IONIS PHARMACEUTICALS, INC.

ISIS 505358-CS3

A Phase 2, Double-Blinded, Randomized, Placebo-Controlled, Dose-Escalation Study to Examine the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of ISIS 505358 in Treatment-Naïve Patients with Chronic Hepatitis B Virus Infection

Protocol Amendment 3 – Korea – 29 January 2019

Sponsor:

Ionis Pharmaceuticals, Inc.
2855 Gazelle Court
Carlsbad, CA 92010

ISIS 505358-CS3

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Protocol History:

Original Protocol:	2 September 2015
Protocol Amendment 1:	19 February 2016
Protocol Amendment 2:	22 November 2016

Sponsor:

Ionis Pharmaceuticals, Inc.
2855 Gazelle Court
Carlsbad, CA 92010

PPD

PPD (Jan 29, 2019)

PPD, MD
Vice President and Head of Clinical Development

Jan 29, 2019

ISIS 505358

Ionis Protocol Number ISIS 505358-CS3

Protocol Amendment 3 – Korea

Clinical Phase: 2

A Phase 2, Double-Blinded, Randomized, Placebo-Controlled, Dose-Escalation Study to Examine the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of ISIS 505358 in Treatment-Naïve Patients with Chronic Hepatitis B Virus Infection

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Date: 29 January 2019

Confidentiality Statement

Protocol Signature Page

Protocol Number: ISIS 505358-CS3

Protocol Title: A Phase 2, Double-Blinded, Randomized, Placebo-Controlled, Dose Escalation Study to Examine the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of ISIS 505358 in Treatment-Naïve Patients with Chronic Hepatitis B Virus Infection

Amendment: Amendment 3 – Korea

Date: 29 January 2019

I hereby acknowledge that I have read and understand the attached clinical protocol, entitled "A Phase 2, Double-Blinded, Randomized, Placebo-Controlled, Dose Escalation Study to Examine the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of ISIS 505358 in Treatment-Naïve Patients with Chronic Hepatitis B Virus Infection," dated 29 January 2019, and agree to conduct the Study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice (E6).

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

Investigator's Signature

Investigator's Name *(please print)* Date (DD Month YYYY)

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PROTOCOL AMENDMENT

Protocol Number: ISIS 505358-CS3

Protocol Title: A Phase 2, Double-Blinded, Randomized, Placebo-Controlled, Dose-Escalation Study to Examine the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of ISIS 505358 in Treatment-Naïve Patients with Chronic Hepatitis B Virus Infection

Amendment Number: 3 – Korea

Amendment Date: 29 January 2019

The main purpose of this amendment is to add Cohort 4 in which patients with chronic hepatitis B and already on a stable regimen of tenofovir or entecavir are treated with ISIS 505358 by the same regimen used in Cohorts 1–3 to dose treatment-naïve HBV patients. This represents an exploratory examination to obtain results that will be used to guide the design of subsequent larger study.

The table below summarizes the modifications made to the ISIS 505358-CS3 Protocol (deletions from *Was* are underlined; additions in the *Now Is* are in bold font). Some minor edits have not been listed

Protocol Section	Description of Change	Rationale
Synopsis: Exploratory Objective(s) Section 1.3 Exploratory Objectives	Added: To explore antiviral activity, safety/tolerability, and PK during concurrent treatment with ISIS 505358 and nucleos(t)ide analogue (i.e., tenofovir or entecavir)	Addition of Cohort 4
Synopsis: Study Design Synopsis: Treatment Groups Section 3.1 Study Design	Added to Cohort 3 text: (note: cohort was studied as 300 mg ISIS 505358 or placebo based on antiviral activity observed in Cohort 2 per Section 3.7)	Update treatment performed in Cohort 3
Synopsis: Study Design Section 3.1 Study Design	Added: One (1) exploratory cohort (Cohort 4) will examine add-on treatment of patients already on stable nucleos(t)ide analogue (i.e., tenofovir or entecavir) regimen with 300 mg ISIS 505358 or placebo (3:1 randomization). Patients enrolled into the study may be either HBeAg positive (+) or negative (-). The target size of Cohort 4 is approximately 8 patients, but size may range from 4 to > 8 depending upon availability of participants. Each patient randomized into Cohort 4 is expected to continue their ongoing nucleos(t)ide analogue regimen and be treated with Study Drug by the above schedule for Cohorts 1–3. After Day 22, the patients are expected to continue their ongoing nucleos(t)ide analogue regimen and will be followed until Day 211. On Day 29, the effects of Study Drug treatment will be assessed.	Addition of Cohort 4

Protocol Section	Description of Change	Rationale
Synopsis: Inclusion Criteria in Study Population Section 5.1 Inclusion Criteria	Was: 4. Plasma HBV DNA ≥ 2 x 10^3 IU/mL Now Is: 4. **Cohort 1–3:** Plasma HBV DNA ≥ 2 x 10^3 IU/mL **Cohort 4: HBV DNA adequately suppressed (e.g., plasma or serum HBV DNA below the lower limit of quantitation)**	Addition of Cohort 4
Synopsis: Inclusion Criteria in Study Population Section 5.1 Inclusion Criteria	Added: 7. Cohort 4 only: Currently taking and have been taking tenofovir or entecavir without changes in drug, dose level and/or frequency of administration for ≥ 12 months and expect to continue taking without change through to the end of their participation in this study (approximately 8 months)	Addition of Cohort 4
Synopsis: Exclusion Criteria in Study Population Section 5.2 Exclusion Criteria	Was: 1. Current or prior receipt of anti-HBV nucleos(t)ide analogue therapy. Patients who have failed prior interferon treatment, greater than 6 months prior to Screening, may be evaluated for possible participation in the study Now Is: 1. **Cohort 1–3 only:** Current or prior receipt of anti-HBV nucleos(t)ide analogue therapy. Patients who have failed prior interferon treatment, greater than 6 months prior to Screening, may be evaluated for possible participation in the study	Addition of Cohort 4
Synopsis: Treatment Groups	Added: Cohort 4: 300 mg ISIS 505358 or placebo (in patients already on stable tenofovir or entecavir regimen)	Addition of Cohort 4
Dose-Escalation section of Synopsis Section 3.6 Data and Safety Monitoring Board	Was: After completion of Study Drug administration and the last Day 29 assessment for Cohort 3, the DSMB will review the cumulative safety and antiviral data …. Now Is: After completion of Study Drug administration and the last Day 29 assessment**s** for Cohort 3 **and for Cohort 4, respectively**, the DSMB will review the cumulative safety and antiviral data ….	Addition of Cohort 4
Synopsis: Statistical Considerations Section 4.4 Unblinding of Treatment Assignment	Was An unblinded interim analysis may be performed after all patients have progressed beyond their Study Day 29 assessments. Now Is: An unblinded interim analysis may be performed **of Cohort 1–3** after all patients **within these cohorts** have progressed beyond their Study Day 29 assessments. **An unblinded interim analysis similarly may be performed of Cohort 4 after all Cohort 4 patients have progressed beyond their Study 29 assessments.**	Addition of Cohort 4
Dose Escalation Flow in Study Design and Treatment Schema	Added: Note: Cohort 3 was 300 mg or placebo (3:1) based on antiviral activity observed in Cohort 2 per Section 3.7	Addition of Cohort 4

Protocol Section	Description of Change	Rationale
Patient Participation Flow in Study Design and Treatment Schema	Added: Cohort 4 only: treatment with tenofovir or entecavir during all periods (i.e., Screen, Treatment, and Post-Study Drug Treatment)	Addition of Cohort 4
Section 2.4.4 Clinical Experience	Added: Section 2.4.4.1 ISIS 505358-CS1	Added subsection header for clarity
Section 2.4.4 Clinical Experience	Added: Section 2.4.4.2 ISIS 505358-CS3 Interim blinded results from Cohort 1 (150 mg or placebo, 6:2 ratio), Cohort 2 (300 mg or placebo, 6:2 ratio), and Cohort 3 (300 mg or placebo, 6 patients, ratio unknown) are available. There has been one SAE in the study—Alanine aminotransferase increased. A patient in Cohort 2 experienced an ALT elevation that peaked at 781 U/L (23.7 x ULN, ULN = 33) on Study Day 38 (15 days after last protocol-specified dose of Study Drug). ALT levels declined to < 5 x ULN within 11 days and became consistently < 3 x ULN by Day 72. The patient had a lesser concurrent AST elevation. Her total bilirubin levels were stable throughout the study. This ALT elevation temporally coincided with HBsAg and HBV DNA reduction to less than the lower limit of quantitation and therefore may have been secondary to the clearance of infected hepatocytes. Three (3) additional patients—2 in Cohort 1 and 1 in Cohort 2—experienced treatment-emergent elevations of ALT to > 3 x ULN. None had changes in total bilirubin levels. Like the aforementioned patient with SAE, the ALT elevations of these 3 patients were temporally related to reductions in HBsAg. However, there were other patients in ISIS 505358-CS3 with HBsAg reductions but without ALT elevation. Therefore, the possibility that the ALT elevations are a direct adverse effect (wholly or partially) of ISIS 505358 on the liver cannot be entirely excluded. Some activity against HBV was observed in the treatment-naïve patients treated ISIS 505358-CS3. Three (3) of the 8 patients in Cohort 1 had HBsAg reductions of 0.39, 1.19, and 1.24 $\log_{10}$ IU/mL. Five of the 8 patients in Cohort 2 had HBsAg reductions of 0.462, 1.19, 1.96, 2.57, and 3.80 $\log_{10}$ IU/mL. A 6th patient in Cohort 2 had a 0.81 $\log_{10}$ IU/mL reduction in HBV DNA although the patient's HBsAg reduction was minimal (0.12 $\log_{10}$ IU/mL). Dose-related reductions in HBV DNA were also observed in the study. This antiviral activity was observed in both HBeAg positive and negative patients. In Cohort 3, one (1) of the patients was observed to have developed a 3.33 log10 IU/mL reduction in HBsAg and 3.53 $\log_{10}$ IU/mL reduction in HBV DNA on Day 29. Two (2) other patients each had 0.43 $\log_{10}$ IU/mL reductions of HBsAg and a 4th patient had a reduction of HBV DNA of 0.77 $\log_{10}$ IU/mL despite minimal HBsAg reduction. Overall, continued clinical development of ISIS 505358 is supported by the results from ISIS 505358-CS1 and the emerging results from ISIS 505358-CS3.	Update clinical experience with interim experience from CS3

Protocol Section	Description of Change	Rationale
Added: Section 2.6 Combination of ISIS 505358 with Nucleos(t)ide Analogues	Added: Section 2.6 Combination of ISIS 505358 with Nucleos(t)ide Analogues The differences in pharmacokinetic behavior between drugs like ISIS 505358 and nucleos(t)ide analogues indicate drug-drug interaction between the 2 drug types is unlikely. The antiviral and safety results from Cohorts 1-2 of ISIS 505358-CS3 are consistent with that expectation. Drugs of the 2′-MOE ASO class, like ISIS 505358, rapidly become highly bound (~95%) to serum proteins upon entry into the blood stream and are then rapidly distributed ($t_{1/2}$ ~2 hr) to tissues where these drugs enter cells through target mediated endocytosis and micropinocytosis (Geary et al. 2008; Bennett and Swayze 2010). 2′-MOE ASOs are eliminated primarily by nucleolytic degradation (Geary et al., 2008). None of these processes are inhibited by small-molecule drugs like nucleos(t)ide analogues. Conversely, tenofovir, entecavir, and other nucleos(t)ide analogues circulated in the blood stream with little binding to serum proteins and are eliminated in urine as intact drug by a combination of glomerular filtration and active secretion (Bristol-Myers Squibb 2018; Gilead 2018). The renal elimination has been shown to be mediated by the organic anion transporters OAT1, OAT3 and OCT2 (Cihlar et al. 2001; Uwai et al. 2007; Xu et al. 2013). 2′-MOE ASO have been shown to be neither substrates nor inhibitors of OAT1, OAT3, or OCT2 (Yu et al. 2016; Shemesh et al. 2017). In Cohorts 1–2 of ISIS 505358-CS3, tenofovir dosing was started on Study Day 29, which is 7-days after the last dosing of ISIS 505358. Since the ASO has a long tissue half-life (2–3 weeks based on the non-clinical studies), there is a period of at least several weeks during which there will be a substantial presence of both drugs in the livers and other tissues of treated patients. For Cohorts 1 and 2, potent reduction of HBV DNA was observed following initiation of tenofovir in all patients. There were no obvious differences in tenofovir potency between the cohorts consistent with the different ISIS 505358 dose levels of the 2 cohorts nor were there differences within cohorts that possibly indicate a difference between ASO and placebo-treated patients. Neither were there any adverse event or clinical laboratory results suggestive of a safety effect due to drug-drug interaction. The results from Cohort 3 to date are consistent with Cohort 2.	Addition of Cohort 4
Section 3.4.1 Screening	Added: Patients in screening for Cohort 4 are expected to continue to take their pre-existing ongoing nucleos(t)ide analogue therapy.	Addition of Cohort 4
Section 3.4.2 Treatment	Added: Patients in Cohort 4 are expected to continue to take their ongoing nucleos(t)ide analogue therapy during this period.	Addition of Cohort 4

Protocol Section	Description of Change	Rationale
Section 3.4.3 Post-Study Drug Treatment Follow-Up	Added: Patients in Cohort 4 are expected to continue their nucleos(t)ide analogue therapy that was ongoing prior to Screening. Follow-up visits will be performed on Study Days 36, 57, 85, 113, and 211. The final study visit will be Study Day 211	Addition of Cohort 4
Added: Section 6.5 Nucleos(t)ide Analogue Plasma Concentration	Added: Section 6.5 Nucleos(t)ide Analogue Plasma Concentration Cohorts 1–3: measurement of tenofovir and entecavir plasma concentrations will be performed following the Study Drug administration period. The measurement for each patient will depend on the nucleos(t)ide analogue they are taking. The specimen collection days are Study Days 29, 36, 57, 85, 113, and 211. Cohort 4: measurement of tenofovir and entecavir plasma concentrations will be performed to explore whether treatment-emergent changes to nucleos(t)ide analogue plasma levels occur during ISIS 505358 administration. The measurement for each patient will depend on the nucleos(t)ide analogue they are taking. The specimen collection days are Study Days 1, 8, 15, 22, 29, 36, 57, 85, 113 and 211. Because there is a known food effect for tenofovir and entecavir, subjects will be asked to be consistent in their eating habits and when they take their doses before the clinic visits on these days. Sites should make reasonable efforts to ensure that the pre-dose blood draws on these visit days generally occur at the same time of day (± 2 hour) to lessen any day-to-day variability.	Addition of Cohort 4
Table 2 Study Drug Dosing Information	Was: Cohort 2 Now Is: Cohort 2**, Cohort 4**	Addition of Cohort 4
Table 2 Study Drug Dosing Information	Added footnote: *Patients in Cohort 3 were treated with total volume of 2.0 mL and total dose of 300 mg or placebo	Addition of Cohort 4
Section 8.2 Other Protocol-Required Drugs	Was: After the conclusion of Study Day 29 assessment, patients are required …. Now is: After the conclusion of Study Day 29 assessment**s**, patients **in Cohorts 1–3** are required ….	Addition of Cohort 4
Section 8.2 Other Protocol-Required Drugs	Added: Patients in Cohort 4 are expected to continue throughout this study their stable nucleos(t)ide analogue regimen that was ongoing prior to their enrollment in this study.	Addition of Cohort 4
Section 8.10.1 Concomitant Therapy	Was: Entecavir and tenofovir are considered concomitant medications in this study. Their usage should be in accordance with the recommendations contained in their respective region-specific prescribing information and should be recorded in each patient's CRF.	Addition of Cohort 4

Protocol Section	Description of Change	Rationale
Section 8.10.1 Concomitant Therapy *Continued*	Now Is: Entecavir and tenofovir are considered concomitant medications in this study. **In Cohort 4, patients should be on stable tenofovir or entecavir therapy prior to Screening and their nucleos(t)ide analogue** usage should be in accordance with the recommendations contained in their respective region-specific prescribing information. **For all cohorts, each patient's nucleos(t)ide analogue usage** should be recorded in **their** CRF.	Addition of Cohort 4
Section 10.5 Interim Analysis	Was: An unblinded interim analysis may be performed after all patients have progressed beyond their Study Day 29 assessments and the database has been locked. Details of the analysis and controlled access to the unblinded data will be specified in the SAP. Now Is: An unblinded interim analysis **for Cohort 1–3** may be performed after all **Cohort 1–3** patients have progressed beyond their Study Day 29 assessments and the database has been locked. **An unblinded interim analysis for Cohort 4 may be performed similarly after all Cohort 4 patients have progressed beyond their Study Day 29 assessments and locking of the data.** Details of the analysis and controlled access to the unblinded data will be specified in the SAP.	Addition of Cohort 4
Section 10.6.4 Pharmacokinetic Analysis	Was: Plasma levels of tenofovir (and entecavir if administered) will be measured on multiple days during the post-Study Drug Treatment Follow-Up period of the study (see Appendix C). Only the nucleos(t)ide analogue that is taken by each patient will be measured. Plasma concentrations of tenofovir (and entecavir) in patients previously treated with ISIS 505358 will be compared to prior treatment with placebo. Details for analysis will be defined in the SAP Now Is: **Cohorts 1–3:** Plasma levels of tenofovir (and entecavir if administered) will be measured on multiple days during the post-Study Drug Treatment Follow-Up period of the study (see Appendix C). Only the nucleos(t)ide analogue that is taken by each patient will be measured. Plasma concentrations of tenofovir (and entecavir) in patients previously treated with ISIS 505358 will be compared to prior treatment with placebo. Details for analysis will be defined in the SAP **Cohort 4: Plasma levels of the tenofovir or entecavir taken by each patient will be measured on multiple days during the study periods before, during, and after Study Drug administration (see Appendix C). Only the nucleos(t)ide analogue that is taken by each patient will be measured. Plasma levels on Day 1 (prior to ISIS 505358) will be compared for each patient to plasma levels taken concurrently with ISIS 505358, matched for time from dose and fed/fasted state, to monitor for potential interactions. Details for analysis will be defined in the SAP.**	Addition of Cohort 4

Protocol Section	Description of Change	Rationale
Section 13 References	Added: Bristol-Myers Squibb et al. 2018 Cihlar et al. 2007 Gilead Sciences 2008 Shemesh et al. 2017 Uwai et al. 2007 Xu et al 2013 Yu et al. 2016	Addition of Cohort 4
Added: Appendix A Schedule of Procedures – Cohort 4	Added: Schedule of Procedures - Cohort 4 Table differs from Cohort 1–3 table in that the Nucleos(t)ide Analogue Concentration row contains additional collections at Screen and Days 1, 8, 15 and 22.	Addition of Cohort 4
Added: Appendix C PK Sampling Schedule – Cohort 4	Added: PK Sampling Schedule – Cohort 4 Schedule differs from Cohort 1–3 in that the Nucleos(t)ide Analogue Sampling Schedule displays Screen, D1, D2, D4, D8, D15, D22 and D23.	Addition of Cohort 4
Synopsis – Pharmacokinetic Evaluation	Added: Cohort 4 only: tenofovir and entecavir additionally during Screen and Study Drug Treatment periods	Addition of Cohort 4

PROTOCOL SYNOPSIS

Protocol Title	A Phase 2, Double-Blinded, Randomized, Placebo-Controlled, Dose Escalation Study to Examine the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of ISIS 505358 in Treatment-Naïve Patients with Chronic Hepatitis B Virus Infection
Study Phase	2
Indication	Nucleos(t)ide analogue treatment-naïve patients with chronic HBV infection
Primary Objective	To examine the safety and tolerability of ISIS 505358 administration to treatment-naive patients with chronic hepatitis B virus infection
Secondary Objective(s)	• To examine the effects of ISIS 505358 administration on plasma HBV DNA concentration • To examine the effects of ISIS 505358 administration on serum HBV surface antigen (HBsAg) concentration • To examine the effect of ISIS 505358 administration on serum HBV e antigen (HBeAg) concentration in the patients that were HBeAg positive at Baseline • To assess plasma pharmacokinetics (PK) of ISIS 505358 in patients with chronic HBV infection • To describe the safety and tolerability of tenofovir (and entecavir if administered) therapy after conclusion of ISIS 505358 administration
Exploratory Objective(s)	• To describe the rate of seroconversion of patients to antibody to HBV surface antigen (anti-HBs antibody) positivity during treatment with ISIS 505358 and then during subsequent treatment with tenofovir (or entecavir) • To describe the rate of seroconversion of HBeAg positive patients to antibody to HBV e antigen (anti-HBe antibody) positivity during treatment with ISIS 505358 and subsequent treatment with tenofovir (or entecavir) • To explore the effect of prior ISIS 505358 exposure on the plasma concentrations of tenofovir (and entecavir) administered after the conclusion of ISIS 505358 administration • To explore antiviral activity, safety/tolerability, and PK during concurrent treatment with ISIS 505358 and nucleos(t)ide analogue (i.e., tenofovir or entecavir)
Study Design	This study examines the effects of ISIS 505358 or placebo (3:1 randomization) administered subcutaneously to treatment-naïve patients who are chronically infected with HBV and the effects of subsequent nucleos(t)ide analogue treatment on these patients. Three (3) ISIS 505358 dose levels will be evaluated through the treatment of 3 sequential cohorts of 8 patients each: • Cohort 1 (150 mg ISIS 505358 or placebo) • Cohort 2 (300 mg ISIS 505358 or placebo) • Cohort 3 (450 mg ISIS 505358 or placebo) (note: cohort was studied as 300 mg ISIS 505358 or placebo based on antiviral activity observed in Cohort 2 per Section 3.7) In these cohorts, 6 patients will be randomized to treatment with ISIS 505358 and 2 patients to treatment with placebo. Patients enrolled into the study may be either HBeAg positive (+) or negative (-).

