Clinical Trial of Entecavir/Peginterferon in Immune Tolerant Children with Chronic HBV Infection

Hepatitis B Research Network (HBRN)

Synopsis

Study aims
To determine the safety and efficacy of treatment with 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a. Efficacy will be assessed by sustained virologic response (HBeAg loss and HBV DNA $\leq$1,000 IU/ml) 48 weeks after cessation of therapy in children ages to 3-<18 years who are in the immune tolerant phase of chronic hepatitis B (CHB) infection. Safety will be assessed by Adverse Events and Serious Adverse Events through the end of treatment and through the end of follow-up.

Type of study
- Single arm treatment study.

1. Background
1.1. Introduction
Chronic hepatitis B virus infection (CHB) affects 360 million people worldwide, including approximately 1.5 million in North America. Most children with CHB in North America acquire their infection by vertical transmission from their infected mothers during the perinatal period or by horizontal transmission during early childhood. They may live in families who originate from countries where CHB is endemic, or have been adopted from such countries. In the absence of neonatal immunization, up to 90% of newborn infants exposed to the hepatitis B virus (HBV) develop CHB (1) (2). Although CHB is usually a mild disease in childhood, liver histology often reveals mild inflammation and fibrosis beginning at an early age (3). The incidence of cirrhosis and hepatocellular carcinoma in children with HBV is up to 1.5% and 0.25-1%, respectively (4, 5); this incidence increases over time to an estimated life-time risk of 30% (6) (7). The risk of hepatocellular carcinoma is increased in children who acquire CHB at an earlier age (8) (2, 9) (10). CHB may have other important consequences for children, including impairment of quality of life (11, 12), transmission to sexual partners, and transmission to the infants of infected teenage mothers. Effective treatment for CHB during childhood is therefore an important goal.

The outcome of treatment of CHB is partly dependent upon the phase of infection present in the patient at the time of treatment. Three phases of disease are recognized in CHB acquired in childhood. The initial “immune tolerant” phase is marked by the presence of HBsAg, HBeAg and high levels of hepatitis B virus (HBV) DNA, but minimal or no elevations in serum alanine and aspartate aminotransferase levels (ALT, AST) and minimal activity or injury on liver biopsy. The immune tolerant phase is variable in duration, but is typically followed during childhood or early adulthood by an “immune activation” phase, marked by rises in serum ALT and AST and inflammation and hepatocellular necrosis on liver biopsy. Although data in children is limited, there are concerns derived from adult studies that increased duration of this immune activation phase in some patients is associated with progression of hepatic fibrosis and development of cirrhosis. However, the immune activation phase will often resolve

spontaneously to an “inactive carrier” phase, marked by a fall in HBV DNA levels, loss of HBeAg, seroconversion to expression of anti-HBe, and normalization of ALT and AST levels with minimal necro-inflammatory activity on liver biopsy. Further progression of fibrosis is unlikely to occur in the inactive carrier phase, although these patients remain at risk of hepatocellular carcinoma. Children infected vertically from their mothers are likely to seroconvert to anti-HBe later than those infected horizontally (13). In a long-term study of Chinese children with CHB, 24% seroconverted to anti-HBe over an average 12 years of follow-up (14). In some patients, the loss of HBeAg is not accompanied by a permanent drop of HBV DNA to low levels and inactivity of liver disease. These patients have HBeAg-negative CHB, which can be progressive and is marked by fluctuating and moderate levels of HBV DNA in serum.

1.2. Current treatment of CHB in children

The current approach to the treatment of CHB in children focuses on the immune active phase, during which necro-inflammatory hepatitis may increase the progression of fibrosis. Two therapies interferon (IFN) and lamivudine are licensed for the treatment of chronic HBV infection in children, and the existing research data suggests that mono-therapy with either is most effective when used in the immune active phase of CHB. In a large clinical trial of IFN-α therapy for chronic HBV infection in children, 114 children were recruited with HBeAg positive CHB and ALT elevation greater than 1.5 times the upper limit of normal (15). Of the 74 children treated for 24 weeks with 6 MU/m^2 thrice weekly, 26% became negative for HBeAg and HBV DNA, compared to 11% of controls. Adverse effects were generally mild and similar to those seen in adults, with the addition that growth in height and weight were slowed during IFN-α therapy in children (16). A meta-analysis of pediatric trials of interferon therapy for CHB published up to November 1995 found six randomized controlled studies that included 240 children (17). Each of these studies was small (20-77 patients) and many methodological flaws were identified. The combined odds ratio was 4.6 (95% confidence intervals 2.4-8.7, $p<0.00001$) favoring IFN treatment for clearance of HBV DNA at end of treatment, and 2.2 (95% CI 1.1-4.2, $p=0.014$) for clearance of DNA persisting at the end of 6-18 months post-treatment follow-up. However, in a long-term follow-up study of 74 children, the overall rate of HBeAg clearance did not differ between 37 treated and 37 untreated children five years after treatment with IFN-α had finished, although the rate of HBsAg loss was significantly greater in treated children (25% vs. 0%) (18).

