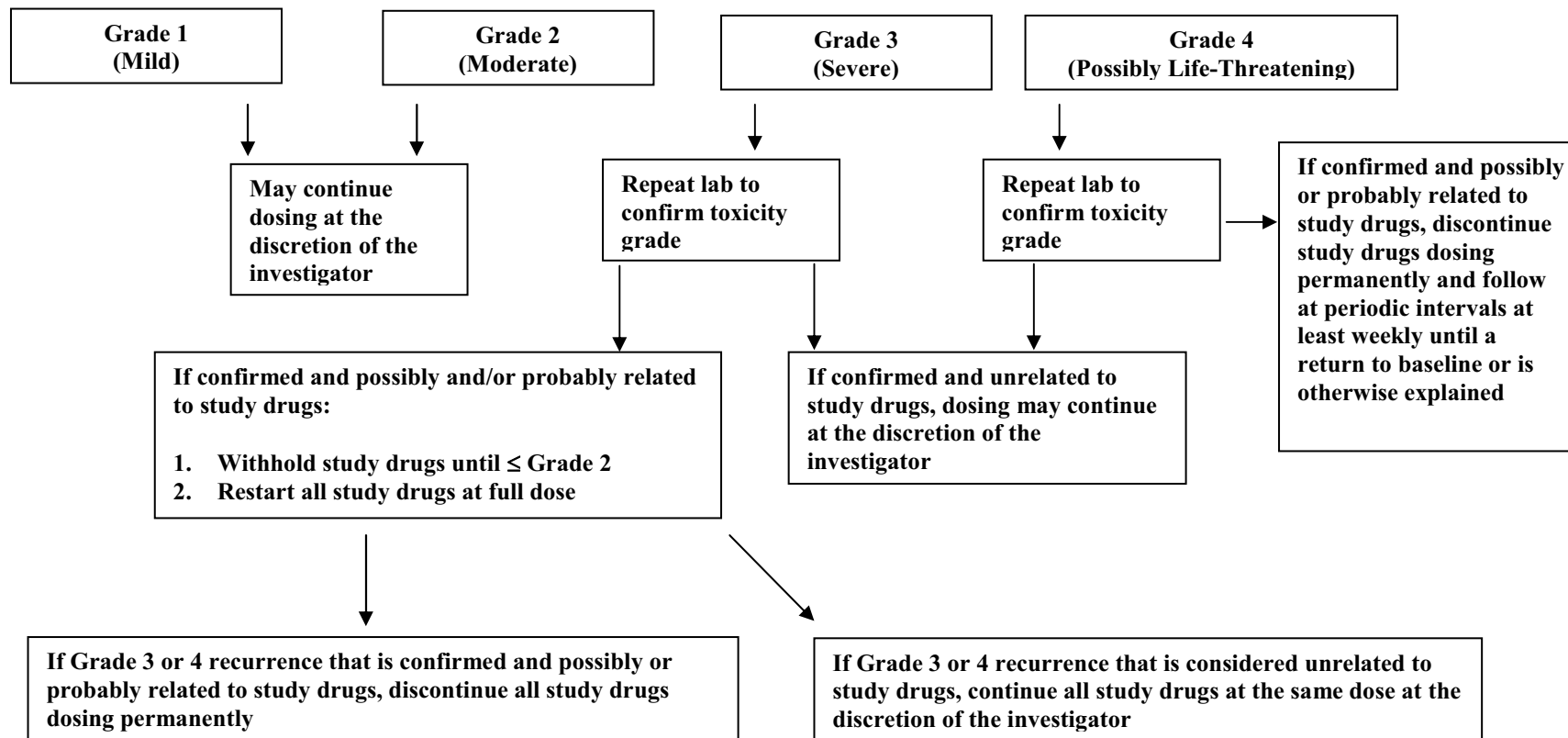
**INFECTION**

	<b>Grade 1</b>	<b>Grade 2</b>	<b>Grade 3</b>	<b>Grade 4</b>
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial 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 (e.g., 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 ( $\geq 2$  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<sub>2</sub>, PCO<sub>2</sub>, bicarbonate, oxygen saturation) should be performed within 48 hours. If the arterial specimen contains lactate at a level more than two times the upper limit of normal, the patient should be discontinued from the study and alternative therapy instituted. Subjects should be monitored weekly until signs and symptoms resolve. Hyperlactatemia should be followed until levels return to below two times the ULN and the patient.

An elevated anion gap in a patient with metabolic acidosis suggests the diagnosis of lactic acidosis. It can be suspected when the sum of cations minus the sum of anions  $[(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)]$  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<sub>2</sub>, PCO<sub>2</sub>, 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.