PROTOCOL SYNOPSIS *Continued*

Study Design *Continued*	For each patient, Study Drug (ISIS 505358 or placebo) will be administered twice in Weeks 1 and 2 (on Days 1, 4, 8, and 11), and then once weekly during Weeks 3 and 4 (i.e., on Days 15 and 22). On Day 29, the effects of Study Drug treatment will be assessed. After these assessments, all patients will then commence chronic daily treatment with tenofovir (entecavir if judged more appropriate by the patient or Investigator) for 6 months. Continuation of nucleos(t)ide analogue therapy after discontinuation from this study will be the decision of the patient in consultation with their physician. One exploratory cohort (Cohort 4) will examine add-on treatment of patients already on stable nucleos(t)ide analogue (i.e., tenofovir or entecavir) regimen with 300 mg ISIS 505358 or placebo (3:1 randomization). Patients enrolled into the study may be either HBeAg positive (+) or negative (-). The target size of Cohort 4 is approximately 8 patients, but size may range from 4 to > 8 depending upon availability of participants. Each patient randomized into Cohort 4 is expected to continue their ongoing nucleos(t)ide analogue regimen and be treated with Study Drug by the above schedule for Cohorts 1-3. After Day 22, the patients are expected to continue their ongoing nucleos(t)ide analogue regimen and will be followed until Day 211. On Day 29, the effects of Study Drug treatment will be assessed. An independent Data and Safety Monitoring Committee (DSMB) will participate in the review of the study results and in the dose-escalation decisions (see below and Sections 3.6 and 3.7)
Number of Patients	Approximately 32 patients will be treated with Study Drug in this study. Additional patients may be treated due to difficulties of coordinating screening and enrollment from multiple sites and/or to replace patients that discontinue from cohorts before Day 29 for non-adverse event reasons but the actual number treated will not exceed 48.
Study Population	**Inclusion Criteria** 1. Have given written informed consent (signed and dated) and any authorizations required by local law and is able to comply with all study requirements 2. Age 18 to 70 years 3. Chronic HBV infection ≥ 6 months (e.g., positive for serum HBsAg ≥ 6 months) 4. Cohorts 1–3: Plasma HBV DNA ≥ 2 x 10^3 IU/mL Cohort 4: HBV DNA adequately suppressed (e.g., plasma or serum HBV DNA below the lower limit of quantitation) 5. Serum HBsAg ≥ 50 IU/mL 6. Satisfy the following a. Females: Non-pregnant and non-lactating; surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy), post-menopausal (defined as 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the postmenopausal range for the laboratory involved) or, if engaged in sexual relations of childbearing potential, subject is using an acceptable contraceptive method (refer to Section 6.4) from the time of signing the informed consent form until at least 13 weeks after the last dose of Study Drug (ISIS 505358 or placebo) b. Males: Surgically sterile or if engaged in sexual relations with a female of child-bearing potential, subject is utilizing an acceptable contraceptive method (refer to Section 6.4) during treatment with Study Drug and for at least 13 weeks after the last dose of Study Drug

PROTOCOL SYNOPSIS *Continued*

Study Population ***Continued***	**Inclusion Criteria** ***Continued*** 7. Cohort 4 only: Currently taking and have been taking tenofovir or entecavir without changes in drug, dose level and/or frequency of administration for ≥ 12 months and expect to continue taking without change through to the end of their participation in this study (approximately 8 months) **Exclusion Criteria** 1. Cohorts 1-3 only: Current or prior receipt of anti-HBV nucleos(t)ide analogue therapy. Patients who have failed prior interferon treatment, greater than 6 months prior to Screening, may be evaluated for possible participation in the study 2. History of liver cirrhosis and/or evidence of cirrhosis as determined by any 1 of the following: a. Liver biopsy (i.e., Metavir Score F4) within 2 years of Screening, or b. Fibroscan > 12 KPa, within 12 months of Screening, or c. AST-to-Platelet Index (APRI) > 2 and Fibrosure result > 0.7 within 12 months of Screening For patients without a test for cirrhosis in the above timeframes, Fibroscan, or APRI and Fibrosure, may be performed during the screening period to rule out cirrhosis 3. History of liver failure as evidenced by ascites, hepatic encephalopathy, and/or gastric or esophageal varices 4. History of liver disease other than Hepatitis B 5. Co-infection with hepatitis C virus (HCV), human immunodeficiency virus (HIV), or hepatitis D virus (HDV) 6. BMI > 35 kg/m^2 7. History of, or suspected presence of vasculitis 8. Received solid organ or bone marrow transplant 9. Currently taking, or took within 3 months of Screening, any immunosuppressing drugs (e.g., prednisone). If the patient received a short course (≤ 1-week), the situation may be discussed with the Ionis Medical Monitor, or designee 10. Diagnosed hepatocellular carcinoma or suspected hepatocellular carcinoma as evidenced by screening alpha-fetoprotein ≥ 200 ng/mL. If the screening alpha-fetoprotein is ≥ 50 ng/mL and < 200 ng/mL, the absence of liver mass must be documented by imaging within 6 months before randomization 11. Screening laboratory results as follows, or any other clinically-significant abnormalities in screening laboratory values that would render a patient unsuitable for inclusion a. ALT or AST > 5 x ULN b. Total bilirubin > 1.1 x ULN c. Serum albumin < 3.5 g/dL d. INR > 1.2 e. Platelet count < 140 k/mm^3 f. Hemoglobin < 12.0 g/dL for males and < 11.0 g/dL for females g. WBC < 3.0 k/mm^3 h. Serum creatinine > 1.1 x ULN i. Urine protein/creatinine (P/C) ratio ≥ 0.2 mg/mg. In the event of P/C ratio above this threshold, eligibility may be confirmed by a quantitative total urine protein measurement of < 150 mg/24-hr j. Positive test (including trace) for blood on urinalysis. In the event of a positive test, eligibility may be confirmed with urine microscopy showing < 5 red blood cells per high power field

PROTOCOL SYNOPSIS *Continued*

Study Population ***Continued***	**Exclusion Criteria** ***Continued*** 12. History of Gilbert's Syndrome or history of laboratory results consistent with Gilbert's Syndrome 13. Clinically-significant abnormalities aside from chronic HBV infection in medical history (e.g., previous acute coronary syndrome within 6 months of Screening, major surgery within 3 months of Screening, uncontrolled diabetes) or physical examination 14. History of bleeding diathesis or coagulopathy 15. History of extrahepatic disorders possibly related to HBV immune complexes (e.g., glomerulonephritis, polyarteritis nodosa) 16. Active infection other than HBV requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1 17. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix that has been successfully treated 18. Treatment with another investigational drug, biological agent or device within 4 weeks of Screening or 5 half-lives of Study Drug, whichever is longer 19. Treatment with any non-ISIS oligonucleotide (including siRNA) at any time 20. Prior treatment with an ISIS oligonucleotide within 9 months of Screening 21. History of excess alcohol consumption within 6 months of Screening as defined as weekly intake of > 14 drinks per week (> 2 drinks per day) for males or > 7 drinks per week (> 1 drink in a day) for females. (One [1] drink is equivalent to 12 g of alcohol, e.g., 12 ounces [360 mL] of beer, 5 ounces [150 mL] of wine or 1.5 ounces [45 mL] of 80 proof distilled spirits) 22. History of drug abuse or dependence, or recreational use of drugs: within 3 months of Screening for soft drugs (such as marijuana) and within 1-year of Screening for hard drugs (such as cocaine, phencyclidine [PCP]) 23. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator 24. Have any other conditions (medical, social or other), which in the opinion of the Investigator would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study 25. Patients with hereditary problems of galactose intolerance, Lapp lactose deficiency, or glucose-galactose malabsorption, unless the patient will be treated with the lactose-free formulation of entecavir during the study
Treatment Groups	Cohort 1: 150 mg ISIS 505358 or placebo Cohort 2: 300 mg ISIS 505358 or placebo Cohort 3: 450 mg ISIS 505358 or placebo (note: cohort was studied as 300 mg ISIS 505358 or placebo based on antiviral activity observed in Cohort 2 per Section 3.7) Cohort 4: 300 mg ISIS 505358 or placebo (in patients already on stable tenofovir or entecavir regimen)
Study Drug Dosage and Administration	ISIS 505358 (150 mg/mL, 1.0 mL) or placebo (1.0 mL), both supplied in 2-mL stoppered glass vials 150 mg dose: single 1.0 mL subcutaneous injection 300 mg dose: two 1.0 mL subcutaneous injections 450 mg dose: two 1.5 mL subcutaneous injections

PROTOCOL SYNOPSIS *Continued*

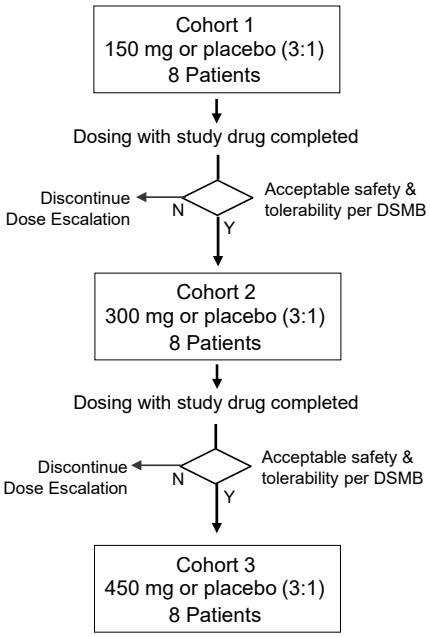
Dose Escalation	Following completion of the last Day 29 assessment in each cohort, the cumulative study results will be reviewed by a DSMB. After their review of Cohort 1, the DSMB may recommend initiation of Cohort 2 provided the committee found the safety and tolerability results to be sufficient and acceptable. Likewise, after their review of Cohort 2, they may recommend initiation of Cohort 3. After completion of Study Drug administration and the last Day 29 assessments for Cohort 3 and for Cohort 4, respectively, the DSMB will review the cumulative safety and antiviral data from the study, advise on any notable treatment-emergent issues, and make recommendations regarding the continuation of the study and periodicity of further DSMB reviews during the remainder of the study. (See Section 3.6 and 3.7 for additional information regarding the DSMB reviews and the dose-escalation criteria.). Study enrollment and dose-escalation will cease in the event that any of the following occur provided there is no alternative explanation other than ISIS 505358 administration as the cause of the event as determined by the DSMB: • Death of 1 patient • Occurrence of dose-limiting toxicity (DLT) (see Section 3.8) of the same character in 2 patients of a dose level (e.g., 2 cases of Division of AIDS (DAIDS) Grade 3 cardiac ischemia) Discontinuation of enrollment and dose-escalation will occur when 1 of the above criteria is met regardless of the number of patients that have been enrolled at the time of the event. For example, enrollment would be discontinued after the second patient to have DAIDS Grade 3 cardiac ischemia related to ISIS 505358 even if there are only 2 patients that had been enrolled into the cohort. If enrollment is halted due to the above, the DSMB will make recommendations concerning the further treatment of the remaining patients already receiving Study Drug in the study. The options include, but are not limited to, ceasing treatment with Study Drug entirely or continuing at reduced or current dose levels (Section 8.7). In the above deliberations on dose escalation and expansion of enrollment, the DSMB will also consider the results from the HBV serological measurements (e.g., HBsAg, HBeAg, and HBV DNA). For example, the DSMB may recommend against further dose escalations despite acceptable safety and tolerability because of obvious attainment of maximum antiviral effect.
Dose Limiting Toxicities	For consistency in the reporting of adverse events between personnel involved in the conduct of this study, adverse events will be described and graded according to the Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014 (see Appendix D) except for responses at subcutaneous injection sties. The occurrence of the following adverse events categorized as Possibly Related or Related to Study Drug administration will be considered as suspected DLTs: 1. Confirmed (by repeated specimen collection) laboratory result meeting 1 of the treatment stopping rules in Section 8.6 2. All DAIDS Grade ≥ 3 non-laboratory adverse events with the exception of flu-like symptoms consistent with the typical acute, transient, responses to 2'-MOE ASO injection (e.g., fever and/or chills beginning within 6 hours of dosing that resolve spontaneously within 36 hours and/or fatigue lasting ≤ 7 days). See the Guidance to the Investigator in the Investigator's Brochure 3. All DAIDS Grade 4 confirmed (by repeated specimen collection) laboratory adverse events other than the test results defined in Section 8.6 Adverse events and laboratory abnormalities will be considered to be DLTs if there is at least a possible association to ISIS 505358 or there is no definite alternative explanation for the safety finding. Since some of the patients will be placebo-treated and adverse events in placebo-treated patients cannot be DLTs, the events identified by Investigators will be considered suspected DLT. The DSMB (unblinded to treatment assignment) will be the arbiter as to which suspected events are to be considered DLTs. However, the DSMB will not recommend continuation or resumption of Study Drug dosing for any patient so as to not potentially make an Investigator aware of a patient's treatment assignment. The DSMB will not disclose to parties outside of the DSMB which of the suspected DLTs are actual DLTs.

PROTOCOL SYNOPSIS *Continued*

Rationale for Dose and Schedule Selection	The dose levels and treatment schedule replicate those tested previously in healthy volunteers in the multiple-dose portion of ISIS 505358-CS1
Study Visit Schedule and Procedures	Treatment with Study Drug on Study Days 1, 4, 8, 11, 15, and 22 Additional on-treatment visit on Study Day 2 Post-Study Drug-treatment follow-up visits on Study Days 23, 29, 36, 57, 85, 113, and 211
Safety and Tolerability Evaluations	Adverse events, clinical chemistry, hematology, and urinalysis, concomitant medication usage, physical examination, ECG, vital signs
Efficacy Evaluations	Quantitative plasma HBV DNA Quantitative serum HBsAg Quantitative serum HBeAg, where applicable Qualitative anti-HBs and anti-HBe antibodies
Pharmacokinetic Evaluations	ISIS 505358 concentrations in plasma Tenofovir and entecavir plasma levels during the post-Study Drug treatment follow-up period Cohort 4 only: tenofovir and entecavir additionally during Screen and Study Drug Treatment periods
Statistical Considerations	There is no statistical rationale for the selected sample size of 8 subjects per cohort. The sample size was based on prior experience with other members of the drug class to ensure an adequate initial assessment of the safety, tolerability and pharmacokinetics of ISIS 505358 while minimizing the number of subjects unnecessarily exposed to the drug. An unblinded interim analysis may be performed of Cohort 1–3 after all patients within these cohorts have progressed beyond their Study Day 29 assessments. An unblinded interim analysis similarly may be performed of Cohort 4 after all Cohort 4 patients have progressed beyond their Study 29 assessments.
Sponsor	Ionis Pharmaceuticals, Inc.

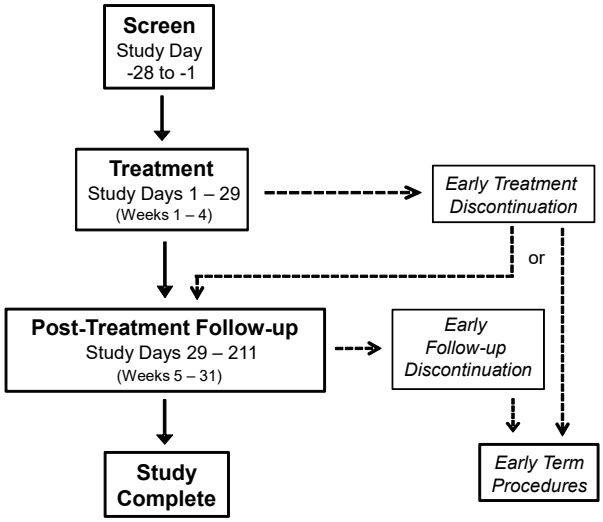
STUDY DESIGN AND TREATMENT SCHEMA

Dose Escalation Flow

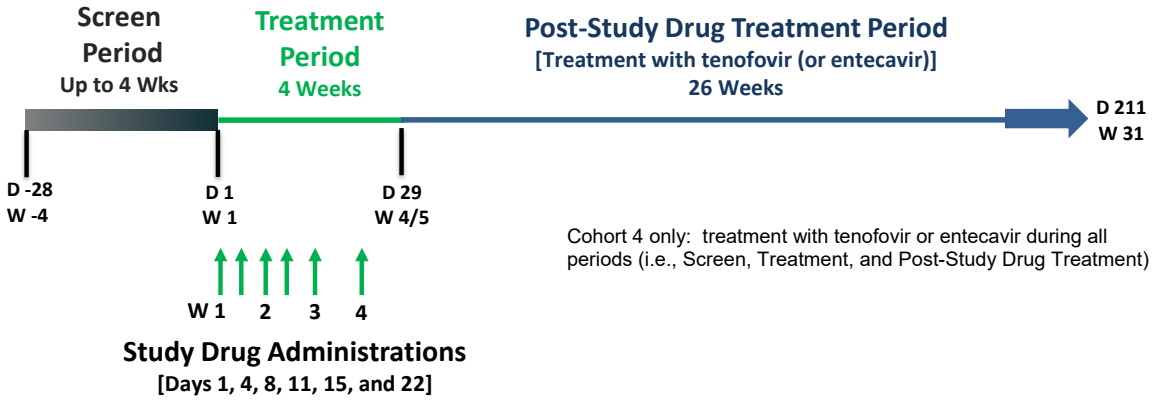


Note: Cohort 3 was 300 mg or placebo (3:1) based on antiviral activity observed in Cohort 2 per Section 3.7

Patient Participation Phases



Patient Participation Flow



Cohort 4 only: treatment with tenofovir or entecavir during all periods (i.e., Screen, Treatment, and Post-Study Drug Treatment)

STUDY GLOSSARY

Abbreviation/Acronym	Definition
2′-MOE	2′-*O*-(2-methoxyethyl)-modified ribonucleotides
AE	Adverse event
Anti-HBe antibody	Antibody to HBV e antigen
Anti-HBs antibody	Antibody to HBV surface antigen
APRI	AST-to-Platelet Index
ASO	Antisense oligonucleotide
cccDNA	Covalently closed circular DNA
C_{max}	Maximum observed drug concentration
CHB	Chronic hepatitis B
CRP	C-reactive protein
DAIDS	Division of AIDS
DLT	Dose-limiting toxicity
DSMB	Data and Safety Monitoring Board
eCRF	Electronic case report form
FAS	Full analysis set
GCP	Good Clinical Practice
HBV	Hepatitis B virus
HBeAg	HBV e antigen
HBsAg	HBV surface antigen
HCC	Hepatocellular cancer
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HED	Human equivalent dose
HIV	Human Immunodeficiency virus
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ITT	intent-to-treat
IXRS	Interactive voice/internet response system
mRNA	Messenger Ribonucleic Acid
$MRT_{0\text{-}24hr}$	Mean plasma residence time
n/a	Not applicable
NCS	Not clinically-significant

PBMC	Peripheral blood mononuclear cell
PK	Pharmacokinetic
RNase H	Ribonuclease H
SAE	Serious adverse event
SAP	Statistical analysis plan
siRNA	Small interfering ribonucleic acid
Study Day 1	Defined as the first day Study Drug product is administered to the patient
Study Drug	ISIS 505358 or Placebo
SUSAR	Suspected unexpected serious adverse reaction
Tenofovir	Tenofovir disoproxil fumarate
T_{max}	Time taken to reach C_{max}
ULN	Upper limit of the laboratory reference range
WHO	World Health Organization

1. OBJECTIVES

1.1 Primary Objective

To examine the safety and tolerability of ISIS 505358 administration to treatment-naive patients with chronic hepatitis B virus infection.

1.2 Secondary Objectives

- To examine the effects of ISIS 505358 administration on plasma HBV DNA concentration
- To examine the effects of ISIS 505358 administration on serum HBV surface antigen (HBsAg) concentration
- To examine the effect of ISIS 505358 administration on serum HBV e antigen (HBeAg) concentration in the patients that were HBeAg positive at Baseline
- To assess plasma pharmacokinetics (PK) of ISIS 505358 in patients with chronic HBV infection
- To describe the safety and tolerability of tenofovir (and entecavir if administered) therapy following conclusion of ISIS 505358 administration

1.3 Exploratory Objectives

- To describe the rate of seroconversion of patients to antibody to HBV surface antigen (anti-HBs antibody) positivity during treatment with ISIS 505358 and then during subsequent treatment with tenofovir (or entecavir)
- To describe the seroconversion of HBeAg positive patients to antibody to HBV e antigen (anti-HBe antibody) positivity during treatment with ISIS 505358 and then during subsequent treatment with tenofovir (or entecavir)
- To explore the effect of prior ISIS 505358 exposure on the plasma concentrations of tenofovir (and entecavir) administered after the conclusion of ISIS 505358 administration
- To explore antiviral activity, safety/tolerability, and PK during concurrent treatment with ISIS 505358 and nucleos(t)ide analogue (i.e., tenofovir or entecavir)

2. BACKGROUND AND RATIONALE

2.1 Overview of Disease

Hepatitis B virus (HBV) infection, especially chronic infection, is a significant worldwide medical problem. More than 2 billion of the world's population has been infected. Of these, the infection became chronic in an estimated 360 million (WHO 2009). Every year, 1 million people die from HBV-related disease complications. For those with chronic infection, up to 20% progress to liver cirrhosis (WHO 2002). Individuals with chronic infection also have a heightened risk for hepatocellular cancer (HCC). It is estimated the lifetime risk for cirrhosis and/or HCC is 40-50% for men and about 15% for women with chronic infection (WHO 2002).

HBV infection is highly endemic in Southeast Asia, the Pacific Basin (except Japan, Australia and New Zealand), Sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the Central Asian Republics, and some of Eastern Europe. In these areas, 70-90% of the population can become HBV infected before the age of 40 years and 8-20% are chronically infected because infection frequently occurs at birth or early in life (WHO 2002). The probability of developing a chronic infection from an acute infection is inversely linked to age at infection (90% chronic infection development when infected at birth, 25-50% when infected at ages 1-5 years, and 1-5% when infected as older children and adults) (WHO 2002).

Chronic hepatitis B (CHB) is a spectrum of disease characterized by the presence of detectable hepatitis B surface antigen (HBsAg) in the blood or serum for longer than 6 months. CHB may be inactive and cause no significant health problems or may progress to liver fibrosis, cirrhosis and HCC. The progression of liver disease is associated with serum HBV DNA levels, serum ALT levels, and the severity of liver disease (EASL 2012). CHB can be broadly divided into 2 major forms—namely, hepatitis B virus e antigen (HBeAg) positive and HBeAg negative. HBeAg negative CHB is also referred to as anti-HBe-positive and precore mutant hepatitis. Patients with HBeAg negative CHB have a naturally occurring mutant form of HBV that does not produce HBeAg because of a mutation in the precore or core promoter region of the HBV genome. Without treatment, both forms have a high risk of progression to advanced hepatic fibrosis, cirrhosis and subsequent complications if they have indicators of active liver disease (Lok and McMahon 2009; EASL 2012).

2.2 Goals of Therapy for CHB

The goal of therapy for CHB is to improve quality of life and survival by preventing progression of the disease to cirrhosis, decompensated liver disease, end-stage liver disease, HCC and death. This goal can be achieved if HBV replication is suppressed in a sustained manner thereby decreasing the histological activity of CHB and reducing the risk of cirrhosis and HCC (Liaw et al. 2004; Feld et al. 2009). Thus, effective therapy for CHB must ensure virological suppression that induces normalization of serum ALT levels, resolution of active necroinflammatory injury on liver biopsy, and prevention of complications.

In both HBeAg positive and HBeAg negative CHB, the ultimate treatment endpoint is loss of detectable serum HBsAg (Lok and McMahon 2009; EASL 2012). HBsAg loss is preceded by a robust immunological response to HBV infection that results in sustained suppression of serum HBV DNA and disease resolution. Natural history studies of CHB conducted over many decades in large patient cohorts have shown that HBsAg loss was durable in most cases and associated with improved survival and reduced risk of HCC (Kim et al. 2014). Patients who achieved HBsAg seroconversion had the absolute lowest risk with a cumulative risk for HCC < 1% over 9 years (Chen et al. 2006), including patients with compensated cirrhosis who had survival of nearly 100% after 14 years (Fattovich et al. 1998).