In a large multinational study of lamivudine in children with immune active CHB (ALT >1.3 times the upper limit of normal range), 23% of 191 treated children lost HBeAg after one year of treatment, compared to 11% of 95 children who received placebo (OR 2.1, 95% CI 1.0-4.1, $p=0.04$) (19). The drug was well tolerated, but mutant HBV was identified in 19% of treated children at one year.

None of the newer antiviral nucleoside or nucleotide analogues for CHB have been licensed for use in children, although pediatric clinical trials are underway or have been recently completed. In a randomized controlled study of adefovir dipivoxil in children with CHB, entry criteria included HBV DNA $>10^5$ copies/ml and ALT >1.5 times the upper limit of normal (20). The primary endpoint (normal ALT and HBV DNA <1000 copies/ml) was achieved by significantly more children receiving adefovir than placebo only in those children 12-18 years old (23% *vs.* 0%, $p<0.007$) but not in those younger than 12 years. HBV mutations that have been previously associated with hepatitis flares were not identified in the treated children.

1.3. Treatment of children with CHB during the immune tolerant phase of infection
A large proportion of children with CHB remain in the immune tolerant phase during much of their childhood. Treatment with IFN alone during this phase of infection is rarely effective and loss of HBV DNA or anti-HBe seroconversion is seen in less than 10% of treated participants (7, 21). However, the potential role of combination therapy with a nucleoside analogue and IFN was examined in a recent pilot study of 23 children (22). The investigators wished to test the hypothesis that initial therapy with lamivudine would reduce the high viral load exhibited by children with immune tolerance to HBV and thus increase the likelihood of response to IFN during a subsequent period of combination therapy. During follow up prior to the study all of these 23 children had mostly normal ALT values, and at study entry only two had elevated ALT (53 and 111 IU/L). Lamivudine was given for 8 weeks and then combined IFN and lamivudine therapy for a further 44 weeks. HBV DNA by PCR was negative in 48% at end of treatment, and 5 (22%) children seroconverted to anti-HBe. Four (17%) children cleared HBsAg and seroconverted to anti-HBs. Six months after treatment, HBV DNA was again positive in all but the children who had seroconverted to anti-HBe and (presumably) anti-HBs.

Previous reports of treatment of immune tolerant children are sparse. Among 24 Chinese children under 5 years of age randomized to receive IFN-α for 12 weeks or an oral treatment, there was no difference in sustained reduction in viral DNA or seroconversion to anti-HBe (21, 23). IFN therapy given to children after an initial steroid course showed a trend to greater effectiveness compared to IFN alone, in a study of 90 Chinese children recruited with high viral load and any value of ALT (23). Analysis of a subgroup of immune tolerant children recruited to a similar multicentre European study revealed a near-significant trend towards response to IFN (6 of 20 children seroconverted to anti-HBe) compared to placebo (1 of 18 seroconverted to anti-HBe, p=0.06) for children with AST <50 IU/L at baseline (24). However, no similar trend was noted when children with normal ALT at baseline were analyzed.

Review of recent literature to determine the normal ALT reference ranges in children showed the following: the European Hepatitis C virus (HCV) Network studied 1293 HCV-uninfected children born to HCV positive mothers (25). In this group normal ALT values for children < 18 months old were 60 IU/L for males and 55 IU/L for females, whereas for children > 18 month old, 40 IU/L for males and 35 IU/l for females were described. In another study, Schwimmer et al (26) analyzed NHANES data in 982 participant 12 – 17 years of age without any risk factor for liver disease. The NHANES data showed that the ALT levels in >95% was 25.8 IU/L for males and 22.1 IU/L for females.