In patients with HBeAg positive CHB, clearance of HBeAg is also associated with improved outcomes, provided that HBeAg loss is sustained after treatment is discontinued and the patient does not subsequently develop HBeAg negative CHB with recurrent disease despite loss of HBeAg (Feld et al. 2009). When HBeAg loss occurs, it is associated with a decrease in HBV DNA to low levels, normalization of serum ALT, improved histology, and an increased

potential for HBsAg seroclearance (Liaw 2009). Accordingly, HBeAg loss has been a major endpoint of antiviral therapy in CHB studies.

First-line therapy for CHB is treatment with a nucleoside or nucleotide (nucleos(t)ide) analogue. At least 6 nucleos(t)ide analogues are available for the treatment of CHB patients (Lok and McMahon 2009; EASL 2012). While these antiviral agents are effective in suppressing HBV replication in both HBeAg positive and HBeAg negative CHB, patients frequently relapse after treatment is discontinued, particularly if HBsAg loss was not achieved. Treatment with a pegylated interferon (PEG-interferon), of which 2 are available, is also approved for CHB (Lok and McMahon 2009; EASL 2012) for a defined treatment duration (usually up to 48 weeks). Because of their frequent and sometimes severe side-effects and high cost vs. a small gain in treatment response, PEG-interferons are less frequently used than the nucleos(t)ide analogues. Unfortunately, with both the nucleos(t)ides and PEG-interferon, HBsAg loss is rarely achieved. Rates of HBsAg loss following 12 months of treatment with either a nucleos(t)ide or PEG-interferon generally ranged from 0-3% in most studies (Lok and McMahon 2009; EASL 2012). HBeAg loss occurs more frequently following treatment with either the nucleos(t)ides or PEG-interferon, approximately 15-30% after 1-2 years of therapy, but off-treatment durability is variable and questions remain as to whether virologic responses can be maintained over an extended follow-up period. Thus, the majority of patients on treatment fail to achieve a sustained off-treatment virological response and require extended and often life-long therapy to suppress HBV DNA.

2.3 Therapeutic Rationale

Among the features that distinguish patients that have resolved their HBV infection from patients with chronic HBV infections is the latter remain positive for circulating HBsAg and negative for free anti-HBs antibody (Robinson 1995). The HBsAg in the serum of infected patients is found in 3 forms: (1) 42 nm, spherical particles (i.e., infectious virus also known as Dane particles); (2) 15-25 nm spherical particles; and (3) 20 x 20-200 nm filaments. The latter 2 represent incomplete virus particles. The amount of HBsAg in incomplete virus particles is more than 100 times of that in infectious particles.

It has been proposed that the continued production of viral antigens by infected hepatocytes interferes with immune clearance of both the infected cells and circulating virus particles (Vanlandschoot and Leroux-Roels 2003). *In vitro* studies with human peripheral blood mononuclear cells (PBMC) have shown HBsAg impairs the functioning of dendritic cells and inhibits the activation of monocytes (Vanlandschoot et al. 2002; Op den Brouw et al. 2009). Most chronically infected patients produce antibody to HBsAg, but these can only be detected as immune complexes due to the vast excess of circulating antigen (Maruyama et al. 1993). HBeAg is also thought to have a role in immune response evasion through down-regulation of the innate immune system (Milich et al. 1998; Wu et al. 2009; Walsh and Locarnini 2012). As noted above, since loss of HBsAg expression is rarely achieved while loss of HBeAg expression occurs in a higher proportion of the patient population, HBsAg appears to be the main antagonist of immune clearance.

Should the viral antigens be instrumental in preventing clearance of persistent infection by the immune system, reducing the expression of these antigens, especially HBsAg, would be expected to permit reconstitution of an immune response against HBV (Boni et al. 2007;

Boni et al. 2012; Bertoletti and Ferrari 2013). Support for this hypothesis is the observation that spontaneous seroconversion and resolution of chronic infection is most likely in patients that have lower serum HBsAg levels (Chen et al. 2012; Höner zu Siederdissen and Cornberg 2014). Similarly, during treatment with nucleos(t)ide analogues, patients with low HBsAg levels are more likely to lose HBsAg and seroconvert to anti-HBs antibody positive than patients with high HBsAg levels (Wursthorn et al. 2010; Jaroszewicz et al. 2011; Boni et al. 2012; Höner zu Siederdissen and Cornberg 2014).

In CHB patients treated with PEG-interferon, those that achieved sustained virologic responses were observed to have substantially more serum HBsAg reduction than non-responders. For HBeAg-positive CHB patients, a mean HBsAg reduction of 3.3 log at the end of treatment was reported for responders compared to 0.69 log for non-responders (Sonneveld et al. 2010). For HBeAg-negative patients, mean HBsAg reductions of 1.1–2.1 log at the end of treatment have been described for responders and < 0.3 log for non-responders (Brunetto et al. 2009; Moucari et al. 2009; Rijckborst et al. 2010). These observations are consistent with the hypothesis that high HBsAg levels inhibit viral clearance.

ISIS 505358 is antisense inhibitor that is designed to inhibit the synthesis of viral proteins (e.g., HBsAg, HBeAg) without having a direct effect on covalently closed circular DNA (cccDNA) or integrated HBV DNA. ISIS 505358 also is not an immune modulator. Therefore, treatment of CHB subjects with ISIS 505358 will permit examination of whether reduction of viral proteins allows resumption of a host immune response against HBV virus and infected cells and can induce HBsAg and/or HBeAg seroclearance leading to sustained suppression of HBV replication after cessation of all treatments for CHB.

2.4 ISIS 505358

2.4.1 Mechanism of Action

ISIS 505358 is a 2′-*O*-(2-methoxyethyl)-modified chimeric phosphorothioate antisense oligonucleotide (2′-MOE chimeric ASO) drug targeted to HBV RNAs. It is complementary to a region present in all 4 of the messenger ribonucleic acid (mRNA) and the pre-genomic RNA produced by the virus. The ISIS 505358 binding site is downstream of the pre-core, core and surface antigen (pre-S1, pre-S2, and S) coding sequences and within the coding sequences for polymerase and X protein. The HBV direct repeat 2 sequence (DR2) is contained within the ISIS 505358 binding site. The binding site is highly conserved across all HBV genotypes (i.e., complete complementarity to 98% of approximately 2900 HBV genomic sequences compiled from publicly available sequences). ISIS 505358 binds to RNA by Watson and Crick base pairing. The hybridization (binding) of ISIS 505358 to the cognate RNA results in the RNase H-mediated degradation of the RNA and thereby prevents the production of the proteins translated from the RNA (Figure 1). Maximal antisense-mediated reduction of target mRNA levels is typically greater than 90% of control levels in sensitive tissues (Crooke and Bennett 1996; Zhang et al. 2010). Furthermore, reduction in target mRNA levels using this approach correlates directly with a subsequent reduction in target protein levels. ISIS 505358 was selected for clinical development based upon its superior potency in inhibiting the HBV surface antigen (HBsAg) levels of transgenic mice that produce HBV. It also reduces HBeAg, total HBV RNA, and HBV DNA in these mice.

2.4.2 Chemistry

Chemically, ISIS 505358 is a synthetic oligomer of 20 nucleotides (i.e., a 20-mer) that are connected sequentially by phosphorothioate linkages. The nucleotide sequence of ISIS 505358 is complementary to a 20-nucleotide sequence present in all 4 mRNAs and the pre-genomic RNAs of HBV. Structurally, the oligonucleotide has 3 regions (Figure 1). Two (2) of them, the 5 nucleotides at the 5′-end (N_1–N_5) and the 5 nucleotides at the 3′-end (N_{16}–N_{20}), are composed of MOE-modified ribonucleotides. These MOE-modified nucleotides confer (1) increased affinity to the target mRNA (Altmann et al. 1996; McKay et al. 1999), (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue) (Geary et al. 2003), and (3) amelioration of some of the high dose toxicities resulting in an improved safety profile compared to first generation antisense drugs containing phosphorothioate-modified oligodeoxynucleotides (DNA) (Henry et al. 2000). The third region, the central portion of the oligonucleotide (N_6–N_{15}), is composed of 10 oligodeoxynucleotides. ASOs with this chimeric design are sometimes referred to as MOE-gapmers. ISIS 505358 employs this chimeric structure to enable use of the RNase H1-mechanism for antisense activity. This is because while the 2′-MOE modification confers increased stability and affinity, it does not support RNase H1 catalysis of RNA hybridized to 2′-MOE-modified nucleotides (McKay et al. 1999). This is caused by conformational changes induced in the heteroduplex by 2′-alkoxy:RNA hybrids that are not recognized by RNase H enzymes (Inoue et al. 1987; Monia et al. 1993). By limiting the 2′-MOE modification to nucleotides flanking the phosphorothioate oligodeoxynucleotide core, the beneficial attributes of the 2′-MOE chemistry are preserved while also retaining RNase H recognition.

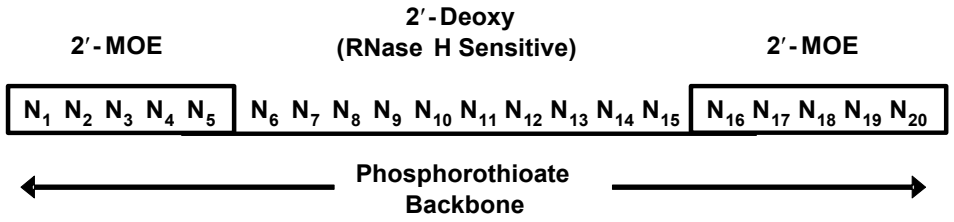


Figure 1 Structure of 2′-MOE Chimeric Phosphorothioate Oligonucleotides. Each N is either A, G, T, or 5-methyl-C

The 2′-MOE chimeric ASO chemical class is the most advanced class of drugs designed to target mRNA. Clinical studies have been performed for 34 of these drugs. In aggregate, more than 130 clinical studies have been performed and approximately 5,600 subjects have been dosed with 2′-MOE chimeric ASO s by intravenous and/or subcutaneous administration. These studies include treatments with doses as high as 1200 mg. With several class members, there is a substantial 3-month duration treatment experience (approximately 1,700 patients in aggregate) and for 1 drug, patients have been treated for more than 4 years. One (1) class member, Kynamro®, received marketing approval from the US Food and Drug Administration in January 2013 for the treatment of patients with homozygous familial hypercholesterolemia.

Other chemical classes of RNA-targeting oligonucleotide drugs are also in clinical development including, but not limited to, locked nucleic acid (LNA) chimeras, fully 2′-*O*-methyl-modified phosphorothioates, morpholinos, and small interfering ribonucleic acids (siRNAs) (Bennett and Swayze 2010). While the clinical trial data for these other chemical classes is more limited than that for 2′-MOE chimeric ASOs, it is clear that each chemical class has a unique profile with regard to efficacy, potency, and adverse events (AEs). There have been reports of renal and hepatic effects for some of these other chemistries that have not been observed with 2′-MOE chimeric ASOs (Goemans et al. 2011; van Poelgeest et al. 2013; Kakiuchi-Kiyota et al. 2014). Therefore, the results from these other chemical classes should not be extrapolated to 2′-MOE chimeric ASOs or vice versa.

2.4.3 Preclinical Experience

Detailed information concerning the preclinical studies conducted with ISIS 505358 can be found in the Investigator's Brochure.

The nonclinical toxicology of ISIS 505358 was examined in 13-week and 6-month repeated-dose general toxicology studies in mice, 13-week and 9-month studies in monkey, 2 *in-vitro* genotoxicity studies (Ames bacterial reverse mutation assay and chromosomal aberration assay in CHO cells), 2 safety pharmacology studies (a combined cardiovascular and respiratory function study in monkey and an Irwin's CNS study in mice) and in segment I and II reproductive toxicology studies. No significant toxicities were observed in the 13-week toxicology studies to preclude development of the doses and durations being proposed for clinical study. Most of the observed effects were minimal-to-mild in severity, occurred at doses above the anticipated human exposure, and are typical for 2′-MOE chimeric ASOs administered at high (30-40 mg/kg) subcutaneous doses (e.g., in monkeys: transient aPTT prolongation, transient increases of complement split products such as Bb, uptake and accumulation of oligonucleotide in tissues as evidenced by the presence of basophilic granules within liver Kupffer cells and kidney proximal tubule epithelial cells, and mild (1.3-1.8 fold) increases in spleen weight).

In the 9-month toxicology study, 6 monkeys were terminated early during the treatment portion and 1 animal was discontinued prematurely during the 3-month recovery phase of the study. These events were either related to monkey-specific responses to high dose 2′-MOE chimeric ASO administration (i.e., related to chronic, repeated, low-level complement pathway activation) or to spontaneous, naturally-occurring events (e.g., gastric ulcer, recurrent rectal prolapse, spontaneous glomerulosclerosis, and pulmonary embolism). With other 2′-MOE chimeric MOE ASOs where both monkey and human treatment experience exists, the complement pathway responses observed in monkeys were not observed in humans (Shen et al. 2014).

The genotoxicity and reproductive toxicology studies were negative, and no adverse effects were observed in the safety pharmacology studies.

The plasma pharmacokinetics (toxicokinetics) and tissue distribution of ISIS 505358 were also investigated in the above general toxicology studies. The results were consistent with those observed for other 2′-MOE chimeric ASOs (Geary et al. 2008). After subcutaneous injection in monkeys, ISIS 505358 rapidly distributed from the injection site to the blood stream and then to tissues within 48 hours. The mean residence time for ISIS 505358 in plasma during this

distribution phase is 5-8 hours. While 2′-MOE chimeric ASOs distribute to almost all tissues (CNS being a notable exception), liver and kidney are the main organs for ASO distribution. The half-life of 2′-MOE chimeric ASO drug class members in tissue is typically 2-4 weeks with nucleolytic degradation as the metabolic mechanism. For monkeys treated with ISIS 505358, the half-life of the drug in kidney cortex was 13.3-15.7 days and in liver was 8.7-19.9 days. Consistent with slow clearance from tissues, the plasma terminal elimination half-life of ISIS 505358 in monkeys was 10.6-43 days.

2.4.4 Clinical Experience

2.4.4.1 ISIS 505358-CS1

Clinical results for ISIS 505358 are available from one completed study, ISIS 505358-CS1, a Phase 1, blinded, placebo-controlled, dose escalation study designed to assess the safety, tolerability and pharmacokinetics of single and multiple doses of ISIS 505358 administered to healthy subjects. A detailed summary of the results from ISIS 505358-CS1 are provided in the Investigator's Brochure.

Briefly, the trial evaluated single-dose treatments at 4 ISIS 505358 dose levels (75, 150, 300, and 450 mg) and multiple-dose treatments at 3 dose levels (150, 300, and 450 mg). For each single and multiple-dose treatment dose level, 4 subjects were randomized, 3 to ISIS 505358 and 1 to placebo. All treatments were administered by subcutaneous injection. For the multiple-dose treatments, there was a 2-week loading regimen (2 doses/wk) followed by a 2-week maintenance schedule (1 dose/wk) such that subjects were to be dosed on Days 1, 4, 8, 11, 15, and 22. All subjects successfully received all Study Drug doses and completed all follow-up assessments. A total of 28 healthy subjects received treatment – 21 with ISIS 505358. The subjects were mostly male (22 vs. 6) and white (24 vs. 4) with a median age of 53 years (range: 27–62 years).

The tolerability for treatment with ISIS 505358 was satisfactory across all dose levels. A total of 198 adverse events were reported. Aside from 2 that were of moderate intensity (MedDRA preferred terms of (i) Productive Cough and (ii) Face Injury), all were of mild intensity. The bulk of the adverse events (72% for the multiple-dose subjects) involved subcutaneous injection sites. Approximately one-half of these injection site events were symptoms of short duration (minutes to hours) including brief muscle twitches, transient discomfort/pain, and short-lived erythema. Local cutaneous reactions at the injection site (LCRIS, defined as those events presenting as pain, tenderness, erythema, pruritus or swelling occurring on the day of injection and persisting for at least 2 days) were limited to the subjects treated with ISIS 505358 at the 300 mg and 450 mg doses. The median duration of an LCRIS was 4 days and subjects varied in the number of doses that were followed by an LCRIS (median of 2 out of 6 doses).

The second most common adverse event was C-Reactive Protein Increased (5 of 12 ISIS 505358 single-dose subjects and 6 of 9 ISIS 505358 multiple-dose subjects). From the laboratory results, elevated C-reactive protein (CRP) levels were observed associated with the initial ISIS 505358 doses of 300 mg and 450 mg in ISIS 505358-CS1. No systemic symptoms consistent with an inflammatory response (e.g., fever, chills, body aches) were reported to coincide with the elevations. In the multiple-dose subjects, the prevalence of CRP increases after the 6^{th} dose of 300 mg and 450 mg ISIS 505358 was much reduced. Similar transient increases in CRP have

been observed following the dosing of some other 2′-MOE chimeric ASOs. The significance of asymptomatic transient CRP elevations following early ISIS 505358 doses is unknown.

There were no serious adverse events (SAEs) or adverse events leading to treatment discontinuation. In the laboratory results, 1 subject in the 300 mg ISIS 505358 dose group had 2 asymptomatic ALT elevations to 106 and 108 U/L (2.6 x upper limit of the laboratory reference range [ULN]) of unknown origin. Despite this patient, the overall liver chemistry results in ISIS 505358-CS1 do not raise a hepatic effect concern. There were no changes in laboratory results to suggest any adverse effects of ISIS 505358 on renal function, hematological cell counts, hemoglobin, or the complement pathway. The known transient non-significant effect of 2′-MOE chimeric ASOs on aPTT was observed. There were no treatment related changes in vital signs or ECG results.

Clinical pharmacokinetics analyses produced results consistent with the profiles of other 2′-MOE chimeric ASOs. The plasma C_{max} of ISIS 505358 was dose-related and was approximately 11.0-15.8 µg/mL following injection of 450 mg. T_{max} occurred 3-8 hour after injection and mean plasma residence time ($MRT_{0\text{-}24hr}$) was approximately 8 hours due to the rapid distribution of drug from blood to tissue. In the multiple-dose subjects, the PK characteristics following the first dose and the 6th dose were highly similar which reflects the known lack of significant drug accumulation in the plasma by 2′-MOE chimeric ASOs.

Overall, continued clinical development of ISIS 505358 is supported by the results from ISIS 505358-CS1.

2.4.4.2 ISIS 505358-CS3

Interim blinded results from Cohort 1 (150 mg or placebo, 6:2 ratio), Cohort 2 (300 mg or placebo, 6:2 ratio), and Cohort 3 (300 mg or placebo, 6 patients, ratio unknown) are available. There has been one SAE in the study—Alanine aminotransferase increased. A patient in Cohort 2 experienced an ALT elevation that peaked at 781 U/L (23.7 x ULN, ULN = 33) on Study Day 38 (15 days after last protocol-specified dose of Study Drug). ALT levels declined to < 5 x ULN within 11 days and became consistently < 3 x ULN by Day 72. The patient had a lesser concurrent AST elevation. Her total bilirubin levels were stable throughout the study. This ALT elevation temporally coincided with HBsAg and HBV DNA reduction to less than the lower limit of quantitation and therefore may have been secondary to the clearance of infected hepatocytes.

Three (3) additional patients—2 in Cohort 1 and 1 in Cohort 2—experienced treatment-emergent elevations of ALT to > 3 x ULN. None had changes in total bilirubin levels. Like the aforementioned patient with SAE, the ALT elevations of these 3 patients were temporally related to reductions in HBsAg. However, there were other patients in ISIS 505358-CS3 with HBsAg reductions but without ALT elevation. Therefore, the possibility that the ALT elevations are a direct adverse effect (wholly or partially) of ISIS 505358 on the liver cannot be entirely excluded.

Some activity against HBV was observed in the treatment-naïve patients treated in ISIS 505358-CS3. Three (3) of the 8 patients in Cohort 1 had HBsAg reductions of 0.39, 1.19, and 1.24 $\log_{10}$ IU/mL. Five (5) of the 8 patients in Cohort 2 had HBsAg reductions of 0.462,

1.19, 1.96, 2.57, and 3.80 log_{10} IU/mL. A 6th patient in Cohort 2 had a 0.81 log_{10} IU/mL reduction in HBV DNA although the patient's HBsAg reduction was minimal (0.12 log_{10} IU/mL). Dose-related reductions in HBV DNA were also observed in the study. This antiviral activity was observed in both HBeAg positive and negative patients. In Cohort 3, one (1) of the patients was observed to have developed a 3.33 log_{10} IU/ml reduction in HBsAg and 3.53 log_{10} IU/ml reduction in HBV DNA on Day 29. Two (2) other patients each had 0.43 log_{10} IU/mL reductions of HBsAg and a 4th patient had a reduction of HBV DNA of 0.77 log_{10} IU/mL despite minimal HBsAg reduction.

Overall, continued clinical development of ISIS 505358 is supported by the results from ISIS 505358-CS1 and the emerging results from ISIS 505358-CS3.

2.5 ISIS 505358 Dose and Schedule of Administration Rationale

2.5.1 Target Dose Levels

The dose of ISIS 505358 that produced 90% reduction of HBsAg levels (i.e., the ED_{90}) in the preclinical HBV transgenic mouse model was 20 mg/kg. For 2′-MOE chimeric ASOs, the human equivalent dose (HED) is most accurately estimated from the mouse dose based on dose-adjusted plasma AUCs (Yu et al. 2015). By this scaling, the HED is 5-fold lower than the mouse dose and therefore the human ED_{90} for ISIS 505358 is estimated to be 4.0 mg/kg or 280 mg for a 70 kg human.

To investigate the role of HBsAg in maintaining the persistence of HBV infection, it is desirable to reduce HBsAg levels as much as possible. Therefore, doses of 300 and 450 mg were included for study in ISIS 505358-CS3 because these 2 dose levels (1) are > 280 mg, (2) were repeatedly administered for 4 weeks to healthy volunteers with acceptable safety and tolerability in ISIS 505358-CS1, (3) are simple multiples of the 150 mg/mL ISIS 505358 drug product, (4) were lower than 840 mg for 70 kg human, the human equivalent of the NOAEL level of 12 mg/kg in monkeys, and (5) are in the range of doses that other 2′-MOE chimeric ASOs have been successfully administered by subcutaneous injection for 13-week durations and found to be generally safe and well-tolerated. Doses higher than 450 mg ISIS 505358 were not selected for study due to the number of injections necessary for administering more than 3 mL of drug product per dose.

2.5.2 Treatment Duration

The 4-week treatment duration of ISIS 505358-CS3 was selected to replicate the 4-week duration of the already completed healthy volunteer study. This duration is less than one-third of the duration of the 13-week IND-enabling preclinical general toxicology studies (see Section 2.4.3 and the Investigator's Brochure, Revision 2 dated 17 February 2015).

2.5.3 Loading/Maintenance Dosing

For drugs such as ISIS 505358 that have tissue concentration half-lives of 2-3 weeks, regular repeated dosing (e.g., once weekly) for 10-15 weeks (i.e., 5 x half-life) is required to achieve steady state drug concentrations in tissues. Such dosing schedules, therefore, would potentially result in prolonged exposure of the resident HBV to sub-therapeutic concentrations of ISIS 505358 which, in turn, could promote proliferation of any potential resistance variants. The

extended time to steady-state also hinders reaching conclusions about the safety and pharmacological effects of ISIS 505358 in short duration studies.

For the pharmacological activity of ISIS 505358 on HBsAg production, the amount of drug in the liver is the important determinant as opposed to the amount of drug in plasma. From PK measurements in monkey, the half-life of ISIS 505358 in the liver was estimated as approximately 2 weeks (Section 2.4.3 and the Investigator's Brochure, Revision 2 dated 17 February 2015). The half-life in human liver is expected to be similar to that measured in monkeys.

In order to accelerate reaching steady-state concentrations in tissues and thereby both reduce the duration of exposure to sub-therapeutic concentrations of ISIS 505358 and permit fuller assessment of the ISIS 505358 effects on safety during this 4-week treatment study, loading doses are included in the dosing schedule in ISIS 505358-CS3. Extra doses are added to Weeks 1 and 2 so that Study Drug will be administered twice in Weeks 1 and 2 (on Days 1, 4, 8, and 11) before switching to once weekly during Weeks 3 and 4. Inclusion of the loading schedule is predicted to result in achievement of hepatic steady-state concentrations following the Week 3 dose (i.e., before the last dose in schedule). The ISIS 505358-CS3 dosing regimen is supported by both the toxicology studies which employed loading regimens and the results from the completed healthy volunteer study, ISIS 505358-CS1, which used the same dosing schedule as in this protocol.

2.6 Combination of ISIS 505358 with Nucleos(t)ide Analogue

The differences in pharmacokinetic behavior between drugs like ISIS 505358 and nucleos(t)ide analogues indicate drug-drug interaction between the 2 drug types is unlikely. The antiviral and safety results from Cohorts 1–2 of ISIS 505358-CS3 are consistent with that expectation.

Drugs of the 2′-MOE ASO class, like ISIS 505358, rapidly become highly bound (~95%) to serum proteins upon entry into the blood stream and are then rapidly distributed ($t_{1/2}$ ~2 hr) to tissues where these drugs enter cells through target mediated endocytosis and micropinocytosis (Geary et al. 2008; Bennett and Swayze 2010). 2′-MOE ASOs are eliminated primarily by nucleolytic degradation (Geary et al. 2008). None of these processes are inhibited by small-molecule drugs like nucleos(t)ide analogues.

Conversely, tenofovir, entecavir, and other nucleos(t)ide analogues circulated in the blood stream with little binding to serum proteins and are eliminated in urine as intact drug by a combination of glomerular filtration and active secretion (Bristol-Myers Squibb 2018; Gilead 2018). The renal elimination has been shown to be mediated by the organic anion transporters OAT1, OAT3 and OCT2 (Cihlar et al. 2001; Uwai et al. 2007; Xu et al. 2013). 2′-MOE ASO have been shown to be neither substrates nor inhibitors of OAT1, OAT3, or OCT2 (Yu et al. 2016; Shemesh et al. 2017).

In Cohorts 1–2 of ISIS 505358-CS3, tenofovir dosing was started on Study Day 29, which is 7-days after the last dosing of ISIS 505358. Since the ASO has a long tissue half-life (2-3 weeks based on the non-clinical studies), there is a period of at least several weeks during which there will be a substantial presence of both drugs in the livers and other tissues of treated patients. For Cohorts 1 and 2, potent reduction of HBV DNA was observed following initiation of tenofovir in

all patients. There were no obvious differences in tenofovir potency between the cohorts consistent with the different ISIS 505358 dose levels of the 2 cohorts nor were there differences within cohorts that possibly indicate a difference between ASO and placebo-treated patients. Neither were there any adverse event or clinical laboratory results suggestive of a safety effect due to drug-drug interaction. The results from Cohort 3 to date are consistent with Cohort 2.

3. EXPERIMENTAL PLAN

3.1 Study Design

This study examines the effects of ISIS 505358 or placebo (3:1 randomization) administered subcutaneously to treatment-naïve patients who are chronically infected with HBV and the effects of subsequent nucleos(t)ide analogue treatment of these patients. Three (3) ISIS 505358 dose levels will be evaluated through the treatment of 3 sequential cohorts of 8 patients each:

- Cohort 1 (150 mg ISIS 505358 or placebo)
- Cohort 2 (300 mg ISIS 505358 or placebo)
- Cohort 3 (450 mg ISIS 505358 or placebo) (note: cohort was studied as 300 mg ISIS 505358 or placebo based on antiviral activity observed in Cohort 2 per Section 3.7)

In these cohorts, 6 patients will be randomized to treatment with ISIS 505358 and 2 patients to treatment with placebo. Patients enrolled into the study may be either HBeAg positive (+) or negative (-).

For each patient, Study Drug (ISIS 505358 or placebo) will be administered twice in Weeks 1 and 2 (on Days 1, 4, 8, and 11), and then once weekly during Weeks 3 and 4 (i.e., on Days 15 and 22). On Day 29, the effects of Study Drug treatment will be assessed. After these assessments, all patients will then commence chronic daily treatment with tenofovir disoproxil fumarate (tenofovir) (entecavir if judged more appropriate by the patient or Investigator) for 6 months. Continuation of nucleos(t)ide analogue after discontinuation from this study will be the decision of the patient in consultation with their physician.

One exploratory cohort (Cohort 4) will examine add-on treatment of patients already on stable nucleos(t)ide analogue (i.e., tenofovir or entecavir) regimen with 300 mg ISIS 505358 or placebo (3:1 randomization). Patients enrolled into the study may be either HBeAg positive (+) or negative (-).

The target size of Cohort 4 is approximately 8 patients, but the size may range from 4 to > 8 depending upon availability of participants. Each patient randomized into Cohort 4 is expected to continue their ongoing nucleos(t)ide analogue regimen and be treated with Study Drug by the above schedule for Cohorts 1–3. After Day 22, the patients are expected to continue their ongoing nucleos(t)ide analogue regimen and be followed until Day 211. On Day 29, the effects of Study Drug treatment will be assessed.

An independent Data and Safety Monitoring Committee (DSMB) will participate in the review of the study results and in the dose-escalation decisions (see Sections 3.6 and 3.7).

3.2 Number of Study Centers

This study will be conducted at multiple centers both in and outside of the United States.

3.3 Number of Subjects

Approximately 32 patients will be treated with Study Drug in this study. Additional patients may be treated due to difficulties of coordinating screening and enrollment from multiple sites and/or to replace patients that discontinue from cohorts before Day 29 for non-adverse event reasons but the actual number treated will not exceed 48.

3.4 Overall Study Duration and Follow-up

The Study will consist of screening, treatment, and post-treatment follow-up periods. Please refer to the Schedule of Procedures in Appendix A.

3.4.1 Screening

Subject eligibility for the Study will be determined within 28 days prior to first Study Drug dose. Patients in screening for Cohort 4 are expected to continue to take their pre-existing ongoing nucleos(t)ide analogue therapy.

3.4.2 Treatment

Eligible subjects will report to the Study Center for treatment with Study Drug on Study Days 1, 4, 8, 11, 15, and 22. They will additionally report to the Study Center for assessment on Study Days 2, 23, and 29.

Patients in Cohort 4 are expected to continue to take their ongoing nucleos(t)ide analogue therapy during this period.

3.4.3 Post-Study Drug Treatment Follow-Up

During the Post-Study Drug Treatment Follow-Up period, patients in Cohorts 1–3 will be treated with tenofovir (entecavir if judged more appropriate by the patient or Investigator) for 6 months commencing after the assessments of Day 29. Subjects are to return to the Study Center for follow-up visits on Study Days 36, 57, 85, 113, and 211. The final study visit will be Study Day 211.

Patients in Cohort 4 are expected to continue their nucleos(t)ide analogue therapy that was ongoing prior to Screening. Follow-up visits will be performed on Study Days 36, 57, 85, 113, and 211. The final study visit will be Study Day 211.

3.5 End of Study

The End-of-Study is defined as last patient, last visit.

3.6 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be assembled to review safety, tolerability and antiviral activity data collected on during this study. Based on the DSMB's regular assessments of the safety and tolerability of ISIS 505358, the DSMB will provide recommendations to the Sponsor for continuing the study as planned or for modifying or stopping cohorts and/or the study.

Following the completion of the last Day 29 assessment in each cohort, the DSMB will review the cumulative safety and tolerability results from the study and make dose-escalation recommendations. Should the committee find the safety and tolerability results to be sufficient and acceptable after their review of results from Cohort 1, the committee may recommend initiation of Cohort 2. Likewise, after their review of Cohort 2, the committee may recommend initiation of Cohort 3. For the Cohort 2 through-Day 29 review, the DSMB will also be provided with the Cohort 1 post-Study Drug treatment results that have emerged since the Cohort 1 dose-escalation review. The DSMB will consider those results in forming their recommendations on study continuation.

After completion of Study Drug administration and the last Study Day 29 assessment for Cohort 3 and for Cohort 4, respectively, the DSMB will again review the cumulative study results, advise on any notable treatment-emergent issues, and make recommendations regarding the continuation of the study and the periodicity of further DSMB reviews during reminder of the study. At any time during the study, the DSMB may also make recommendation regarding the frequency of their reviews, increases or decreases to the sizes of Cohorts 1-3, and/or introduction of cohorts to evaluated intermediate dose levels.

Additional details on the assessments, frequency of review, meeting schedules and controlled access to unblinded data will be outlined in the DSMB Charter and Statistical Analysis Plan (SAP).

3.7 Dose Escalation

Study enrollment and dose-escalation will cease in the event that any of the following occur provided there is no alternative explanation other than ISIS 505358 administration as the cause of the event as determined by the DSMB:

- Death of 1 patient
- Occurrence of dose-limiting toxicity (DLT) (see Section 3.8) of the same character in 2 patients of a dose level (e.g., 2 cases of Division of AIDS (DAIDS) Grade 3 cardiac ischemia)

Discontinuation of enrollment and dose-escalation will occur when 1 of the above criteria is met regardless of the number of patients that have been enrolled at the time of the event. For example, enrollment would be discontinued after the second patient to have DAIDS Grade 3 cardiac ischemia related to ISIS 505358 even if there are only 2 patients that had been enrolled into the cohort.

If enrollment is halted due to the above, the DSMB will make recommendations concerning the further treatment of the remaining patients already receiving Study Drug in the study. The options include, but are not limited to, ceasing treatment with Study Drug entirely or continuing at reduced or current dose levels (Section 8.7).

In the above deliberations on dose escalation and expansion of enrollment, the DSMB will also consider the results from the HBV serological measurements (e.g., HBsAg, HBeAg, and HBV DNA). For example, the DSMB may recommend against further dose escalations despite acceptable safety and tolerability because of obvious attainment of maximum antiviral effect.

3.8 Dose Limiting Toxicity

For consistency in the reporting of adverse events between personnel involved in the conduct of this study, adverse events will be described and graded according to the Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014 (see Appendix D) except for responses at subcutaneous injections sites. Responses at injection sites will be graded according to the mild, moderate, and severe criteria defined in Section 9.4.3.2 to allow comparison to the results from this study to those obtained in ISIS 505358-CS1 and to the clinical study results from other 2′-MOE chimeric ASOs. The occurrence of the following adverse events categorized as Possibly Related or Related to Study Drug administration will be considered as suspected DLTs:

1. Confirmed (by repeated specimen collection) laboratory result meeting 1 of the treatment stopping rules in Section 8.6
2. All DAIDS Grade ≥ 3 non-laboratory adverse events with the exception of flu-like symptoms consistent with the typical acute, transient, responses to 2′-MOE ASO injection (e.g., fever and/or chills beginning within 6 hours of dosing that resolve spontaneously within 36 hours and/or fatigue lasting ≤ 7 days). See the Guidance to the Investigator in the Investigator's Brochure.
3. All DAIDS Grade 4 confirmed (by repeated specimen collection) laboratory adverse events except the test results defined in Section 8.6

Adverse events and laboratory abnormalities will be considered to be DLTs if there is at least a possible association to ISIS 505358 or there is no definite alternative explanation for the safety finding. Since some of the patients will be placebo-treated and adverse events in placebo-treated patients cannot be DLTs, the events identified by Investigators will be considered suspected DLT. The DSMB (unblinded to treatment assignment) will be the arbiter as to which suspected events are to be considered DLTs. However, the DSMB will not recommend continuation or resumption of Study Drug dosing for any patient so as to not potentially make an Investigator aware of a patient's treatment assignment. The DSMB will not disclose to parties outside of the DSMB which of the suspected DLTs are actual DLTs.

4. SUBJECT ENROLLMENT

4.1 Screening

Before patients may be enrolled into the study, the Sponsor or designee requires a copy of the Study Center's written Institutional Review Board (IRB) approval of the protocol, informed consent form, and all other patient information and/or recruitment material.

Patients must sign the consent form before any screening tests or assessments are performed. At the time of consent, the patient will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including screening procedures, are performed. At the time of randomization, patients will be assigned a unique patient identification number. This number will be used to identify the patient throughout the trial and must be used on all study documentation related to that patient. The screening number and patient identification number must remain constant throughout the entire trial. In the event the patient is re-consented and re-screened, the patient must be given a new screening number and, if

re-randomized, a new patient identification number. Screening numbers and patient identification numbers, once assigned, will not be re-used.

If a patient has an exclusionary result(s) in their Screening laboratory tests, and the Investigator has reason to believe the result is erroneous, the Investigator may discuss his reasons with the Ionis Medical Monitor or designee. The Ionis Medical Monitor or designee may give approval for retesting and will specify the number of retests, or alternative tests, and the testing schedule that must be met in order to qualify the patient for inclusion in the study.

4.2 Randomization

Patients will be randomized prior to the first dose of Study Drug (ISIS 505358 or placebo), after all screening assessments have been completed and after the Investigator has verified that they are eligible per criteria in Sections 5.1 and 5.2. No patient may begin treatment prior to randomization and assignment of a unique patient identification number.

The Investigator or designee will obtain the patient's identification number and cohort, and Study Drug kit number from an Interactive Voice/Internet Response System (IXRS). The system will randomize eligible patients 3:1 to receive ISIS 505358 or placebo, respectively.

A permuted block schedule will be used for the randomizations. The Sponsor's Quality Assurance department or designee will hold a copy of the randomization lists generated by the IXRS vendor.

4.3 Replacement of Subjects

Subjects withdrawn early from the Study before completion of Study Day 29 procedures may be replaced at the discretion of the Sponsor. The additional subjects will be assigned to the same Study Drug (ISIS 505358 or placebo) as the subjects who are being replaced.

Subjects whose randomization code has been broken will not be replaced.

4.4 Unblinding of Treatment Assignment

All patients, study monitors, Study Center personnel and contract research organization (CRO) personnel will be blinded to Study Drug assignment (ISIS 505358 or placebo) throughout the study. However, if a patient has suffered a Serious Adverse Event (as defined in Section 9.3.3), and/or when knowledge of the treatment assignment will impact the clinical management of the patient, the Investigator will have the ability to unblind the treatment assignment for that patient using the IXRS. If time permits, the Investigator may contact the Sponsor's Medical Monitor, or designee, about the situation before unblinding. Regardless, the Sponsor or designee will be informed of the unblinding of a patient within 24 hours. In addition, all suspected unexpected serious adverse reactions (SUSARs) will be unblinded as necessary by the Sponsor's Drug Safety and Quality Assurance personnel for the purpose of regulatory reporting (see Section 9.2). Every reasonable attempt should be made to complete the early termination study procedures and observations (see Appendices A and B) prior to unblinding, as knowledge of the treatment arm could influence patient assessment.

The DSMB may be unblinded when reviewing safety and tolerability results. Sponsor and contract personnel supporting the DSMB, and not in communication with Investigators and/or investigational site staff, may also be unblinded.

An unblinded interim analysis for Cohorts 1–3 may be performed after all Cohort 1–3 patients have progressed beyond their Study Day 29 assessments. For such an analysis, select Sponsor personnel that have no direct communication with the Investigational Centers may become unblinded to individual patient treatment assignments. An unblinded interim analysis for Cohort 4 may be performed similarly after all Cohort 4 patients have progressed beyond their Study Day 29 assessments.

5. PATIENT ELIGIBILITY

To be eligible to participate in this study candidates must meet the following eligibility criteria within 28 days prior to Study Day 1 or at the time-point specified in the individual eligibility criterion listed.

5.1 Inclusion Criteria

1. Have given written informed consent (signed and dated) and any authorizations required by local law and is able to comply with all study requirements
2. Age 18 to 70 years
3. Chronic HBV infection ≥ 6 months (e.g., positive for serum HBsAg ≥ 6 months)
4. Cohorts 1–3: Plasma HBV DNA ≥ 2 x 10^3 IU/mL
   Cohort 4: HBV DNA adequately suppressed (e.g., plasma or serum HBV DNA below the lower limit of quantitation)
5. Serum HBsAg > 50 IU/mL
6. Satisfy the following:
   a. Females: Non-pregnant and non-lactating; surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy), post-menopausal (defined as 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the postmenopausal range for the laboratory involved) or, if engaged in sexual relations of child-bearing potential, subject is using an acceptable contraceptive method (refer to Section 6.4) from the time of signing the informed consent form until at least 13 weeks after the last dose of Study Drug (ISIS 505358 or placebo)
   b. Males: Surgically sterile or if engaged in sexual relations with a female of childbearing potential, subject is utilizing an acceptable contraceptive method (refer to Section 6.4) during treatment with Study Drug and for at least 13 weeks after the last dose of Study Drug
7. Cohort 4 only: Currently taking and have been taking tenofovir or entecavir without changes in drug, dose level and/or frequency of administration for ≥ 12 months and expect to continue taking without change through to the end of their participation in this study (approximately 8 months)

5.2 Exclusion Criteria

1. Cohort 1–3 only: Current or prior receipt of anti-HBV nucleos(t)ide analogue therapy. Patients who have failed prior interferon treatment, greater than 6 months prior to Screening, may be evaluated for possible participation in the study.
2. History of liver cirrhosis and/or evidence of cirrhosis as determined by any 1 of the following:
   a. Liver biopsy (i.e., Metavir Score F4) within 2 years of Screening, or
   b. Fibroscan > 12 KPa, within 12 months of Screening, or
   c. AST-to-Platelet Index (APRI) > 2 and Fibrosure result > 0.7 within 12 months of Screening

   For patients without a test for cirrhosis in the above timeframes, Fibroscan, or APRI and Fibrosure, may be performed during the screening period to rule out cirrhosis
3. History of liver failure as evidenced by ascites, hepatic encephalopathy, and/or gastric or esophageal varices
4. History of liver disease other than Hepatitis B
5. Co-infection with hepatitis C virus (HCV), human immunodeficiency virus (HIV), or hepatitis D virus (HDV)
6. BMI > 35 kg/m^2
7. History of, or suspected presence of vasculitis
8. Received solid organ or bone marrow transplant
9. Currently taking, or took within 3 months of Screening, any immunosuppressing drugs (e.g., prednisone). If the patient received a short course (≤ 1-week), the situation may be discussed with the Ionis Medical Monitor, or designee
10. Diagnosed hepatocellular carcinoma or suspected hepatocellular carcinoma as evidenced by screening alpha-fetoprotein ≥ 200 ng/mL. If the screening alpha-fetoprotein is ≥ 50 ng/mL and < 200 ng/mL, the absence of liver mass must be documented by imaging within 6 months before randomization
11. Screening laboratory results as follows, or any other clinically-significant abnormalities in screening laboratory values that would render a patient unsuitable for inclusion
    a. ALT or AST > 5 x ULN
    b. Total bilirubin > 1.1 x ULN
    c. Serum albumin < 3.5 g/dL
    d. INR > 1.2
    e. Platelet count < 140 k/mm^3
    f. Hemoglobin < 12.0 g/dL for males and < 11.0 g/dL for females
    g. WBC < 3.0 k/mm^3

h. Serum creatinine > 1.1 x ULN

i. Urine protein/creatinine (P/C) ratio ≥ 0.2 mg/mg. In the event of P/C ratio above this threshold, eligibility may be confirmed by a quantitative total urine protein measurement of < 150 mg/24-hr

j. Positive test (including trace) for blood on urinalysis. In the event of a positive test, eligibility may be confirmed with urine microscopy showing < 5 red blood cells per high power field

12. History of Gilbert's Syndrome or history of laboratory results consistent with Gilbert's Syndrome
13. Clinically-significant abnormalities aside from chronic HBV infection in medical history (e.g., previous acute coronary syndrome within 6 months of Screening, major surgery within 3 months of Screening, uncontrolled diabetes) or physical examination
14. History of bleeding diathesis or coagulopathy
15. History of extrahepatic disorders possibly related to HBV immune complexes (e.g., glomerulonephritis, polyarteritis nodosa)
16. Active infection other than HBV requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Study Day 1
17. Malignancy within 5 years, except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix that has been successfully treated
18. Treatment with another investigational drug, biological agent or device within 4 weeks of screening or 5 half-lives of Study Drug, whichever is longer
19. Treatment with any non-ISIS oligonucleotide (including siRNA) at any time
20. Prior treatment with an ISIS oligonucleotide within 9 months of Screening
21. History of excess alcohol consumption within 6 months of Screening as defined as weekly intake of > 14 drinks per week (> 2 drinks per day) for males or > 7 drinks per week (> 1 drink in a day) for females. (One [1] drink is equivalent to 12 g of alcohol, e.g., 12 ounces [360 mL] of beer, 5 ounces [150 mL] of wine or 1.5 ounces [45 mL] of 80 proof distilled spirits)
22. History of drug abuse or dependence, or recreational use of drugs: within 3 months of Screening for soft drugs (such as marijuana) and within 1-year of Screening for hard drugs (such as cocaine, phencyclidine [PCP])
23. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator
24. Have any other conditions (medical, social or other), which in the opinion of the Investigator would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study
25. Patients with hereditary problems of galactose intolerance, Lapp lactose deficiency, or glucose-galactose malabsorption, unless the patient will be treated with the lactose-free formulation of entecavir during the study

6. STUDY PROCEDURES

6.1 Study Schedule

All required study procedures are outlined in Appendices A, B, and C.

6.1.1 Screening

For patients without history of cirrhosis and without liver biopsy within 2 years of Screening or Fibroscan or Fibrosure and APRI within 1-year of Screening, Fibroscan, or APRI and Fibrosure, may be performed during Screening in order to answer Exclusion Criteria #2 (Section 5.2).