1.4. Previous experience of combination treatment with lamivudine and IFN for children with CHB
Studies in adults have demonstrated a greater virological response whilst on treatment with combination therapy with lamivudine and IFN for HBeAg positive CHB compared to IFN monotherapy, but this benefit is transient and no difference is found on longer term follow-up after treatment. Several studies have examined this question in children, *but only among children who were in the immune active phase of infection* (typical inclusion criteria include an ALT greater than 1.5 times the upper limit of normal). The treatment schedule varied between studies, including some groups of patients given a period of monotherapy with either lamivudine or IFN first, followed by combined treatment, and then sometimes monotherapy again thereafter, and other groups given combined therapy from the outset. In studies comparing combined therapy to monotherapy, no differences in post-treatment outcome were found between groups, although early on-

treatment responses (normalization of ALT, reduction in HBV DNA) were sometimes better in the combined therapy group (27, 28) (29) (30, 31). In studies that compared different combination therapy regimens, most showed no differences between study groups given different regimens or treatment durations(31-33) (34, 35). However, one Turkish randomized study of 177 children showed a greater proportion of children who started lamivudine and IFN simultaneously underwent seroconversion to anti-HBe (64 of 112 children at 24 months, 57%) compared to children given lamivudine for 2 months before IFN was added (21 of 65 children, 32%, $p<0.05$) (36).

2. Study objectives

2.1. Primary objective

The primary objective of this study is to examine safety and efficacy of treatment with 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a in children ages 3 to <18 years who are in the immune tolerant phase of HBeAg positive chronic hepatitis B infection.

2.2. Primary endpoints

2.2.1 Safety: Adverse Events and Serious Adverse Events through the end of treatment and through the end of follow-up.

2.2.2 Efficacy: HBeAg loss (lack of detectable HBeAg) and HBV DNA ≤1,000 IU/mL at the time of last follow-up 48 weeks after end-of-treatment (week 96).

2.3. Secondary endpoints

2.3.1. At the end of treatment (week 48):

a. Cumulative HBsAg loss
b. HBeAg loss
c. HBeAg seroconversion
d. HBsAg seroconversion
e. ALT ≤40 IU/L for males, ≤35 IU/L for females
f. HBV DNA ≤1000 IU/mL
g. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test)
h. Absence of detectable antiviral drug-resistance HBV mutations
i. Growth parameters (weight, height, BMI, Tanner scores)

2.3.2. **Sustained end of follow-up responses** at 48 weeks after end-of-treatment (week 96):

a. Cumulative HBsAg loss
b. HBeAg loss
c. HBeAg seroconversion
d. HBsAg seroconversion
e. ALT ≤40 IU/L for males, ≤35 IU/L for females
f. HBV DNA ≤1000 IU/mL
g. HBV DNA <20 IU/mL (LLOQ of COBAS Ampliprep/COBAS TaqMan HBV v2.0 test)
h. Growth parameters (weight, height, BMI, Tanner scores)

3. Study design

This single arm treatment study will be conducted by the pediatric centers within the NIH Hepatitis B Research Network. Children ages 3 to <18 years with immune tolerant CHB

infection (ALT ≤60 U/l in males and ≤40 U/l in females) who fulfill the entry criteria will be recruited to the study, after obtaining parental/guardian consent and participant assent**.**

Participants will receive entecavir as monotherapy for 8 weeks and then combination therapy with entecavir and peginterferon alfa-2a by weekly subcutaneous injection until week 48. Participants will be followed until week 96 (48 weeks after discontinuation of therapy). Screening can include historical values up to 52 weeks prior to baseline visit (i.e. initiation of treatment), but there must be a screening visit within 6 weeks of the baseline visit.

Assessments will be undertaken at baseline, weeks 4, 8, 10, 12, 14, 16 then every 4 weeks until week 48, and then at week 52, 56, 60, 72, 84 and 96. Data collected will describe baseline demographics, symptoms of liver disease, intercurrent illnesses, and findings on physical examination. Blood work will be drawn to measure markers of viral and liver disease status, assessment of drug adverse effects, and for research biospecimen banking.