6.1.2 Treatment Period

All reasonable attempts should be made to ensure compliance with the visit schedule as outlined in Appendix A. Aside from Study Day 2 occurring on the day after Day 1, Day 23 occurring on the day after Day 22, and Day 29 occurring 7 days after Day 22, occasional variances are permitted to accommodate holidays, patient needs, and scheduling conflicts. A window of ± 1 day is allowed for the Study Day 4–22 visits. However, the administration of Study Drug doses should be separated by at least 36 hours. Following a variance, the patient should return to the schedule based on elapsed days from the actual Day 1 (i.e., variances do not permanently shift the schedule).

6.1.3 Post-Treatment Period

The permitted variance for the Study Day 36 visit is ± 1 day and for the Day 57 visit is ± 3 days. For other post-treatment visits (Days 85, 113, and 211), a ± 7 day variance is permitted.

6.1.4 Early Termination

If a decision is made to withdraw a patient from Study Drug treatment, or if a patient on their own accord chooses to withdraw from treatment, all reasonable attempts should be made to have the patient participate in the post-treatment schedule beginning with the Day 29 (at 7 days after the last Study Drug dose or at the earliest time thereafter). If this is not possible, every attempt should be made for the patient to attend an Early Termination visit as soon as possible prior to withdrawal.

If a decision is made to withdraw a patient from post-treatment follow-up, or if a patient on their own accord chooses to withdraw from post-treatment follow-up, every attempt should be made for the patient to attend an Early Termination visit as soon as possible prior to withdrawal.

6.2 Laboratory Assessments

Laboratory analyte samples will be collected throughout the study. A list of these analytes is contained in Appendix B.

In addition, serum and plasma specimens will be collected at a number of time-points (see Appendix A) and stored frozen for retrospective clinical safety, immunologic and/or virologic assays. Examples include, but are not limited to, retrospective LDH or CPK isoform measurements, measurement of cytokine, chemokine, and/or autoantibodies, or measurement of additional markers of kidney function, or mutation analysis of breakthrough HBV in order to follow-up effects observed in the current study or in another study of ISIS 505358. The selection

of time points balanced supporting examination of effects that may occur at any time during the study against an excessive amount of blood collection.

Peripheral blood mononuclear cell preparation at selected time points (see Appendix A) stored frozen for future cellular immunity investigations.

6.3 HBV Resistance Mutation Monitoring

Serum HBV DNA levels for each patient will be measured throughout the study (see Appendix A). If evidence of virologic failure is observed, patients will be interviewed regarding treatment compliance and concomitant usage of medication that might affect virus replication (e.g., corticosteroids), and other potentially relevant parameters. Additional blood samples will be collected for measurement of ISIS 505358 concentration in plasma (and nucleos(t)ide analogue concentration if applicable) and for HBV resistance mutation analysis where the viral genome will be DNA sequenced to determine whether mutations have occurred the ISIS 505358 binding region (and, if applicable, whether any known the nucleos(t)ide analogue resistance mutations are present in the polymerase coding region).

Virologic failure is defined as the occurrence of either virologic breakthrough (i.e., a confirmed increase of ≥ 1 $\log_{10}$ HBV DNA copies/mL above nadir or becoming quantifiable after being below the lower limit of quantitation) or virologic nonresponse (i.e., failure to achieve HBV DNA levels below 10^3 copies/mL)

6.4 Restriction on the Lifestyle of Patients

6.4.1 Contraception Requirements

All male subjects and women of childbearing potential must refrain from sperm/egg donation and practice effective contraception from the time of signing the informed consent form until at least 13 weeks after their last dose of study treatment.

Male subjects must also encourage their female partner to use effective contraception from the time of signing the informed consent until 13 weeks after the subject's last dose of study treatment.

For the purposes of this Study, women of childbearing potential are defined as any female who has experienced menarche, and who does not meet 1 of the following conditions:

- Postmenopausal: 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years, 12 months of spontaneous amenorrhea without an alternative medical cause and FSH levels in the postmenopausal range for the laboratory involved
- 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Post hysterectomy

For the purposes of the Study, effective contraception is defined as follows:

For male subjects:

- Effective male contraception includes a vasectomy with negative semen analysis at Follow-up, or the use of condoms together with spermicidal foam/gel/film/ cream/suppository
- Male subjects with partners that are pregnant must use condoms as contraception to ensure that the fetus is not exposed to the Study Drug

For female subjects and female partners of male subjects:

- Using 1 or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation), hormonal contraception, intrauterine contraception/device, or any 2 barrier methods (a combination of male or female condom* with diaphragm, sponge, or cervical cap) together with spermicidal foam/gel/film/cream/suppository

***Note: A female condom and a male condom should not be used together as friction between the two can result in either or both products failing.**

6.5 Nucleos(t)ide Analogue Plasma Concentrations

Cohorts 1–3: measurement of tenofovir and entecavir plasma concentrations will be performed following the Study Drug administration period. The measurement for each patient will depend on the nucleos(t)ide analogue they are taking. The specimen collection days are Study Days 29, 36, 57, 85, 113, and 211.

Cohort 4: measurement of tenofovir and entecavir plasma concentrations will be performed to explore whether treatment-emergent changes to nucleos(t)ide analogue plasma levels occur during ISIS 505358 administration. The measurement for each patient will depend on the nucleos(t)ide analogue they are taking. The specimen collection days are Study Days 1, 8, 15, 22, 29, 36, 57, 85, 113 and 211.

Because there is a known food effect for tenofovir and entecavir, subjects will be asked to be consistent in their eating habits and when they take their doses before the clinic visits on these days. Sites should make reasonable efforts to ensure that the pre-dose blood draws on these visit days generally occur at the same time of day (± 2 hour) to lessen any day-to-day variability.

7. STUDY DRUG

7.1 Study Drug Description

Study Drug (ISIS 505358 or placebo) characteristics are listed in Table 1.

The Study Drug is contained in 2-mL stoppered glass vials and will be provided to the Study Centers by the Sponsor. The Study Drug storage and preparation instructions will be provided by the Sponsor. For long term storage at clinical sites, the Study Drug must be stored securely at 2–8 °Celsius and be protected from light.

Table 1 Study Drug Characteristics

Study Drug	ISIS 505358	Placebo
Strength	150 mg/mL	Not Applicable
Volume/Formulation	1 mL solution per vial	1 mL solution per vial
Route of Administration	SC	SC

7.2 Packaging and Labeling

The Sponsor will provide the Investigator with packaged Study Drug labeled in accordance with specific country regulatory requirements.

7.3 Study Drug Accountability

The Study Center staff is required to document the receipt, dispensing, and return of Study Drug supplies provided by the Sponsor. The Study Center must return all used and unused Study Drug vials to the Sponsor or designee.

8. TREATMENT OF PATIENTS

8.1 Study Drug Administration

Study Drug will be administered by subcutaneous injection by trained study center personnel. The recommended sites for injection are the abdomen, upper arm, and thigh.

The volume of injection for each dose level is shown in Table 2. For doses ≥ 300 mg, the dose will be split into 2 equal injections (i.e., 2 x 1.0 mL for 300 mg dose and 2 x 1.5 mL for 450 mg dose) and administered to 2 separate anatomical sites (e.g., upper arm, thigh, abdomen quadrants). The site(s) of injection should be changed administration-to-administration and injection into areas with ongoing injection site responses should be avoided.

Please refer to the Study Drug Manual provided by the Sponsor or designee for more detailed instructions for Study Drug preparation and administration.

Table 2 Study Drug Dosing Information

Cohort	Total Volume to Administer	Total Dose
Cohort 1	1.0 mL	150 mg or placebo
Cohort 2, Cohort 4	2.0 mL	300 mg or placebo
Cohort 3	3.0 mL*	450 mg or placebo*

* Patients in Cohort 3 were treated with total volume of 2.0 ml and total dose of 300 mg or placebo

In the event a patient experiences difficulty with the number or volume of the injections or skin responses at the injection site, the Investigator should contact the Ionis Medical Monitor to

discuss possible mitigations that may enable the patient to complete the dosing period. These mitigations include, but are not limited to, subdividing a dose into smaller injection volumes, and splitting the administration of a dose to more than 1 day.

8.2 Other Protocol-Required Drugs

After the conclusion of Study Day 29 assessments, patients in Cohorts 1–3 are required to commence treatment with tenofovir. Patients and/or Investigators may opt for treatment with entecavir if they deem that more appropriate.

Patients in Cohort 4 are expected to continue throughout this study their stable nucleos(t)ide analogue regimen that was ongoing prior to their enrollment in this study.

8.3 Other Protocol-Required Treatment Procedures

There are no other protocol-required treatment procedures.

8.4 Treatment Precautions

There are no required specific treatment precautions.

8.5 Safety Monitoring Rules

Please refer also to the 'Guidance for Investigator' section of the Investigator's Brochure.

For the purposes of safety monitoring, Baseline is defined as the pre-dose value on Day 1 or, if no pre-dose Day 1 result is available, it is the pre-dose test performed closest to Day 1.

In addition to the standard monitoring of clinical safety parameters, the following guidelines are provided for the monitoring of selected parameters chosen based on preclinical and clinical observations.

Confirmation Guidance: At any time during the study (Treatment or Post-Treatment Periods), the initial clinical laboratory results meeting the safety monitoring criteria presented below **must be confirmed** by performing measurements (ideally in the same laboratory that performed the initial measurement) on new specimens. All new specimen collections should take place as soon as possible (within 48 hours of receiving initial result). If the repeat testing collection is not performed by 7 days of the initial collection, the initial result will be assumed as confirmed. If the initial laboratory result meeting a stopping rule is observed during the Treatment Period, the results from the retest **must be available** prior to administration of the next dose of Study Drug.

Re-dosing Guidance: Patients with initial laboratory test values that reach a stopping rule must not be re-dosed until the re-test results are available. In general, patients who do not meet the stopping rules based upon retest may continue dosing. However, the Investigator and the Ionis Medical Monitor (or appropriately qualified designee) should confer as to whether additional close monitoring of the patient is appropriate. If any of the stopping criteria described below (refer to Sections 8.6.1 to 8.6.4) are met and are confirmed, treatment of the patient with Study Drug will be stopped and patients will be followed as described below.

8.5.1 Safety Monitoring Rules for Liver Chemistry Tests

The following safety monitoring rules for liver chemistry tests are adapted from the draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," issued by the U.S. Department of Health and Human Services, Food and Drug Administration, July 2009. For a definition of Baseline please refer to guidance in Section 8.5 above.

In the event of an ALT or AST measurement that is > 3 x ULN or > 2 x Baseline value, whichever is greater, at any time during the study (Treatment or Post-Treatment Period), the initial measurement(s) should be confirmed as described above (i.e., blood collection for retest should be performed within 48 hours of receiving the initial result). Additional confirmatory measurements should also be performed if ALT or AST levels increase to the greater of 5 x ULN or 3 x Baseline.

Frequency of Repeat Measurements: Patients with confirmed ALT or AST levels > 3 x ULN or > 2 x Baseline value, whichever is greater, that are continuing to rise should have their liver chemistry tests (ALT, AST, ALP, INR and total bilirubin) retested twice weekly until levels stabilize and begin to recover. Thereafter, the frequency of further testing will be discussed between the Investigator and Ionis Medical Monitor, or designee, and performed as clinically indicated. Once ALT and AST levels become ≤ 1.2 x ULN (or 1.2 x Baseline value if the Baseline value was > ULN), the frequency of testing can return to that specified in Appendix A - Schedule of Procedures for the relevant Study Day.

Further Investigation into Liver Chemistry Elevations: For patients with confirmed ALT or AST levels > 3 x ULN or > 2 x Baseline value, whichever is greater, the following evaluations should be performed:

1. Obtain a more detailed history of symptoms and prior and concurrent diseases
2. Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
3. Obtain a history for exposure to environmental chemical agents and travel
4. Serology for viral hepatitis sources other than HBV (HAV IgM, HCV antibody, HDV IgM, HEV IgM, CMV IgM, and EBV antibody panel)
5. Serology for autoimmune hepatitis (i.e., antinuclear antibody (ANA), anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibody, total IgG.)
6. Collect and store plasma sample for PK analysis (of nucleos(t)ide analogue and ISIS 505358) as soon as possible following occurrence of an event
7. Collect and store plasma samples for HBV DNA quantitation and resistance mutation analysis
8. Collect and store serum sample for serum acetaminophen adduct HPLC assay

Additional liver evaluations, including gastroenterology/hepatology consultations, hepatic CT or MRI scans, may be performed at the discretion of the Investigator, in consultation with the

Sponsor Medical Monitor. Repetition of the above evaluations should be considered if a patient's ALT and/or AST levels continue to increase.

8.6 Study Drug (ISIS 505358 or Placebo) Stopping Rules

8.6.1 Stopping Rules for Liver Chemistry Elevations

Upon receipt of laboratory results meeting stopping criteria, Study Drug administration should be suspended while awaiting the results from confirmation testing. In the event of **confirmed laboratory results** (by testing of new specimens) meeting any of the following criteria, and the event is **without a probable cause other than Study Drug-induced liver injury** as discussed with the Ionis Medical Monitor, or designee, dosing of a patient with Study Drug will be stopped permanently.

Table 3 Study Drug Stopping Rules for ALT or AST Elevations

Baseline ALT ≤ 2x ULN	Baseline ALT > 2 x ULN
If confirmed ALT or AST ≥ 8 x ULN, permanently discontinue Study Drug	If confirmed ALT or AST ≥ 4 x Baseline, or confirmed ALT or AST ≥ 20 x ULN permanently discontinue Study Drug
If confirmed ALT or AST ≥ 3 to < 8 x ULN, permanently discontinue Study Drug if any of the following apply: • appearance or worsening of symptoms felt by the Investigator to be potentially related to worsening of hepatic inflammation such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (> ULN) • total bilirubin > 1.5 x ULN and direct bilirubin > 35%	If confirmed ALT or AST ≥ 2 to < 4 x baseline, permanently discontinue Study Drug if any of the following apply: • appearance or worsening of symptoms felt by the Investigator to be potentially related to worsening of hepatic inflammation such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (> ULN) • total bilirubin > 1.5 x ULN and direct bilirubin > 35%

Note: Baseline is latest laboratory result prior to first dose of Study Drug (ISIS 505358 or placebo)

The investigations described above in Section 8.5.1 should be performed as soon as possible for any patient meeting 1 of these stopping rules unless the Investigator and Ionis Medical Monitor, or designee, agree on another schedule.

The Investigator and Ionis Medical Monitor, or designee, should discuss the advisability of continuing Study Drug administration in situations where ALT or AST are elevated in ways not covered by the above rules (e.g., rising ALT levels > 2 x ULN but < 3 x ULN coupled with rising total bilirubin > 1.1 x ULN but < 1.5 x ULN). In situations where alternative probable causes exist for the above laboratory results (e.g., Gilbert's syndrome diagnosis and/or direct bilirubin < 35% of total; physical trauma and elevated creatine kinase coincident with elevated ALT and/or AST), the Investigator and Ionis Medical Monitor should confer about the possibility of continuing/resuming Study Drug treatment.

8.6.2 Stopping Rules for Hematologic Test Results

In the event of a **confirmed laboratory results** (by testing of new specimens) meeting any of the following criteria, and the event is **without a probable cause other than Study Drug** as

discussed with the Ionis Medical Monitor, or designee, dosing of a patient with Study Drug will be stopped permanently.

1. Hemoglobin < 9.0 g/dL (i.e., ≥ DAIDS Grade 3)
2. White Blood Cell count < 1.5 k/mm^3 (i.e., ≥ DAIDS Grade 3)
3. Absolute neutrophil count < 0.75 k/mm^3 (i.e., ≥ DAIDS Grade 3)
4. Platelet count < 75 k/mm^3

The follow-up schedule for events meeting these events will be determined by the Investigator in consultation with the Ionis Medical Monitor, or designee. The Ionis Medical Monitor, or designee, should also be consulted as to further investigations into alternative sources for these events. In situations where the hematologic changes are unlikely related to the Study Drug, the Investigator and Ionis Medical Monitor, or designee, will conference on the possibility of continuing/resuming treatment with Study Drug.

8.6.3 Stopping Rules for Renal Function Test Results

In the event of **confirmed laboratory results** (by testing of new specimens) for either of the following criteria, and the event is **without a probable cause other than Study Drug** as discussed with the Ionis Medical Monitor, or designee, dosing of a patient with Study Drug will be stopped permanently.

1. Confirmed serum creatinine increase that is both ≥ 0.3 mg/dL and ≥ 40% above Baseline creatinine values (defined as the average of the Screening and Study Day 1 results) and above upper limit of the reference range (i.e., > ULN)
2. Confirmed urine protein/creatinine ratio ≥ 0.90
3. Evidence of glomerular injury on urine microscopic exam.

The follow-up schedule for any events meeting either of these criteria will be determined by the Investigator in consultation with the Ionis Medical Monitor, or designee. The Ionis Medical Monitor, or designee, should also be consulted as to further investigations into alternative sources for the changes in renal function tests. In situations where the changes in renal function tests are unlikely related to Study Drug alone or in combination with nucleos(t)ide analogues, the Investigator and Ionis Medical Monitor, or designee, will confer about the possibility of continuing resuming treatment with Study Drug.

8.6.4 Stopping Rules for Significant Inflammatory Reaction

In the event of clinical symptoms (e.g., constellation of symptoms such as severe fever, chills, and myalgia) or confirmed laboratory findings (e.g., substantial decrease in serum complement protein levels) consistent with a significant inflammatory reaction, and the event is **without a probable cause other than Study Drug** as discussed with the Ionis Medical Monitor, or designee, dosing of the patient with Study Drug will be stopped permanently.

8.7 Adjustment of Dose and/or Treatment Schedule

Should one or more Study Drug doses not be administered due to events not or unlikely related to Study Drug (e.g., weather prevents travel to the study center, recuperation from rhinovirus

infection, recovery from injury), the patient will resume dosing with Study Drug at the point in the schedule corresponding to the patient's actual elapsed time from Study Day 1. Treatment will resume at the original assigned dose level. Missed doses and time off from treatment will not be made up. Treatment will end with the dose on Study Day 22 (see Appendix A – Schedule of Procedures).

After its review of a suspected DLT, the DSMB may determine the event was not an actual DLT (e.g., patient treated with placebo) and may recommend resumption of dosing with Study Drug be considered. The DSMB will not disclose the actual treatment assignment of the patient. Any resumption of treatment will be as described above.

If the number of patients with DLTs exceeds the limits described in Section 3.8 and further enrollment to that dose level is halted, 1 of the options available to the DSMB is to recommend some or all of the patients already receiving Study Drug have their dose level reduced by 1 dose step. That is, patients on treatment at 450 mg could be reduced to 300 mg, those at 300 mg could be reduced to 150 mg, and those on at 150 mg could be reduced to 75 mg.

8.8 Discontinuation of Study Treatment

A patient must permanently discontinue study treatment for any of the following:

- The patient becomes pregnant. Report the pregnancy according to instructions in Section 9.5.4
- The patient withdraws consent
- The patient experiences an adverse event that necessitates permanent discontinuation of study treatment
- The patient experiences an AE that necessitates unblinding of the Investigator to the patient's treatment assignment

A patient must stop treatment with Study Drug, at least temporarily, if the patient develops laboratory test abnormalities that meet any of the stopping rules listed in Sections 8.6.1 to 8.6.4.

The reason for discontinuation of study treatment must be recorded in the CRF and source documentation.

Patients that are discontinued from treatment with Study Drug will enter the Post-Treatment Period (see Section 6.1.4; tenofovir/entecavir treatment omitted if contraindicated) unless consent is withdrawn. Every effort should be made to complete the early termination study procedures and observations if the patient does not enter post-treatment follow-up.

8.9 Withdrawal of Patients from the Study

Patients must be withdrawn from the study for any of the following:

- Withdrawal of consent
- The patient is unwilling or unable to comply with the protocol

Other reasons for withdrawal of patients from the study might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from Study, must be recorded in the CRF.

Any patient who withdraws consent to participate in the study will be removed from further treatment and study observation immediately upon the date of request. These patients should be encouraged to complete the early termination study procedures and observations at the time of withdrawal (Appendix A).

For patients withdrawn for reasons other than withdrawal of consent every effort should be made to complete the early termination study procedures and observations at the time of withdrawal (see Appendix A).

8.10 Concomitant Therapy and Procedures

The use of concomitant therapies or procedures defined below must be recorded on the patient's CRF. AEs related to administration of these therapies or procedures must also be documented on the appropriate CRF.

8.10.1 Concomitant Therapy

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications and vitamin supplements) administered between signing informed consent and last post-treatment visit.

Entecavir and tenofovir are considered concomitant medications in this study. In Cohort 4, patients should be on stable tenofovir or entecavir therapy prior to Screening and their nucleos(t)ide analogue usage should be in accordance with the recommendations contained in their respective region-specific prescribing information. For all cohorts, each patient's nucleos(t)ide analogue usage should be recorded in their CRF.

Allowed Concomitant Therapy

Aside from the disallowed medication described below, Physician Investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care.

Disallowed Concomitant Therapy

Immunosuppressive/immunomodulatory therapy (e.g., use of > 5 mg systemic prednisone or its equivalent per day) is not permitted at any time during the study.

Initiation of treatment with additional anti-HBV agents (e.g., additional nucleos(t)ide analogues, interferons, vaccines) is not permitted during the study except where necessary in the opinion of the Investigator for re-establishing HBV DNA suppression following viral breakthrough.

Tenofovir and entecavir are principally eliminated by the kidney. Therefore, the concurrent use of drugs that may impair renal function (e.g., high-dose or multiple NSAIDs) should be avoided as recommended in the prescribing information for tenofovir.

Treatments that may increase ALT or AST (e.g., Augmentin, > 2 g acetaminophen/day, initiating treatment with an HMG-CoA Reductase Inhibitor [statin]) should be avoided. The use of these drugs, if necessary, should be discussed with the Ionis Medical Monitor, or designee, preferably before initiation of their administration.

Treatment with herbal medicines thought to possess anti-HBV activity is not permitted.

8.10.2 Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between signing informed consent and last post-treatment visit.

8.11 Treatment Compliance

Treatment dosing is to be performed and recorded by Study Center staff.

9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING

9.1 Sponsor Review of Safety Information

Safety information will be collected, reviewed, and evaluated by the Sponsor or designee in accordance with the Safety Management Plan throughout the conduct of the clinical trial.

9.2 Regulatory Requirements

The Sponsor or designee is responsible for regulatory submissions and reporting to the Investigators of serious adverse events (SAEs) including suspected unexpected serious adverse reactions (SUSARs) per the International Conference on Harmonization (ICH) guidelines E2A and ICH E6. Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

IRBs will be notified of any SAE according to applicable regulations. The DSMB will be notified of any SAE as specified in the DSMB charter.