Participants will continue on therapy until week 48 and complete the full study follow-up protocol thereafter, including those who undergo seroconversion to anti-HBe or anti-HBs before reaching week 48. Participants who experience a sustained elevation of ALT, during which the ALT rises to >60 U/L for males and >40 U/L for females for longer than 24 weeks, will be eligible to receive treatment as recommended by their hepatologist and will continue to complete the study follow-up protocol. Participants who exhibit adverse effects to therapy will undergo dose adjustment or discontinuation of therapy as detailed in the protocol, and will continue to complete the study follow-up protocol.

4. Study population

4.1. Inclusion criteria

1. Enrolled in the Hepatitis B Research Network (HBRN) Cohort Study or completed the necessary components of the Cohort baseline evaluation by the end of the baseline visit for this study.
2. 3 to <18 years at time of the baseline visit (day 0).
3. Documented chronic HBV infection as evidenced by detection of HBsAg in serum for ≥ 24 weeks prior to the baseline visit **or** positive HBsAg and negative anti-HBc IgM within 24 weeks of the baseline visit.
4. Presence of HBeAg in serum at the last screening visit within 6 weeks of the baseline visit
5. Serum HBV DNA level $>10^7$ IU/mL on at least two occasions at least 12 weeks apart during the 52 weeks before the baseline visit. One of the two HBV DNA levels must be within 6 weeks of the baseline visit.
6. ALT ≤60 U/l in males or ≤40 U/l in females, measured on at least 2 occasions: one taken at screening (i.e. within 6 weeks prior to the baseline visit), and another taken at least 12 weeks prior to the screening visit and within the 52 weeks prior to the baseline visit .
7. Compensated liver disease, with normal total bilirubin (except if Gilbert's syndrome), direct bilirubin ≤0.5 mg/dL, INR ≤1.5, and serum albumin ≥3.5 g/dL.
8. Creatinine clearance ≥90 ml/min (see method of calculation in section **5.2.3.2**).
9. Absence of hepatocellular carcinoma on liver ultrasound in the past 52 weeks
10. Parent/guardian provides informed consent and willing to adhere to the requirements of the study.

4.2. Exclusion criteria

1. Presence of infection with HCV-RNA or anti-HCV, anti-HDV, or HIV at screening
2. Presence of another cause of liver disease or HCC (serum alpha-fetoprotein >50ng /ml).
3. Evidence of decompensated liver disease (Childs B-C).
4. History or other evidence of a medical condition associated with chronic liver disease (e.g., hemochromatosis, autoimmune hepatitis, alcoholic liver disease, toxin exposures).
5. Females who are pregnant or breastfeeding.
6. Adolescent females unwilling or unable to use an acceptable method of contraception if sexually active during the treatment period.
7. Children currently breastfeeding while their mother is taking lamivudine, or those who were exposed to lamivudine for ≥24 weeks via maternal lamivudine treatment during pregnancy and/or while breastfeeding.
8. Malignancy or other significant medical or psychiatric illness which, in the opinion a study physician, may interfere with participant treatment, assessment or compliance with the study protocol.
9. Previous liver or other organ transplantation including engrafted bone marrow transplant.
10. Hematological abnormalities during the screening period that contraindicate full dosing with study drugs, e.g absolute neutrophil count < 1.5×10^9 cells/L or platelet count < 120×10^9 cells/L.
11. Known allergy to study drugs; peginterferon alfa-2a or entecavir.
12. Treatment with systemic acyclovir or famciclovir within the previous 6 months.
13. Need for ongoing use of any antivirals with activity against HBV during the course of the study or history of receiving treatment for HBV.
14. Any use of illegal drugs OR use of alcoholic beverages which in the opinion of a study physician is sufficient to prevent adequate compliance with study procedures or increase the risk of pancreatitis or hepatotoxicity.
15. History of immunologically mediated disease (e.g. inflammatory bowel disease, idiopathic thrombocytopenic purpura, lupus erythematosus, autoimmune haemolytic anemia, scleroderma, severe psoriasis, rheumatoid arthritis).
16. History or other evidence of bleeding from esophageal varices or consistent with decompensated liver disease.
17. History or other evidence of chronic pulmonary disease associated with functional limitation.
18. History of significant cardiovascular diseases.
19. History of a severe seizure disorder or current anticonvulsant use.
20. History or other evidence of severe retinopathy.
21. History of thyroid disease poorly controlled on prescribed medications. Participants with elevated thyroid stimulating hormone concentrations with elevation of antibodies to thyroid peroxidase and any clinical manifestations of thyroid disease are excluded.
22. Concomitant use or use during ≤ 6 months prior to the first dose of study drug of anti-neoplastic, immunosuppressive, nephrotoxic or hepatotoxic medication, methadone, theophylline or medications that may affect renal excretion or hepatic metabolism are not permitted.
23. Concomitant use of complementary or alternative medications purported to have antiviral activity.

24. A participant may not be co-enrolled in another clinical trial where an investigational drug is administered.
25. Any other condition or situation that in the opinion of a study physician would make the participant unsuitable for enrollment or could interfere with the participant participating in and completing the study.

5. Study drugs and drug management
5.1. Dosage and administration

Entecavir 0.015 mg/kg/day (up to 0.5 mg maximum daily dose) (based on BMS AI463028 PK trial) (liquid or tablet depending on the weight of the participant, specified in Entecavir dosing table and notes in Appendix I) once daily for 48 weeks and Peginterferon alfa-2a 180 µg/1.73m^2 subcutaneously times the participant's body surface area (BSA) once weekly for 40 weeks beginning 8 weeks after entecavir monotherapy).

Participants will be instructed to take entecavir once daily (tablet or liquid). Entecavir should be taken on an empty stomach, at a time that is at least 2 hours after a meal and 2 hours before the next meal, approximately the same time each day. Participants will be instructed to take peginterferon alfa-2a once weekly. The participant will be instructed to inject the peginterferon alfa-2a at the same time on the same day each week.

5.2. Entecavir
5.2.1. General information
Entecavir is a nucleoside analogue that is primarily cleared by the kidneys. In participants with renal impairment, the apparent clearance of oral entecavir decreases as creatinine clearance decreases if deteriorating renal function should occur (creatinine clearance <30 mL/min) during study participation, discontinuation of study drug is required. The medication is stored at room temperature.

5.2.2. Pharmacokinetic considerations
Entecavir will be dosed at 0.015 mg/kg/day up to a maximum of 0.5 mg/day (liquid or tablet referring to dosing table in Appendix I).

5.2.3. Safety
5.2.3.1. Participant management for ALT/AST elevations or hepatic clinical events during the 8 weeks of monotherapy with entecavir.
Certain ALT/AST elevations or hepatic clinical events may represent a clinically relevant progression of hepatitis. Non-HBV related potential causes of worsening hepatitis also need to be considered (hepatitis A, C, concomitant medications, etc.). Guidelines for management for all ALT/AST elevations (ALT/AST >2x baseline) or hepatic clinical events during the first 8 weeks of entecavir monotherapy will be as follows:

<u>Without clinical or laboratory complications</u>
If the participant is asymptomatic and other liver function tests are (total bilirubin, albumin, INR) stable, and lactic acidosis (plasma lactate >5mmol/L with a ph <7.35) is not suspected by the investigator– monitor participant at least weekly. If persists x 3 weeks, check serum HBV DNA. If >1 $\log_{10}$ decrease from point at which last assessed, continue therapy and monitor weekly until ALT/AST at baseline. If HBV DNA values do not decrease, consideration will be taken to discontinue the participant's current

treatment regimen. The participant will be assessed for possible development of resistance.

If participant is symptomatic, assess for other causes of liver dysfunction.

With clinical or laboratory complications
Monitor serum HBV DNA and lactate and assess for other causes of liver dysfunction.

For management of ALT/AST elevations once combination therapy with entecavir and peginterferon alfa-2a begins follow management algorithm in 6.12.

5.2.3.2. Management of renal impairment
In participants with renal impairment, the apparent clearance of oral entecavir is decreased as creatinine clearance decreased. Treatment will be discontinued with creatinine clearance < 30 mL/min.

In the unlikely event that a subject develops inadequate renal function while on study (estimated glomerular filtration rate of < 50 mL/min/1.73 m^2), the recommendation for dose reduction will follow the established approach used in studies of adult CHB patients. The following table shows the recommendation for dose reduction based upon estimated glomerular filtration rate (GFR).