The Sponsor or designee will evaluate the available information and decide if there is a reasonable possibility that the Study Drug caused the AE and, therefore, meets the definition of a SUSAR. Appropriate personnel at the Sponsor or designee will unblind SUSARs for the purpose of regulatory reporting. The Sponsor or designee will submit SUSARs to Regulatory Agencies in blinded or unblinded fashion according to local law. The Sponsor or designee will submit SUSARs to Investigators in a blinded fashion.

9.3 Definitions

9.3.1 Adverse Event

An adverse event is any unfavorable and unintended sign (including a clinically-significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the Study or use of investigational drug product, whether or not the AE is considered related to the investigational drug product.

9.3.2 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction is any AE caused by the Study Drug.

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

9.3.3 Serious Adverse Event (SAE)

A serious adverse event is any adverse event that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event

  An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization

  Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in a congenital anomaly or birth defect in the offspring of the subject (whether the subject is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

9.4 Monitoring and Recording Adverse Events

Any pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the Study (i.e., before informed consent) should be recorded as Medical History and not recorded as

AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible.

9.4.1 Serious Adverse Events

In the interest of subject safety, and in order to fulfill regulatory requirements**,** all SAEs (regardless of their relationship to Study Drug) should be reported to the Sponsor or designee within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the subject signs the informed consent form and stop at the end of the subject's follow-up period which is defined as the Study Week 30 visit unless the patient withdrew earlier. When the Investigator is reporting by telephone, it is important to speak to someone in person vs. leaving a message. An Initial Serious Adverse Event Form should be completed and a copy should be faxed to the Sponsor or designee. The fax number for reporting SAEs can be found in the Study Reference Manual provided by the Sponsor to each study center.

Detailed information should be actively sought and included on Follow-Up Serious Adverse Event Forms as soon as additional information becomes available. All SAEs will be followed until resolution. SAEs that remain ongoing past the subject's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and Sponsor agree the subject's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

9.4.2 Non-Serious Adverse Events

The recording of non-serious AEs will begin after the subject signs the informed consent form and will stop at the end of the subject's follow-up period, which is defined as the Study Week 30 visit unless the patient withdrew earlier. The Investigator will monitor each subject closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

9.4.3 Evaluation of Adverse Events (Serious and Non-Serious)

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form:

9.4.3.1 Relationship to the Study Drug

The event's relationship to the Study Drug (ISIS 505358 or placebo) is characterized by 1 of the following:

- **Related:** There is clear evidence that the event is related to the use of Study Drug, e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and Study Drug (ISIS 505358 or placebo) administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to Study Drug (ISIS 505358 or placebo) administration and/or exposure suggests that a causal relationship is unlikely (for reporting purposes, Unlikely/Remote will be grouped together with Not Related)

- **Not Related:** The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and Study Drug

9.4.3.2 Severity

The event's severity is graded according to the Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014 (see Appendix D) except for responses at subcutaneous injections sites. Responses at injection sites will be graded according to the mild, moderate, and severe criteria defined below. As noted in the DAIDS criteria, clinical adverse events not specifically identified in the table are to be characterized by 1 of the following:

Grade 1 (Mild): Symptoms causing no or minimal interference with usual social and functional activities

Grade 2 (Moderate): Symptoms causing greater than minimal interference with usual social and functional activities

Grade 3 (Severe): Symptoms causing inability to perform usual social and functional activities

Grade 4 (Potentially Life Threatening): Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability or death

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in Section 9.3.3).

9.4.3.3 Action Taken with Study Drug

Action taken with Study Drug (ISIS 505358 or placebo) due to an adverse event is characterized by 1 of the following.

- **None:** No changes were made to Study Drug (ISIS 505358 or placebo) administration and dose
- **Permanently Discontinued:** Study Drug was discontinued and not restarted
- **Temporarily Interrupted, Restarted – Same Dose:** Dosing was temporarily interrupted or delayed due to the AE and restarted at the same dose
- **Reduced Dose:** Dosing was reduced, temporarily interrupted or delayed due to the AE and restarted at the next lower dose

9.4.3.4 Treatment Given for Adverse Event

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form (treatment should also be recorded on the concomitant treatment or ancillary procedures eCRF as appropriate).

9.4.3.5 Outcome of the Adverse Event

If the event is a non-serious AE, then the event's outcome is characterized by 1 of the following:

- **AE Persists:** Patient terminates from the trial and the AE continues
- **Recovered:** Patient recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity (if applicable):** AE severity changed

If the event is an SAE, then the event's outcome is characterized by 1 of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Patient has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE eCRF (the SAE resolution date should be entered as the date of onset of that AE)
- **Recovered:** Patient recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Fatal:** Patient died (the date of death should be entered as the SAE resolution date)

9.5 Procedures for Handling Special Situations

9.5.1 Abnormalities of Laboratory Tests

Clinically-significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment (e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia). Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically-significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Sponsor Medical Monitor. Laboratory abnormalities deemed not clinically-significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should not be deemed NCS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents and should include the Investigator's assessment of clinical significance of out of range/abnormal laboratory values.

9.5.2 Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the Study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the Study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

9.5.3 Dosing Errors

Study Drug (ISIS 505358 or placebo) errors should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional.

Dosing details should be captured on the Dosing Case Report Form. If the subject takes a dose of Study Drug (ISIS 505358 or placebo) that exceeds protocol specifications and the subject is symptomatic, then the symptom(s) should be documented as an AE and be reported per Section 9.4.

Should an overdose occur, the Investigator or designee should refer to the Guidance to Investigator's section of the Investigator's Brochure and contact the Sponsor or designee within 24 hours.

9.5.4 Contraception and Pregnancy

Male patients and female patients of childbearing potential must continue to use appropriate contraception with their partners, or refrain from sexual activity, as described in Section 6.3.

If a patient becomes pregnant or a pregnancy is suspected, or if a male patient makes or believes that he has made someone pregnant during the study, then the Study Center staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported on Follow-up Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the subject's responsibility.

Female patients: If a suspected pregnancy occurs while on the study (including follow-up), a pregnancy test will be performed. The patient with a confirmed pregnancy will be immediately withdrawn from treatment with Study Drug. However, the patient will be encouraged to complete the post-treatment follow-up portion of the study to the extent that study procedures do not interfere with the pregnancy. Regardless of continued study participation, the study physician will assist the patient in getting obstetrical care and the progress of the pregnancy will be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, the Study Center and

Sponsor may require access to the mother and infant's medical records for an additional 8 weeks after birth.

Male patients: The progress of the pregnancy in a male patient's partner should be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, additional follow-up information may be requested for the mother and infant. Follow-up will be performed to the extent permitted by relevant guidelines (e.g., HIPAA) and privacy considerations.

10. STATISTICAL CONSIDERATIONS

10.1 Study Endpoints, Subsets, and Covariates

10.1.1 Primary Endpoints

The safety endpoints include:

- Adverse events
- Clinical laboratory tests (e.g., serum chemistry, hematology, urinalysis, coagulation, complement, antibodies, pregnancy test for women with childbearing potential)
- Vital signs and body weight
- Physical examination
- ECG
- Concomitant medication usage

There is no primary efficacy endpoint.

10.1.2 Secondary Endpoints

The efficacy endpoints include:

- Change from Baseline to Day 29 in plasma HBV DNA concentration
- Change from Baseline to Week 31 in plasma HBV DNA concentration
- Change from Baseline to Day 29 in serum HBsAg concentration
- Change from Baseline to Week 31 in serum HBsAg concentration
- Proportion of patients with HBsAg loss at Day 29 and at Week 31
- Change from Baseline to Day 29 and to Week 31 in serum HBeAg concentration in patients that were HBeAg positive at Baseline
- Proportion of patients with HBeAg loss at Day 29 and at Week 31 in patients that were HBeAg positive at Baseline
- Plasma pharmacokinetics of ISIS 505358 in patients with chronic hepatitis B virus infection

10.1.3 Exploratory Endpoints

- Proportion of patients with seroconversion to anti-HBs antibody positivity at Day 29 and at Week 31
- Proportion of patients with seroconversion to anti-HBe antibody positivity at Day 29 and at Week 31 in patients that were HBeAg positive at Baseline
- Plasma concentrations of tenofovir and entecavir

10.2 Sample Size Considerations

There is no statistical rationale for the selected sample size of 8 subjects per cohort. The sample size was based on prior experience with other members of the drug class to ensure an adequate initial assessment of the safety, tolerability and pharmacokinetics of ISIS 505358 while minimizing the number of subjects unnecessarily exposed to the drug.

10.3 Populations

The Safety Set will include all randomized patients who received at least 1 dose of ISIS 505358 or placebo.

The Full Analysis Set (FAS), which represents the practically-feasible intent-to-treat (ITT) population as delineated in ICH Guideline E9, will include the subset of the Safety Set with a Baseline and at least 1 post-Baseline plasma HBV DNA concentration.

The Per-Protocol Set will include the subset of the FAS who have:

- received at least 5 doses of ISIS 505358 or placebo during the 4-week Treatment Period
- had plasma HBV DNA concentration measure at Day 29, and
- had no significant protocol deviations that would be expected to affect efficacy assessments

PK Population will include all subjects who were randomized and received at least 1 dose of Study Drug.

10.4 Definition of Baseline

Baseline ECG will be the average of the triplicate taken on Day 1 Pre-dose, if only 1 or 2 assessments are available, the single assessment or average of the 2 assessments will be used.

Baseline creatinine will be the average of all measurements taken between Screening and Day 1 Pre-dose.

For other assessments, baseline will be the last non-missing measurement prior to the first dose of Study Drug.

10.5 Interim Analysis

To ensure patient safety, blinded data including the adverse events and safety laboratory data will be reviewed by the Sponsor on an ongoing basis.

An unblinded interim analysis or Cohort 1–3 may be performed after all Cohort 1–3 patients have progressed beyond their Study Day 29 assessments and the database has been locked. An unblinded interim analysis for Cohort 4 may be performed similarly after all Cohort 4 patients have progressed beyond their Study Day 29 assessments and locking of the data. Details of the analysis and controlled access to the unblinded data will be specified in the SAP.

10.6 Planned Methods of Analysis

All eCRF data, lab data transfers, as well as any outcomes derived from the data, will be provided in the patient data listings. Patient data listings will be presented for all patients enrolled into the study. Descriptive summary statistics including n, mean, median, standard deviation, interquartile range (25^{th} percentile, 75^{th} percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data. Where appropriate, p-values will be reported. All statistical tests will be conducted using 2-sided tests with 5% type I error rates unless otherwise stated.

For data summaries and statistical analyses, the placebo subjects from all 3 cohorts will be pooled. The efficacy endpoints will be assessed on the FAS and Per-Protocol Set. The safety analyses will be performed on the Safety Set.

10.6.1 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized descriptively by treatment group. All patients enrolled will be included in the summary of patient disposition.

10.6.2 Safety Analysis

The safety analyses will be conducted on the Safety Population.

Treatment duration and amount of Study Drug received will be summarized by treatment group. Subject incidence rates of all adverse events will be tabulated by MedDRA system organ class, and by MedDRA preferred term. Tables of "on-study" deaths, serious and significant adverse events, including early withdrawals due to adverse events, will also be provided.

All treatment-emergent AEs, all treatment-emergent AEs potentially related to Study Drug, all treatment-emergent serious AEs, and all treatment-emergent serious AEs potentially related to Study Drug will be summarized.

Laboratory tests including chemistry panel, complete blood count with differential, coagulation panel, complement etc., will be summarized by study visits for each treatment group. These safety variables will also be presented as change and percent change from Baseline over time after Study Drug administration, as appropriate.

Vital sign and ECG measures will be tabulated by treatment group. In addition, the number of patients who experience abnormalities in clinical laboratory evaluations will be summarized by treatment group.

10.6.3 Efficacy Analysis

There are no formal hypotheses in this study; therefore, all the efficacy endpoints will be summarized using descriptive statistics. Additional analyses for comparison between

ISIS 505358 groups and pooled placebo group will be performed in an exploratory manner, and will be detailed in the SAP.

The main efficacy analysis will be the changes from Baseline to Day 29 (1-week after last dose of Study Drug) for plasma HBV DNA and serum HBsAg, and change from Baseline to Day 29 in serum HBeAg concentrations for patients that were HBeAg positive at Baseline. These concentrations will be logarithmic transformed in this analysis. The LOCF method will be used to impute missing values in this analysis. Comparison between the ISIS 505358 and placebo treatment groups will be performed using an ANCOVA model with baseline as a covariate, and treatment group as a factor (if data departs substantially from normality, the Wilcoxon Rank Sum test will be used.

Additional efficacy analyses will include:

- Changes from Baseline to Week 31 in plasma HBV DNA (except Cohort 4), serum HBsAg, and changes from Baseline to Week 31 in serum HBeAg concentrations for patients that were HBeAg positive at Baseline. These concentrations will be logarithmic transformed. Comparison between the ISIS 505358 and placebo treatment groups will be performed using an ANCOVA model with baseline value as a covariate, and treatment group as a factor (if data departs substantially from normality, the Wilcoxon Rank Sum test will be used).
- Proportions of patients with at least 1 logarithmic reduction in plasma HBV DNA (except Cohort 4), serum HBsAg, and serum HBeAg concentrations (for patients that were HBeAg positive at Baseline) at Day 29 and Week 31 by treatment group. Responder analysis with other response thresholds (e.g., 0.5, 1.5, and 2.0 logarithmic reduction) will also be conducted. Comparison between ISIS 505358 and placebo treatment groups will be performed using the Pearson's Chi-square test or Fisher exact test as appropriate.

Exploratory analyses:

- Proportion of patients with seroconversion to anti-HBs antibody positivity at Day 29 and Week 31. Comparison between ISIS 505358 and placebo treatment groups will be performed using the Pearson's Chi-square test or Fisher exact test as appropriate.
- Proportion of patients with seroconversion to anti-HBe antibody positivity at Day 29 and Week 31 in patients that were HBeAg positive at Baseline. Comparison between ISIS 505358 and placebo treatment groups will be performed using the Pearson's Chi-square test or Fisher exact test as appropriate.

10.6.4 Pharmacokinetic Analysis

The plasma exposure and PK of ISIS 505358 will be assessed following single (i.e., first dose) and multiple SC administrations of ISIS 505358.

Non-compartmental PK analysis of ISIS 505358 will be carried out for (Day 1) and the end (Day 22) of ISIS 505358 treatment. Calculated PK parameters may include, but may not necessarily be limited to, the following:

- The maximum observed drug concentration (C_{max}) will be obtained directly from the observed concentration-time profile
- The time of the C_{max} (T_{max}) will be obtained directly from the observed concentration-time profile
- Following single or multiple dosing, area under the plasma concentration-time curve from zero time (pre-dose) to select selected times (t) after dose administration (AUC_t), or over the duration of a dosing interval (tau,τ) after the last administered multiple-dose (AUC_τ), will be calculated using the linear trapezoidal rule
- Following the last administered multiple-dose, the plasma disposition half-life ($t_{1/2\lambda z}$) associated with the apparent terminal elimination phase will be calculated from the equation, $t_{1/2\lambda z} = 0.693/\lambda_z$, where λ_z is the rate constant associated with the apparent terminal elimination phase, when appropriate data exist and at the discretion of the PK scientist

All other plasma PK parameters may be summarized using descriptive statistics.

Cohorts 1–3: Plasma levels of tenofovir (and entecavir if administered) will be measured on multiple days during the post-Study Drug Treatment Follow-Up period of the study (see Appendix C). Only the nucleos(t)ide analogue that is taken by each patient will be measured. Plasma concentrations of tenofovir (and entecavir) in patients previously treated with ISIS 505358 will be compared to prior treatment with placebo. Details for analysis will be defined in the SAP.

Cohort 4: Plasma levels of the tenofovir or entecavir taken by each patient will be measured on multiple days during the study periods before, during, and after Study Drug administration (see Appendix C). Only the nucleos(t)ide analogue that is taken by each patient will be measured. Plasma levels on Day 1 (prior to ISIS 505358) will be compared for each patient to plasma levels taken concurrently with ISIS 505358, matched for time from dose and fed/fasted state, to monitor for potential interactions. Details for analysis will be defined in the SAP.

10.6.5 Additional Analyses

Additional exploratory analyses could be performed based on observed data determined by the study team. Details for additional analyses will be defined in the SAP.

11. INVESTIGATOR'S REGULATORY OBLIGATIONS

11.1 Informed Consent

The written informed consent document should be prepared in the language(s) of the potential patient population, based on an English version provided by the Sponsor or designee.

Before a patient's participation in the trial, the Investigator is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any Study Drug are administered. The patient must be given sufficient time to consider whether to participate in the study.

The acquisition of informed consent and the patient's agreement or refusal to notify his/her primary care physician should be documented in the patient's medical records and the informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion (not necessarily an Investigator). The original signed informed consent form should be retained in the Study Master File and in any other locations required by institutional policy, and a copy of the signed consent form should be provided to the patient.

11.2 Ethical Conduct of the Study

The Guidelines of the World Medical Association (WMA) Declaration of Helsinki dated October 2002 the applicable regulations and guidelines of current Good Clinical Practice (GCP) as well as the demands of national drug and data protection laws and other applicable regulatory requirements will be strictly followed.

11.3 Institutional Review Board

A copy of the protocol, proposed informed consent forms, other written patient information, and any proposed advertising material must be submitted to the IRB for written approval. A copy of the written approval of the protocol and informed consent form must be received by the Sponsor or designee before recruitment of patients into the study and shipment of Study Drug. A copy of the written approval of any other items/materials that must be approved by the Study Center or IRB must also be received by the Sponsor or designee before recruitment of patients into the study and shipment of Study Drug. The Investigator's Brochure must be submitted to the IRB for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent document. The Investigator should notify the IRB of deviations from the protocol in accordance with ICH GCP Section 4.5.2. The Investigator should also notify the IRB of serious adverse events occurring at the Study Center and other adverse event reports received from the Sponsor or designee, in accordance with local procedures.

The Investigator will be responsible for obtaining annual IRB approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all IRB submissions and the IRB continuance of approval must be sent to the Sponsor or designee.

11.4 Patient Confidentiality

The Investigator must ensure that the patient's confidentiality is maintained. On the case report forms or other documents submitted to the Sponsor or designee, patients should be identified by unique initials and a patient study number only. Documents that are not for submission to the Sponsor or designee (e.g., signed informed consent forms) should be kept in strict confidence by the Investigator.

In compliance with national and local regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB direct access to review the patient's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the

study. The Investigator is obligated to inform and obtain the consent of the patient to permit named representatives to have access to his/her study-related records without violating the confidentiality of the patient.

12. ADMINISTRATIVE AND LEGAL OBLIGATIONS

12.1 Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor or designee. Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent document. The regulatory authority and IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the patients or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the IRB to the Sponsor or designee.

12.2 Study Termination

Both the Sponsor or designee and the Investigator reserve the right to terminate the study, according to the terms of the study contract. The Investigator/Sponsor or designee should notify the IRB in writing of the trial's completion or early termination and send a copy of the notification to the Sponsor or designee.

12.3 Study Documentation and Storage

An electronic case report form (eCRF) utilizing an Electronic Data Capture (EDC) application will be used for this study.

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the patient's case report form data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, imaging, and correspondence. In this study, CRFs may not be used as source documents.

The Investigator and Study Center staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with Section 8 of the ICH Guidelines (E6), suitable for inspection at any time by representatives from the Sponsor or designee and/or applicable regulatory authorities. Elements should include:

- Patient files containing completed case report forms, informed consents, and supporting copies of source documentation
- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the IRB and the Sponsor or designee
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction, Final Study Drug Product Reconciliation Statement, and all drug-related correspondence

In addition, all original source documents supporting entries in the case report forms must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor or designee and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor or designee.

12.4 Study Monitoring

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., case report forms and other pertinent data) provided that subject confidentiality is respected.

The Sponsor monitor or designee is responsible for inspecting the case report forms at regular intervals throughout the Study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the case report forms.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing case report forms, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this Study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department (or designees). Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data a clinical data management review will be performed on subject data received by the Sponsor or designee. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor or designee.

The Principal Investigator will sign and date the indicated places on the case report form. These signatures will indicate that the Principal Investigator inspected or reviewed the data on the case report form, the data queries, and the Study Center notifications, and agrees with the content.

12.5 Language

Case report forms must be completed in English. Generic names for concomitant medications should be recorded in English if possible, unless it is a combination drug, then record the trade name in English.

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood.