Recommended dosing of entecavir in participants who develop renal impairment on study (creatinine clearance)

Estimated GFR	**Usual Dose (0.015 mg/kg/day up to a maximum of 0.5 mg/daily)**[a]
≥ 50	Usual Dose
30 to < 50	Dose reduction by 50 % **OR** Usual dose every 48 hours
< 30	discontinue both Entecavir and Peginterferon alfa-2a

[a] For doses less than 0.5 mg daily, the oral solution is recommended.
[b] Subjects receiving entecavir on the day of hemodialysis should receive entecavir after the session.

For participant <18 years of age: To estimate CrCl from a serum creatinine, use the participant's height (cm), and a proportionality constant using the Schwartz method:

CrCl = (k * Ht) / Cr

*For males 3 to < 13 years of age: k = 0.55; For males ≥ 13 to ≤ 17 years of age: k = 0.70; for all females <18 years of age : k = 0.55

Once a participant turns 18 years of age, estimated CrCl is calculated using the Cockcroft-Gault equation as follows:

In males:

$$\text{Creatinine clearance} = \frac{(140 - \text{age}) \times \text{weight in kg}}{(72 \times \text{serum creatinine})}$$

In females:

$$\text{Creatinine clearance} = \frac{140 - \text{age}) \times \text{weight in kg} \times 0.85}{(72 \times \text{serum creatinine})}$$

5.2.3.3. Reproductive toxicity
Entecavir is classified by the US Food and Drug Administration (FDA) as Pregnancy "Category C" (Animal reproduction studies have shown adverse effect on the fetus and there are no adequate and well-controlled studies in pregnant females).

In studies in rats and rabbits, entecavir doses producing systemic drug exposure levels approximately 28 and 212 times those expected in humans at the highest recommended dose (1 mg/day) showed no embryotoxicity or maternal toxicity, respectively. At exposure 3100 times those in humans, maternal toxicity, embryo-fetal toxicity (resorptions), lower fetal body weights, tail and vertebral malformations, reduced ossification (vertebrae, sternebrae, and phalanges), and extra lumbar vertebrae and ribs were observed in rats. At exposure 833 times those in humans, embryo-fetal toxicity (resorptions), reduced ossification (hyoid), and an increased incidence of 13th rib were observed in rabbits. In the pre- and post-natal development assessment in rats, entecavir, at maternally toxic drug exposure levels > 94 times those achieved in humans at the highest recommended dose (1 mg/day), no adverse effects on offspring were seen.

There are no adequate and well-controlled studies in pregnant women. Entecavir should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. A cumulative search of the safety database through 28 September 2011, inclusive, identified 435 reports of entecavir exposure during pregnancy (including within 3 months prior to conception.) Among these pregnancies, 232 were patients who were not in a clinical trial, 202 were clinical trial participants, and 1 was from the published literature. Entecavir exposure was confirmed by a healthcare professional for 341 of the reported pregnancies and 94 were self-reported. The majority of reports (289) involved antenatal maternal exposure to entecavir. An additional 146 involved pregnancies in female partners of male patients treated with entecavir. Among the 289 reported pregnancies with antenatal maternal exposure, there were 72 normal newborns and 14 live births with various disorders including prematurity, small for gestational age with fetal growth retardation, transient respiratory distress, staphylococcal sepsis with jaundice, and various congenital anomalies. In the remainder of the reported entecavir-exposed pregnancies, the patients were lost to follow-up or had not yet delivered at the time of the database search.

Labor and Delivery
There are no studies in pregnant women and no data on the effect of entecavir on transmission of HBV from mother to infant. Therefore, appropriate interventions should be used to prevent neonatal acquisition of HBV.

Nursing Mothers
Entecavir is excreted in the milk of rats. It is not known whether this drug is excreted in human milk. Mothers should be instructed not to breast-feed if they are taking entecavir.

In the current study, all females of childbearing potential will be asked to utilize an effective form of contraception during therapy.

5.3. Peginterferon alfa-2a

5.3.1. General description

Peginterferon alfa-2a is a covalent conjugate of recombinant alfa-2a interferon with a single branched molecule of polyethylene glycol with a molecular weight of approximately 40,000 daltons. Peginterferon alfa-2a is produced using recombinant DNA technology and contains 180 µg/1.0 mL in vial form and the same amount per 0.5 ml in a prefilled syringe. The drug must be kept refrigerated at +4C until use.