12.6 Compensation for Injury

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Subjects will be treated and/or compensated for any study-related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

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14. APPENDICES

Appendix A Schedule of Procedures

Appendix A Schedule of Procedures – Cohorts 1–3

	Screen	Treatment (4 wks)								Post-Treatment Follow-Up (26 wks)						Early Term
Study Week (W) and/or Study Day	**S-28 to S-1**	**W1 D1**	**W1 D2**	**W1 D4**	**W2 D8**	**W2 D11**	**W3 D15**	**W4 D22**	**W4 D23**	**W5 D29**	**W6 D36**	**W9 D57**	**W13 D85**	**W17 D113**	**W31 D211**	
Allowed Variance Window *	**n/a**	**n/a**	**^**	**± 1**	**± 1**	**± 1**	**± 1**	**± 1**	**^**	**§**	**± 1**	**± 3**	**± 7**	**± 7**	**± 7**	**n/a**
Study Drug Administration		X		X	X	X	X	X								
Tenofovir (or entecavir) Daily Administration										X [e]	X	X	X	X	X	
Informed Consent	X															
Inclusion/Exclusion, Medical History	X															
Body Weight and Height [1]	X	X [a]						X		X[a]		X		X	X	X
Physical Exam	X									X[a]				X	X	X
Vital Signs [2]	X	X[a]	X		X[a]	X[a]	X[a]	X[a]	X	X[a]	X	X	X	X	X	X
HIV, Hepatitis C & D	X															
Pregnancy Test [3]	X	X							X		X		X	X	X	X
Alpha-fetoprotein	X															
Chemistry, Hematology, Urinalysis [5]	X	X[a]	X [b]		X[a]		X[a]		X[b]	X[a]	X	X	X	X	X	X
Complement C3	X	X[a]			X[a]		X[a]	X[a]		X[a]	X	X		X	X	X
Complement split products		X [c]	X[b]					X [c]	X[b]		X	X		X	X	X
PT, INR, aPTT	X	X[c]	X[b]					X[c]	X[b]		X	X		X	X	X
C-reactive protein	X	X[a]	X[b]	X[a]	X[a]		X[a]	X[a]	X[b]	X[a]	X	X		X	X	X
Quantitative HBsAg, HBeAg, HBV DNA	X	X[a]					X[a]		X	X[a]	X	X	X	X	X	X
Anti-HBs and anti-HBe antibodies	X	X[a]								X[a]		X		X	X	X
HBsAg genotype		X[a]														
HBV Drug Binding Sites DNA Sequence		X[a]								X[a]				X		X
Archived Serum & Plasma Samples [6]	X	X[a]	X[b]		X[a]		X[a]	X[a]	X[b]	X[a]	X	X	X	X	X	X
Archived PBMC Samples[7]		X[a]					X[a]			X[a]		X		X	X	X
ECG (12-Lead) in triplicate at each time point	X	X[c]	X[b]					X[c]	X[b]	X[a]				X		X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tenofovir (or entecavir) concentration [8]										X[a]	X	X	X	X	X	X
ISIS 505358 PK Blood Sampling [9]		X [d]	X[b]	X[a]	X[a]		X[a]	X[d]	X[b]	X[a]	X	X	X	X	X	X

Appendix A Schedule of Procedures – Cohort 4

	Screen	Treatment (4 wks)								Post-Treatment Follow-Up (26 wks)						Early Term
Study Week (W) and/or Study Day	**S-28 to S-1**	**W1 D1**	**W1 D2**	**W1 D4**	**W2 D8**	**W2 D11**	**W3 D15**	**W4 D22**	**W4 D23**	**W5 D29**	**W6 D36**	**W9 D57**	**W13 D85**	**W17 D113**	**W31 D211**	
Allowed Variance Window *	**n/a**	**n/a**	**^**	**± 1**	**± 1**	**± 1**	**± 1**	**± 1**	**^**	**§**	**± 1**	**± 3**	**± 7**	**± 7**	**± 7**	**n/a**
Study Drug Administration		X		X	X	X	X	X								
Nucleos(t)ide Analogue Daily Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Informed Consent	X															
Inclusion/Exclusion, Medical History	X															
Body Weight and Height [1]	X	X [a]						X		X[a]		X		X	X	X
Physical Exam	X									X[a]				X	X	X
Vital Signs [2]	X	X[a]	X		X[a]	X[a]	X[a]	X[a]	X	X[a]	X	X	X	X	X	X
HIV, Hepatitis C & D	X															
Pregnancy Test [3]	X	X							X		X		X	X	X	X
Alpha-fetoprotein	X															
Chemistry, Hematology, Urinalysis [5]	X	X[a]	X [b]		X[a]		X[a]		X[b]	X[a]	X	X	X	X	X	X
Complement C3	X	X[a]			X[a]		X[a]	X[a]		X[a]	X	X		X	X	X
Complement split products		X [c]	X[b]					X [c]	X[b]		X	X		X	X	X
PT, INR, aPTT	X	X[c]	X[b]					X[c]	X[b]		X	X		X	X	X
C-reactive protein	X	X[a]	X[b]	X[a]	X[a]		X[a]	X[a]	X[b]	X[a]	X	X		X	X	X
Quantitative HBsAg, HBeAg, HBV DNA	X	X[a]					X[a]		X	X[a]	X	X	X	X	X	X
Anti-HBs and anti-HBe antibodies	X	X[a]								X[a]		X		X	X	X
HBsAg genotype		X[a]														
HBV Drug Binding Sites DNA Sequence		X[a]								X[a]				X		X
Archived Serum & Plasma Samples [6]	X	X[a]	X[b]		X[a]		X[a]	X[a]	X[b]	X[a]	X	X	X	X	X	X
Archived PBMC Samples[7]		X[a]					X[a]			X[a]		X		X	X	X
ECG (12-Lead) in triplicate at each time point	X	X[c]	X[b]					X[c]	X[b]	X[a]				X		X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nucleos(t)ide Analogue concentration [8]	X[10]	X			X		X	X		X[a]	X	X	X	X	X	X
ISIS 505358 PK Blood Sampling [9]		X [d]	X[b]	X[a]	X[a]		X[a]	X[d]	X[b]	X[a]	X	X	X	X	X	X

Appendix A Schedule of Procedures *Continued*

* See Section 6.1.2 and 6.1.3 for additional details
^ Day 2 and Day 23 visits must occur on the day after the actual Day 1 and Day 22 visits, respectively
§ Every attempt should be made for the Study Day 29 visit to occur 7 days after the actual occurrence of Study Day 22
n/a Not applicable

Note: If not specifically labeled, "X" means anytime

1 Height measured at Screen visit only
2 BP, HR, RR, temp
3 Women who are not surgically sterile or post-menopausal
4 Women who are post-menopausal and not surgically sterile
5 If hematuria or 2 + proteinuria is observed, see confirmation guidance in Section 8.5
6 Stored at -80° C for retrospective clinical safety, immunologic, and/or virologic assays (e.g., retrospective LDH or CPK isoform measurements, measurement of cytokine and/or chemokine levels, and/or measurement of additional markers of kidney function, or mutation analysis of breakthrough HBV) in this or subsequent clinical studies of ISIS 505358
7 PBMC samples prepared from whole blood, frozen and stored for analysis at future time to be determined based upon results from HBV tests
8 Time of nucleos(t)ide administration and fed/fasted status at time of dose ingestion must be recorded. Time and fed/fasted status does not need to be controlled, but should be similar on each blood draw day
9 See also Appendix C
10 Measurement result not required for Screen pass/fail decision

Time (time is in reference to Study Drug administration):

a Pre-dose (for Day 29, is before first nucleos(t)ide analogue dose)
b 24-hour
c Pre-dose and 3 and 5 hours post-dose
d 0.5, 1, 1.5, 2, 3, 4, 5, and 6 hours post-dose
e Initiate dosing after specimen collections for Day 29 laboratory tests

Appendix B List of Laboratory Analytes

Appendix B List of Laboratory Analytes

Clinical Chemistry Panel

- Sodium
- Potassium
- Chloride
- Bicarbonate
- Total protein
- Albumin
- Calcium
- Magnesium
- Phosphorus
- Glucose
- BUN
- Creatinine
- Cholesterol
- Uric Acid
- Total bilirubin
- Direct (conjugated) bilirubin
- Indirect (unconjugated) bilirubin
- ALT
- AST
- Alkaline phosphatase
- Creatinine kinase
- GGT
- LDH

Screening Tests

- Hepatitis D antibody
- Hepatitis C antibody
- HIV antibody
- Serum βhCG
- Alpha-fetoprotein

Coagulation

- aPTT (sec)
- PT (sec)
- INR

Complement

- C5a
- Bb
- C3

Biomarkers

- C-reactive protein

Pharmacokinetics[1]

- ISIS 505358 levels in plasma
- Tenofovir levels in plasma
- Entecavir levels in plasma

Hematology

- Red blood cells
- Hemoglobin
- Hematocrit
- MCV, MCH, MCHC
- Platelets
- White blood cells
- WBC Differential (% and absolute)
  - Neutrophils
  - Eosinophils
  - Basophils
  - Lymphocytes
  - Monocytes

HBV Tests, Quantitative

- HBsAg
- HBeAg
- HBV DNA

HBV Tests, Qualitative

- Anti-HBs antibody
- Anti-HBe antibody
- HBsAg genotype
- HBV DNA sequence at drug binding sites

Urinalysis

- Color
- Appearance
- Specific gravity
- pH
- P/C Ratio
- Protein
- Blood
- Ketones
- Urobilinogen
- Glucose
- Bilirubin
- Leukocyte esterase
- Nitrate
- Microscopic examination
- Albumin/creatinine ratio

1 Plasma PK samples may also be used for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity testing (or possibly for purposes of immunogenicity assay development and/or validation), or to assess other actions of ISIS 505358 with plasma constituents

Appendix C Plasma PK Sampling Schedules

Appendix C PK Sampling Schedules – Cohorts 1–3

ISIS 505358 Plasma PK Blood Sampling Schedule

D1	D2	D4	D8	D15	D22	D23	D29
Blood: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6 hours post SC Injection	Blood: 24-hour	Blood: Pre-dose	Blood: Pre-dose	Blood: Pre-dose	Blood: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6 hours post SC injection	Blood: 24-hour	Blood: anytime

D36	D57	D85	D113	D211
Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime

Nucleos(t)ide Analogue Sampling Schedule

D29	D36	D57	D85	D113	D211
Blood: before first dose	Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime

Pre-dose refers to prior to administration of ISIS 505358.

Time of nucleos(t)ide administration and fed/fasted at time of dose ingestion must be recorded. Time and fed/fasted (due to food effect for both nucleos(t)ide analogues) does not need to be controlled, but should be similar for each dose on blood draw days, and blood draw time should be similar for each day.

Appendix C PK Sampling Schedules – Cohort 4

ISIS 505358 Plasma PK Blood Sampling Schedule

D1	D2	D4	D8	D15	D22	D23	D29
Blood: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6 hours post-SC Injection	Blood: 24-hour	Blood: Pre-dose	Blood: Pre-dose	Blood: Pre-dose	Blood: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6 hours post-SC injection	Blood: 24-hour	Blood: anytime

D36	D57	D85	D113	D211
Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime

Nucleos(t)ide Analogue Sampling Schedule

Screen	D1	D2	D4	D8	D15	D22	D23
Blood: anytime	Blood: pre-dose	---	---	Blood: pre-dose	Blood: pre-dose	Blood: pre-dose	---

D29	D36	D57	D85	D113	D211
Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime	Blood: anytime

Pre-dose and 24-hour refers to administration of ISIS 505358.

Time of nucleos(t)ide administration and fed/fasted at time of dose ingestion must be recorded. Time and fed/fasted (due to food effect for both nucleos(t)ide analogues) does not need to be controlled, but should be similar for each dose on blood draw days, and blood draw time should be similar for each day.

Appendix D Division of AIDS Table for Grading Severity of Adult and Pediatric Adverse Events Version 2.0, November 2014

Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events

Version 2.0
November 2014

Division of AIDS
National Institute of Allergy and Infectious Diseases
National Institutes of Health
US Department of Health and Human Services

Table of Contents

Glossary and Acronyms

AE	Adverse event; Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.
ALT (SGPT)	Alanine aminotransferase (serum glutamic pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)
AV	Atrioventricular
Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer or movement, continence, and feeding. Young Children Activities that are age and culturally appropriate, such as feeding one's self with culturally appropriate eating implements.
BMI z-score	Body mass index z- score; A body reference norm. Specifically, the number of standard deviations a participant's BMI differs from the average BMI for their age, sex, and ethnicity.
BMD t-score	Bone mineral density t-score; The number of standard deviations above or below the mean bone mineral density of a healthy 30 year old adult of the same sex and ethnicity as the participant.
BMD z-score	Bone mineral density z-score; The number of standard deviations a participant's BMD differs from the average BMD for their age, sex, and ethnicity.
BPAP	Bilevel positive airway pressure; A mode used during noninvasive positive pressure ventilation.
Chemical Pregnancy	A pregnancy in which a positive pregnancy test is followed by a negative pregnancy test without evidence of a clinical pregnancy loss.
CNS	Central nervous system
CPAP	Continuous positive airway pressure
DAERS	DAIDS Adverse Experience Reporting System; An internet-based system developed for clinical research sites to report Expedited Adverse Events (EAEs) to DAIDS. It facilitates timely EAE report submission and serves as a centralized location for accessing and processing EAE information for reporting purposes.
Disability	A substantial disruption of a person's ability to conduct normal life functions.
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
Hospitalization	Does not include the following hospital admissions: under 24 hours, unrelated to an adverse event (e.g., for labor and delivery, cosmetic surgery, social or administrative for temporary placement [for lack of a place to sleep]), protocol-specified, and for diagnosis or therapy of a condition that existed before the receipt of a study agent and which has not increased in severity or frequency.
INR	International normalized ratio

Glossary and Acronyms

Intervention	Medical, surgical, or other procedures recommended or provided by a healthcare professional for the treatment of an adverse event.
IV	Intravenous
IVIG	Intravenous immune globulin
LDL	Low density lipoprotein
LLN	Lower limit of normal
Life-threatening AE	Any adverse event that places the participant, in the view of the investigator, at immediate risk of death from the reaction when it occurred (i.e., it does not include a reaction that would have caused death if it had occurred in a more severe form).
NA	Not applicable
Participant ID	The identification number assigned to a study participant which is used to track study-related documentation, including any reported AEs.
PR Interval	The interval between the beginning of the P wave and the beginning of the QRS complex of an electrocardiogram that represents the time between the beginning of the contraction of the atria and the beginning of the contraction of the ventricles.
PT	Prothrombin time
PTT	Partial thromboplastin time
QTc Interval	The measure of time between the onset of ventricular depolarization and completion of ventricular repolarization corrected for ventricular rate.
RBC	Red blood cell
SI	Standard international unit
ULN	Upper limit of normal
Usual Social & Functional Activities	Activities which adults and children perform on a routine basis and those which are part of regular activities of daily living, for example: <u>Adults</u> Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, or pursuing a hobby. <u>Young Children</u> Activities that are age and culturally appropriate, such as social interactions, play activities, or learning tasks.
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits

Introduction

The Division of AIDS (DAIDS) oversees clinical trials throughout the world which it sponsors and supports. The clinical trials evaluate the safety and efficacy of therapeutic products, vaccines, and other preventive modalities. Adverse event (AE) data collected during these clinical trials form the basis for subsequent safety and efficacy analyses of pharmaceutical products and medical devices. Incorrect and inconsistent AE severity grading can lead to inaccurate data analyses and interpretation, which in turn can impact the safety and well-being of clinical trial participants and future patients using pharmaceutical products.

The DAIDS AE grading table is a shared tool for assessing the severity of AEs (including clinical and laboratory abnormalities) in participants enrolled in clinical trials. Over the years as scientific knowledge and experience have expanded, revisions to the DAIDS AE grading table have become necessary.

The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* replaces the grading table published in 2004 and updated in 2009. In version 2.0, AEs not previously included, but which now are deemed medically important events, are included while other AEs have been removed. Some AE severity grading descriptions have been revised to more appropriately reflect the presentation of these events in clinical settings and their impact on clinical trials. For example, DAIDS performed an extensive literature search and reviews of select DAIDS clinical trial data in revising certain hematology parameters (i.e., hemoglobin, white cell counts, and absolute neutrophil counts). DAIDS also took into consideration the U.S. Food and Drug Administration's guidance regarding the use of local laboratory reference values and ethnic differences among certain healthy adolescent and adult populations in defining parameter limits. Finally, the revised DAIDS AE grading table also contains an updated glossary and acronyms section, an expanded instructions for use section, and an appendix that provides more age-specific information for an AE of concern to DAIDS.

DAIDS is grateful to the DAIDS Grading Table Working Group, numerous government and non-government affiliated medical subject matter experts and reviewers who were instrumental in the revision of the DAIDS AE grading table.

Instructions for Use

General Considerations

The *Division of AIDS* (*DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* consists of parameters, or AEs, with severity grading guidance that are to be used in DAIDS clinical trials for safety data reporting to maintain accuracy and consistency in the evaluation of AEs. The term "severe" is not the same as the term "serious" in classifying AEs. The severity of a specific event describes its intensity, and it is the intensity which is graded. Seriousness, which is not graded, relates to an outcome of an AE and is a regulatory definition.

Clinical sites are encouraged to report parameters in the DAIDS AE grading table as they are written to maintain data consistency across clinical trials. However, since some parameters can be reported with more specificity, clinical sites are encouraged to report parameters that convey additional clinical information. For example, diarrhea could be reported as neonatal diarrhea; seizures, as febrile seizures; and pain, as jaw pain.

The DAIDS AE grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

- Grade 1 indicates a mild event
- Grade 2 indicates a moderate event
- Grade 3 indicates a severe event
- Grade 4 indicates a potentially life-threatening event
- Grade 5 indicates death (*Note*: This grade is not specifically listed on each page of the grading table).

Other points to consider include:

- Use parameters defined by age and sex values as applicable.
- Male and female sex are defined as sex at birth.
- Unless noted, laboratory values are for term neonates. Preterm neonates should be assessed using local laboratory normal ranges.
- Where applicable, Standard International (SI) units are included in italics.

Selecting and Reporting a Primary AE Term

When selecting a primary AE term to report, sites should select the term that best describes what occurred to the participant. For example, a participant may present with itching, urticaria, flushing, angioedema of the face, and dyspnea. If the underlying diagnosis is determined to be an acute allergic reaction, sites should report "Acute Allergic Reaction" as the primary AE term.

Primary AE terms should be reported using the DAIDS Adverse Experience Reporting System (DAERS) only if they meet expedited reporting criteria. However, all primary AE terms should be reported using protocol-specific case report forms (CRFs). Because the reported information is stored in different databases (i.e., safety and clinical), sites should report primary AE terms using the same terminology for data consistency.

Instructions for Use

When reporting using DAERS, other clinically significant events associated with a primary AE term that more fully describe the nature, severity, or complications of the primary AE term should be entered in the "Other Events" section. However, the severity grade for these events must be lower than or equal to the severity grade of the primary AE term. In the example above, dyspnea and angioedema of the face may be entered in the "Other Events" section, because they are more descriptive and provide additional information on the severity of the acute allergic reaction. However, their severity grades must be lower than or equal to the severity grade of the primary AE term of "Acute Allergic Reaction".

Differences exist in the reporting and recording of information (e.g., signs and symptoms, clinically significant events) in DAERS and CRFs. Therefore, sites should refer to their protocols and CRF requirements for further instructions.

Grading Adult and Pediatric AEs
When a single parameter is not appropriate for grading an AE in both adult and pediatric populations, separate parameters with specified age ranges are provided. If no distinction between adult and pediatric populations has been made, the listed parameter should be used for grading an AE in both populations.

Reporting Pregnancy Outcomes
In the *Pregnancy, Puerperium, and Perinatal* section, all parameters are pregnancy outcomes and should be reported using the mother's participant ID. If an infant is not enrolled in the same study as the mother, any identified birth defects should be reported using the mother's participant ID. However, if an infant is enrolled in the same study as the mother or in another study, any identified birth defects should be reported using the infant's participant ID. Sites should refer to the applicable network standards for reporting abnormal pregnancy outcomes on the CRFs.

Determining Severity Grade for Parameters between Grades
If the severity of an AE could fall in either one of two grades (i.e., the severity of an AE could be either grade 2 or grade 3), sites should select the higher of the two grades.

Laboratory Values
General. An asymptomatic, abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited timeframe unless it meets protocol-specific reporting requirements. Sites should refer to the applicable network standards for reporting abnormal laboratory findings on the CRFs.

Values below Grade 1. Any laboratory value that is between the ULN and grade 1 (for high values) or the LLN and grade 1 (for low values) should not be graded or reported as an AE. Sites should consult the *Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0* and their protocol when making an assessment of the need to report an AE.

Overlap of Local Laboratory Normal Values with Grading Table Ranges. When local laboratory normal values fall within grading table laboratory ranges, the severity grading is based on the ranges in the grading table unless there is a protocol-specific grading criterion for the laboratory

Instructions for Use

value. For example, “Magnesium, Low" has a grade 1 range of 1.2 to < 1.4 mEq/L, while a particular laboratory’s normal range for magnesium may be 1.3 to 2.8 mEq/L. If a study participant’s magnesium laboratory value is 1.3 mEq/L, the laboratory value should be graded as grade 1.

Appendix Usage
Appendix A takes priority over the main grading table in all assessments of total bilirubin for term and preterm neonates.

Using Addenda 1-3: Grading Tables Used in Microbicide Studies
In protocols involving topical application of products to the female and male genital tracts or rectum, strong consideration should be given to using Addenda 1-3 (see below) as the primary grading tables for these areas. Although these grading tables are used specifically in microbicide studies, they may be used in other protocols as adjuncts to the main grading table (i.e., the *Division of AIDS (AIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0*). It should be clearly stated in a protocol which addendum is being used as the primary grading table (and thus takes precedence over the main grading table) and which addendum is being used in a complementary fashion.

- Addendum 1 – Female Genital Grading Table for Use in Microbicide Studies – PDF
- Addendum 2 – Male Genital Grading Table for Use in Microbicide Studies – PDF
- Addendum 3 – Rectal Grading Table for Use in Microbicide Studies – PDF

Estimating Severity Grade for Parameters Not Identified in the Grading Table
The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as grade 5.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Major Clinical Conditions
Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arrhythmia (by ECG or physical examination) *Specify type, if applicable*	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities[1] ***Hypertension*** *(with the lowest reading taken after repeat testing during a visit)* *≥ 18 years of age*	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	≥ 160 to < 180 mmHg systolic OR ≥ 100 to < 110 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
< 18 years of age	> 120/80 mmHg	≥ 95th to < 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	≥ 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated

[1] Blood pressure norms for children < 18 years of age can be found in: Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents. *Pediatrics* 2011;128;S213; originally published online November 14, 2011; DOI: 10.1542/peds.2009-2107C.

Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Prolonged PR Interval or AV Block *Report only one* *> 16 years of age*	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
≤ 16 years of age	1st degree AV block (PR interval > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
Prolonged QTc Interval[2]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)

[2] As per Bazett's formula.

Dermatologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Alopecia (scalp only)	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	NA	NA
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Cellulitis	NA	Non-parenteral treatment indicated (e.g., oral antibiotics, antifungals, antivirals)	IV treatment indicated (e.g., IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g., sepsis, tissue necrosis)
Hyperpigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Hypopigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Petechiae	Localized to one area	Localized to more than one area	Generalized	NA
Pruritus[3] (without skin lesions)	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
Rash *Specify type, if applicable*	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis

[3] For pruritus associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).

Endocrine and Metabolic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Diabetes Mellitus	Controlled without medication	Controlled with medication OR Modification of current medication regimen	Uncontrolled despite treatment modification OR Hospitalization for immediate glucose control indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma, end organ failure)
Gynecomastia	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing pain with greater than minimal interference with usual social & functional activities	Disfiguring changes AND Symptoms requiring intervention or causing inability to perform usual social & functional activities	NA
Hyperthyroidism	No symptoms AND Abnormal laboratory value	Symptoms 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	No symptoms AND Abnormal laboratory value	Symptoms 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[4]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
Lipohypertrophy[5]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA

[4] Definition: A disorder characterized by fat loss in the face, extremities, and buttocks.
[5] Definition: A disorder characterized by abnormal fat accumulation on the back of the neck, breasts, and abdomen.

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
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, total parenteral nutrition)
Ascites	No symptoms	Symptoms AND Intervention indicated (e.g., diuretics, therapeutic paracentesis)	Symptoms recur or persist despite intervention	Life-threatening consequences
Bloating or Distension *Report only one*	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
Cholecystitis	NA	Symptoms AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis, 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 *≥ 1 year of age*	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
< 1 year of age	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Dysphagia or Odynophagia *Report only one and specify location*	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake
Gastrointestinal Bleeding	Not requiring intervention other than iron supplement	Endoscopic intervention indicated	Transfusion indicated	Life-threatening consequences (e.g., hypotensive shock)

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Mucositis or Stomatitis *Report only one and specify location*	Mucosal erythema	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Life-threatening consequences (e.g., aspiration, choking) OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptoms with hospitalization not indicated	Symptoms with hospitalization indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Perforation (colon or rectum)	NA	NA	Intervention indicated	Life-threatening consequences
Proctitis	Rectal discomfort with 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)
Rectal Discharge	Visible discharge	Discharge requiring the use of pads	NA	NA
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)

Musculoskeletal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arthralgia	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	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
Myalgia (generalized)	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	No symptoms but with radiographic findings AND No operative intervention indicated	Bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
Osteopenia[6] *≥ 30 years of age*	BMD t-score -2.5 to -1	NA	NA	NA
< 30 years of age	BMD z-score -2 to -1	NA	NA	NA
Osteoporosis[6] *≥ 30 years of age*	NA	BMD t-score < -2.5	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences
< 30 years of age	NA	BMD z-score < -2	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences

[6] BMD t and z scores can be found in: Kanis JA on behalf of the World Health Organization Scientific Group (2007). Assessment of osteoporosis at the primary health-care level. Technical Report. World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. 2007: Printed by the University of Sheffield.

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute CNS Ischemia	NA	NA	Transient ischemic attack	Cerebral vascular accident (e.g., stroke with neurological deficit)
Altered Mental Status (for Dementia, see *Cognitive, Behavioral, or Attentional Disturbance* below)	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	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions
Cognitive, Behavioral, or Attentional Disturbance (includes dementia and attention deficit disorder) *Specify type, if applicable*	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
Developmental Delay *< 18 years of age* *Specify type, if applicable*	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated OR Headache with significant impairment of alertness or other neurologic function

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Neuromuscular Weakness (includes myopathy and neuropathy) *Specify type, if applicable*	Minimal muscle weakness causing no or minimal interference with usual social & functional activities OR No symptoms with decreased strength on examination	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 (includes paresthesia and painful neuropathy) *Specify type, if applicable*	Minimal paresthesia causing no or minimal interference with usual social & functional activities OR No symptoms with sensory alteration on examination	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
Seizures ***New Onset Seizure*** *≥ 18 years of age*	NA	NA	1 to 3 seizures	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
< 18 years of age (includes new or pre-existing febrile seizures)	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Pre-existing Seizure	NA	Increased frequency from previous level of control without change in seizure character	Change in seizure character either in duration or quality (e.g., severity or focality)	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA

Pregnancy, Puerperium, and Perinatal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Fetal Death or Stillbirth (report using mother's participant ID) *Report only one*	NA	NA	Fetal loss occurring at ≥ 20 weeks gestation	NA
Preterm Delivery[7] (report using mother's participant ID)	Delivery at 34 to < 37 weeks gestational age	Delivery at 28 to < 34 weeks gestational age	Delivery at 24 to < 28 weeks gestational age	Delivery at < 24 weeks gestational age
Spontaneous Abortion or Miscarriage[8] (report using mother's participant ID) *Report only one*	Chemical pregnancy	Uncomplicated spontaneous abortion or miscarriage	Complicated spontaneous abortion or miscarriage	NA

[7] Definition: A delivery of a live-born neonate occurring at ≥ 20 to < 37 weeks gestational age.
[8] Definition: A clinically recognized pregnancy occurring at < 20 weeks gestational age.

Psychiatric

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Insomnia	Mild difficulty falling asleep, staying asleep, or waking up early	Moderate difficulty falling asleep, staying asleep, or waking up early	Severe difficulty falling asleep, staying asleep, or waking up early	NA
Psychiatric Disorders (includes anxiety, depression, mania, and psychosis) *Specify disorder*	Symptoms with intervention not indicated OR Behavior causing no or minimal interference with usual social & functional activities	Symptoms with intervention indicated OR Behavior causing greater than minimal interference with usual social & functional activities	Symptoms with hospitalization indicated OR Behavior causing inability to perform usual social & functional activities	Threatens harm to self or others OR Acute psychosis OR Behavior causing inability to perform basic self-care functions
Suicidal Ideation or Attempt *Report only one*	Preoccupied with thoughts of death AND No wish to kill oneself	Preoccupied with thoughts of death AND Wish to kill oneself with no specific plan or intent	Thoughts of killing oneself with partial or complete plans but no attempt to do so OR Hospitalization indicated	Suicide attempted

Respiratory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Bronchospasm	Forced expiratory volume in 1 second or peak flow reduced to ≥ 70 to < 80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 second or peak flow 50 to < 70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 second or peak flow 25 to < 50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 second or peak flow < 25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress *Report only one*	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR 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 ventilator support indicated (e.g., CPAP, BPAP, intubation)

Sensory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hearing Loss *≥ 12 years of age*	NA	Hearing aid or intervention not indicated	Hearing aid or intervention indicated	Profound bilateral hearing loss (> 80 dB at 2 kHz and above) OR Non-serviceable hearing (i.e., >50 dB audiogram and <50% speech discrimination)
< 12 years of age (based on a 1, 2, 3, 4, 6 and 8 kHz audiogram)	> 20 dB hearing loss at ≤ 4 kHz	> 20 dB hearing loss at > 4 kHz	> 20 dB hearing loss at ≥ 3 kHz in one ear with additional speech language related services indicated (where available) OR Hearing loss sufficient to indicate therapeutic intervention, including hearing aids	Audiologic indication for cochlear implant and additional speech-language related services indicated (where available)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Uveitis	No symptoms AND Detectable on examination	Anterior uveitis with symptoms OR Medicamylasal intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
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
Visual Changes (assessed 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)

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of 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
Cytokine Release Syndrome[9]	Mild signs and symptoms AND Therapy (i.e., antibody infusion) interruption not indicated	Therapy (i.e., antibody infusion) interruption indicated AND Responds promptly to symptomatic treatment OR Prophylactic medications indicated for ≤ 24 hours	Prolonged severe signs and symptoms OR Recurrence of symptoms following initial improvement	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Fatigue or Malaise *Report only one*	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 symptoms of fatigue or malaise causing inability to perform basic self-care functions
Fever (non-axillary temperatures only)	38.0 to < 38.6°C or 100.4 to < 101.5°F	≥ 38.6 to < 39.3°C or ≥ 101.5 to < 102.7°F	≥ 39.3 to < 40.0°C or ≥ 102.7 to < 104.0°F	≥ 40.0°C or ≥ 104.0°F
Pain[10] (not associated with study agent injections and not specified elsewhere) *Specify location*	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 indicated
Serum Sickness[11]	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (e.g., antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (e.g., steroids or IV fluids)	Life-threatening consequences (e.g., requiring pressor or ventilator support)

[9] Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and/or shortness of breath.
[10] For pain associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).
[11] Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Underweight[12] *> 5 to 19 years of age*	NA	WHO BMI z-score < -2 to ≤ -3	WHO BMI z-score < -3	WHO BMI z-score < -3 with life-threatening consequences
2 to 5 years of age	NA	WHO Weight-for-height z-score < -2 to ≤ -3	WHO Weight-for-height z-score < -3	WHO Weight-for-height z-score < -3 with life-threatening consequences
< 2 years of age	NA	WHO Weight-for-length z-score < -2 to ≤ -3	WHO Weight-for-length z-score < -3	WHO Weight-for-length z-score < -3 with life-threatening consequences
Weight Loss (excludes postpartum weight loss)	NA	5 to < 9% loss in body weight from baseline	≥ 9 to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)

[12] WHO reference tables may be accesed by clicking the desired age range or by accessing the following URLs: http://www.who.int/growthref/who2007_bmi_for_age/en/ for participants > 5 to 19 years of age and http://www.who.int/childgrowth/standards/chart_catalogue/en/ for those ≤ 5 years of age.

Urinary

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Urinary Tract Obstruction	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

Site Reactions to Injections and Infusions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[13] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*
Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

[13] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

Laboratory Values
Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acidosis	NA	pH ≥ 7.3 to < LLN	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, Low (g/dL; *g/L*)	3.0 to < LLN *30 to < LLN*	≥ 2.0 to < 3.0 *≥ 20 to < 30*	< 2.0 *< 20*	NA
Alkaline Phosphatase, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Alkalosis	NA	pH > ULN to ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT or SGPT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Amylase (Pancreatic) or Amylase (Total), High *Report only one*	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
AST or SGOT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Bicarbonate, Low (mEq/L; *mmol/L*)	16.0 to < LLN *16.0 to < LLN*	11.0 to < 16.0 *11.0 to < 16.0*	8.0 to < 11.0 *8.0 to < 11.0*	< 8.0 *< 8.0*
Bilirubin ***Direct Bilirubin[14], High*** *> 28 days of age*	NA	NA	> ULN	> ULN with life-threatening consequences (e.g., signs and symptoms of liver failure)
≤ 28 days of age	ULN to ≤ 1 mg/dL	> 1 to ≤ 1.5 mg/dL	> 1.5 to ≤ 2 mg/dL	> 2 mg/dL
Total Bilirubin, High *> 28 days of age*	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
≤ 28 days of age	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates
Calcium, High (mg/dL; *mmol/L*) *≥ 7 days of age*	10.6 to < 11.5 *2.65 to < 2.88*	11.5 to < 12.5 *2.88 to < 3.13*	12.5 to < 13.5 *3.13 to < 3.38*	≥ 13.5 *≥ 3.38*
< 7 days of age	11.5 to < 12.4 *2.88 to < 3.10*	12.4 to < 12.9 *3.10 to < 3.23*	12.9 to < 13.5 *3.23 to < 3.38*	≥ 13.5 *≥ 3.38*

[14] Direct bilirubin > 1.5 mg/dL in a participant < 28 days of age should be graded as grade 2, if < 10% of the total bilirubin.

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Calcium (Ionized), High (mg/dL; *mmol/L*)	> ULN to < 6.0 *> ULN to < 1.5*	6.0 to < 6.4 *1.5 to < 1.6*	6.4 to < 7.2 *1.6 to < 1.8*	≥ 7.2 *≥ 1.8*
Calcium, Low (mg/dL; *mmol/L*) *≥ 7 days of age*	7.8 to < 8.4 *1.95 to < 2.10*	7.0 to < 7.8 *1.75 to < 1.95*	6.1 to < 7.0 *1.53 to < 1.75*	< 6.1 *< 1.53*
< 7 days of age	6.5 to < 7.5 *1.63 to < 1.88*	6.0 to < 6.5 *1.50 to < 1.63*	5.50 to < 6.0 *1.38 to < 1.50*	< 5.50 *< 1.38*
Calcium (Ionized), Low (mg/dL; *mmol/L*)	< LLN to 4.0 *< LLN to 1.0*	3.6 to < 4.0 *0.9 to < 1.0*	3.2 to < 3.6 *0.8 to < 0.9*	< 3.2 *< 0.8*
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10 x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	> 1.8 to < 3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline
Creatinine Clearance[15] or eGFR, Low *Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m^2 OR 10 to < 30% decrease from baseline	< 60 to 30 ml/min or ml/min/1.73 m^2 OR ≥ 30 to < 50% decrease from baseline	< 30 ml/min or ml/min/1.73 m^2 OR ≥ 50% decrease from baseline or dialysis needed
Glucose (mg/dL; *mmol/L*) ***Fasting, High***	110 to 125 *6.11 to < 6.95*	> 125 to 250 *6.95 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Nonfasting, High	116 to 160 *6.44 to < 8.89*	> 160 to 250 *8.89 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Glucose, Low (mg/dL; *mmol/L*) *≥ 1 month of age*	55 to 64 *3.05 to 3.55*	40 to < 55 *2.22 to < 3.05*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
< 1 month of age	50 to 54 *2.78 to 3.00*	40 to < 50 *2.22 to < 2.78*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
Lactate, High	ULN to < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences

[15] Use the applicable formula (i.e., Cockroft-Gault in mL/min or Schwatrz in mL/min/1.73m^2).

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Lipase, High	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
Lipid Disorders (mg/dL; *mmol/L*)				
Cholesterol, Fasting, High				
≥ 18 years of age	200 to < 240 *5.18 to < 6.19*	240 to < 300 *6.19 to < 7.77*	≥ 300 *≥ 7.77*	NA
< 18 years of age	170 to < 200 *4.40 to < 5.15*	200 to < 300 *5.15 to < 7.77*	≥ 300 *≥ 7.77*	NA
LDL, Fasting, High				
≥ 18 years of age	130 to < 160 *3.37 to < 4.12*	160 to < 190 *4.12 to < 4.90*	≥ 190 *≥ 4.90*	NA
> 2 to < 18 years of age	110 to < 130 *2.85 to < 3.34*	130 to < 190 *3.34 to < 4.90*	≥ 190 *≥ 4.90*	NA
Triglycerides, Fasting, High	150 to 300 *1.71 to 3.42*	>300 to 500 *>3.42 to 5.7*	>500 to < 1,000 *>5.7 to 11.4*	> 1,000 *> 11.4*
Magnesium[16], Low (mEq/L; *mmol/L*)	1.2 to < 1.4 *0.60 to < 0.70*	0.9 to < 1.2 *0.45 to < 0.60*	0.6 to < 0.9 *0.30 to < 0.45*	< 0.6 *< 0.30*
Phosphate, Low (mg/dL; *mmol/L*)				
> 14 years of age	2.0 to < LLN *0.81 to < LLN*	1.4 to < 2.0 *0.65 to < 0.81*	1.0 to < 1.4 *0.32 to < 0.65*	< 1.0 *< 0.32*
1 to 14 years of age	3.0 to < 3.5 *0.97 to < 1.13*	2.5 to < 3.0 *0.81 to < 0.97*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
< 1 year of age	3.5 to < 4.5 *1.13 to < 1.45*	2.5 to < 3.5 *0.81 to < 1.13*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
Potassium, High (mEq/L; *mmol/L*)	5.6 to < 6.0 *5.6 to < 6.0*	6.0 to < 6.5 *6.0 to < 6.5*	6.5 to < 7.0 *6.5 to < 7.0*	≥ 7.0 *≥ 7.0*
Potassium, Low (mEq/L; *mmol/L*)	3.0 to < 3.4 *3.0 to < 3.4*	2.5 to < 3.0 *2.5 to < 3.0*	2.0 to < 2.5 *2.0 to < 2.5*	< 2.0 *< 2.0*
Sodium, High (mEq/L; *mmol/L*)	146 to < 150 *146 to < 150*	150 to < 154 *150 to < 154*	154 to < 160 *154 to < 160*	≥ 160 *≥ 160*
Sodium, Low (mEq/L; *mmol/L*)	130 to < 135 *130 to < 135*	125 to < 130 *125 to < 135*	121 to < 125 *121 to < 125*	≤ 120 *≤ 120*
Uric Acid, High (mg/dL; *mmol/L*)	7.5 to < 10.0 *0.45 to < 0.59*	10.0 to < 12.0 *0.59 to < 0.71*	12.0 to < 15.0 *0.71 to < 0.89*	≥ 15.0 *≥ 0.89*

[16] To convert a magnesium value from mg/dL to mmol/L, laboratories should multiply by 0.4114.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Absolute CD4+ Count, Low (cell/mm^3; *cells/L*)				
> 5 years of age (not HIV infected)	300 to < 400 *300 to < 400*	200 to < 300 *200 to < 300*	100 to < 200 *100 to < 200*	< 100 *< 100*
Absolute Lymphocyte Count, Low (cell/mm^3; *cells/L*)				
> 5 years of age (not HIV infected)	600 to < 650 *0.600 x 10^9 to < 0.650 x 10^9*	500 to < 600 *0.500 x 10^9 to < 0.600 x 10^9*	350 to < 500 *0.350 x 10^9 to < 0.500 x 10^9*	< 350 *< 0.350 x 10^9*
Absolute Neutrophil Count (ANC), Low (cells/mm^3; *cells/L*)				
> 7 days of age	800 to 1,000 *0.800 x 10^9 to 1.000 x 10^9*	600 to 799 *0.600 x 10^9 to 0.799 x 10^9*	400 to 599 *0.400 x 10^9 to 0.599 x 10^9*	< 400 *< 0.400 x 10^9*
2 to 7 days of age	1,250 to 1,500 *1.250 x 10^9 to 1.500 x 10^9*	1,000 to 1,249 *1.000 x 10^9 to 1.249 x 10^9*	750 to 999 *0.750 x 10^9 to 0.999 x 10^9*	< 750 *< 0.750 x 10^9*
≤ 1 day of age	4,000 to 5,000 *4.000 x 10^9 to 5.000 x 10^9*	3,000 to 3,999 *3.000 x 10^9 to 3.999 x 10^9*	1,500 to 2,999 *1.500 x 10^9 to 2.999 x 10^9*	< 1,500 *< 1.500 x 10^9*
Fibrinogen, Decreased (mg/dL; *g/L*)	100 to < 200 *1.00 to < 2.00* OR 0.75 to < 1.00 x LLN	75 to < 100 *0.75 to < 1.00* OR ≥ 0.50 to < 0.75 x LLN	50 to < 75 *0.50 to < 0.75* OR 0.25 to < 0.50 x LLN	< 50 *< 0.50* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin[17], Low (g/dL; *mmol/L*)[18]				
≥ 13 years of age (male only)	10.0 to 10.9 *6.19 to 6.76*	9.0 to < 10.0 *5.57 to < 6.19*	7.0 to < 9.0 *4.34 to < 5.57*	< 7.0 *< 4.34*
≥ 13 years of age (female only)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*

[17] Male and female sex are defined as sex at birth.
[18] The conversion factor used to convert g/dL to mmol/L is 0.6206 and is the most commonly used conversion factor. For grading hemoglobin results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for the particular laboratory.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
57 days of age to < 13 years of age (male and female)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*
36 to 56 days of age (male and female)	8.5 to 9.6 *5.26 to 5.99*	7.0 to < 8.5 *4.32 to < 5.26*	6.0 to < 7.0 *3.72 to < 4.32*	< 6.0 *< 3.72*
22 to 35 days of age (male and female)	9.5 to 11.0 *5.88 to 6.86*	8.0 to < 9.5 *4.94 to < 5.88*	6.7 to < 8.0 *4.15 to < 4.94*	< 6.7 *< 4.15*
8 to ≤ 21 days of age (male and female)	11.0 to 13.0 *6.81 to 8.10*	9.0 to < 11.0 *5.57 to < 6.81*	8.0 to < 9.0 *4.96 to < 5.57*	< 8.0 *< 4.96*
≤ 7 days of age (male and female)	13.0 to 14.0 *8.05 to 8.72*	10.0 to < 13.0 *6.19 to < 8.05*	9.0 to < 10.0 *5.59 to < 6.19*	< 9.0 *< 5.59*
INR, High (not on anticoagulation therapy)	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN
Methemoglobin (% hemoglobin)	5.0 to < 10.0%	10.0 to < 15.0%	15.0 to < 20.0%	≥ 20.0%
PTT, High (not on anticoagulation therapy)	1.1 to < 1.66 x ULN	1.66 to < 2.33 x ULN	2.33 to < 3.00 x ULN	≥ 3.00 x ULN
Platelets, Decreased (cells/mm^3; *cells/L*)	100,000 to < 124,999 *100.000 x 10^9 to < 124.999 x 10^9*	50,000 to < 100,000 *50.000 x 10^9 to < 100.000 x 10^9*	25,000 to < 50,000 *25.000 x 10^9 to < 50.000 x 10^9*	< 25,000 *< 25.000 x 10^9*
PT, High (not on anticoagulation therapy	1.1 to < 1.25 x ULN	1.25 to < 1.50 x ULN	1.50 to < 3.00 x ULN	≥ 3.00 x ULN
WBC, Decreased (cells/mm^3; *cells/L*)				
> 7 days of age	2,000 to 2,499 *2.000 x 10^9 to 2.499 x 10^9*	1,500 to 1,999 *1.500 x 10^9 to 1.999 x 10^9*	1,000 to 1,499 *1.000 x 10^9 to 1.499 x 10^9*	< 1,000 *< 1.000 x 10^9*
≤ 7 days of age	5,500 to 6,999 *5.500 x 10^9 to 6.999 x 10^9*	4,000 to 5,499 *4.000 x 10^9 to 5.499 x 10^9*	2,500 to 3,999 *2.500 x 10^9 to 3.999 x 10^9*	< 2,500 *< 2.500 x 10^9*

Urinalysis

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to ≤ 500 mg	> 2+ or > 500 mg	NA
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	6 to < 10 RBCs per high power field	≥ 10 RBCs per high power field	Gross, with or without clots OR With RBC casts OR Intervention indicated	Life-threatening consequences
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher	NA

Appendix A.
Total Bilirubin Table for Term and Preterm Neonates

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Total Bilirubin[19], High (mg/dL; *µmol/L*)[20]				
Term Neonate[21] *< 24 hours of age*	4 to < 7 *68.4 to < 119.7*	7 to < 10 *119.7 to < 171*	10 to < 17 *171 to < 290.7*	≥ 17 *≥ 290.7*
24 to < 48 hours of age	5 to < 8 *85.5 to < 136.8*	8 to < 12 *136.8 to < 205.2*	12 to < 19 *205.2 to < 324.9*	≥ 19 *≥ 324.9*
48 to < 72 hours of age	8.5 to < 13 *145.35 to < 222.3*	13 to < 15 *222.3 to < 256.5*	15 to < 22 *256.5 to < 376.2*	≥ 22 *≥ 376.2*
72 hours to < 7 days of age	11 to < 16 *188.1 to < 273.6*	16 to < 18 *273.6 to < 307.8*	18 to < 24 *307.8 to < 410.4*	≥ 24 *≥ 410.4*
7 to 28 days of age (breast feeding)	5 to < 10 *85.5 to < 171*	10 to < 20 *171 to < 342*	20 to < 25 *342 to < 427.5*	≥ 25 *≥ 427.5*
7 to 28 days of age (not breast feeding)	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
Preterm Neonate[20] *35 to < 37 weeks gestational age*	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).
32 to < 35 weeks gestational age and < 7 days of age	NA	NA	10 to < 14 *171 to < 239.4*	≥ 14 *≥ 239.4*
28 to < 32 weeks gestational age and < 7 days of age	NA	NA	6 to < 10 *102.6 to < 171*	≥ 10 *≥ 171*
< 28 weeks gestational age and < 7 days of age	NA	NA	5 to < 8 *85.5 to < 136.8*	≥ 8 *≥ 136.8*
7 to 28 days of age (breast feeding)	5 to < 10 *85.5 to < 171*	10 to < 20 *171 to < 342*	20 to < 25 *342 to < 427.5*	≥ 25 *≥ 427.5*
7 to 28 days of age (not breast feeding)	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN

[19] Severity grading for total bilirubin in neonates is complex because of rapidly changing total bilirubin normal ranges in the first week of life followed by the benign phenomenon of breast milk jaundice after the first week of life. Severity grading in this appendix corresponds approximately to cut-offs for indications for phototherapy at grade 3 and for exchange transfusion at grade 4.
[20] A laboratory value of 1 mg/dL is equivalent to 17.1 µmol/L.
[21] Definitions: Term is defined as ≥ 37 weeks gestational age; near-term, as ≥ 35 weeks gestational age; preterm, as < 35 weeks gestational age; and neonate, as 0 to 28 days of age.