Interferon inhibits viral replication by inducing an antiviral state in cells. Interferon does not enter the hepatocyte, but rather binds to specific receptors on the cell surface, which initiates intracellular signaling that leads to rapid activation of multiple "interferon-stimulated genes (ISGs); the encoded proteins of these genes inhibit viral replication in infected hepatocytes by multiple mechanisms (including inhibition of viral protein synthesis and breakdown of viral RNA). Peginterferon alfa-2a also has immunomodulatory activity that is thought to be important in obtaining a virological response. Steady-state serum concentrations of peginterferon alfa-2a are reached within 5 to 8 weeks using once weekly dosing. The mean terminal half-life after subcutaneous dosing is 160 hours compared to 5 hours for standard, non-peginterferon alfa-2a.

5.3.2. Pharmacokinetic considerations

In participants with end-stage renal disease undergoing hemodialysis, there is a 25% to 45% reduction in clearance of peginterferon alfa-2a. The effect of milder renal impairment has not been studied but it is advised that in participants with impaired renal function, signs and symptoms of interferon toxicity should be closely monitored. It is also recommended that peginterferon alfa-2a be used with caution in participants with creatinine clearance < 50 mL/min.

There is no known effect of peginterferon alfa-2a on the pharmacokinetics of drugs metabolized by the cytochrome P-450 system. There are no known clinically significant interactions with nucleos(t)ide analogue therapy, but a phase IV registration trial of peginterferon alfa-2a and telbivudine was prematurely discontinued due to a higher than anticipated incidence of peripheral neuropathy.

5.3.3. Safety

As with standard alpha interferon, treatment with peginterferon alfa-2a is associated with many troublesome and occasionally serious, or even life-threatening, side effects. Dose discontinuation has been reported in 6% to 9% and dose modification in 31% to 47% of participants treated in the peginterferon alfa-2a registration trials for hepatitis B. The most frequent causes of dose adjustment were laboratory abnormalities such as thrombocytopenia or leucopenia. These side effects are particularly common in participants with advanced liver disease and hypersplenism. Peginterferon alfa-2a is absolutely contraindicated in participants with decompensated cirrhosis due to the possibility of serious infections, flares of disease and worsening decompensation.

The majority of clinical experience in the use of peginterferon alfa-2a has been in chronic hepatitis C in which peginterferon alfa-2a is combined with ribavirin. In the few studies of peginterferon alfa-2a in chronic hepatitis B it has appeared that side effects are less frequent than in chronic hepatitis C. The overall incidence of serious adverse events was less in studies in hepatitis B (4%-5%) than in those with hepatitis C (7% to 16%), and fewer drug withdrawals were reported (6% to 8% versus 17% to 33%, respectively) despite similar doses and durations of therapy. Depression was also less frequently reported in studies done in hepatitis B (4%) than hepatitis C (22%, $p < 0$.

001).

Peginterferon alfa-2a should be avoided in participants with other serious co-morbid illnesses, including, but not limited to, coronary artery disease, cerebrovascular disease, serious autoimmune conditions and severe depression.

The major safety results from the four monotherapy adult studies are as follows: the incidences and types of adverse events were similar among participants treated with peginterferon and peginterferon alfa-2a. The most frequently reported adverse events were those often observed for interferon, including headache, fatigue, myalgia, pyrexia, rigors, arthralgia, nausea, alopecia, insomnia, diarrhea, abdominal pain, depression, and injection site reaction. Most of these events were of mild to moderate intensity. The most common serious adverse events were infections, psychiatric disorders, and gastrointestinal disorders and were seen with equal frequency in the peginterferon (9%) and IFN (7%) groups. For any given treatment-related serious adverse event, the incidence was <1% in any group. Nine participants died and only two of the nine deaths were considered to be possibly or probably related to the study drug. Approximately 20% of both groups needed dose modification during treatment, most frequently due to neutropenia. Treatment was discontinued prematurely in 10% of the participants in both groups. Depression and fatigue were the two most common adverse events and thrombocytopenia and elevation in serum ALT concentration were the two most common laboratory abnormalities leading to premature treatment discontinuation. Most treatment-related serious adverse events resolved without sequelae.

The intention of the protocol is that participants demonstrating a response to therapy remain on study drug until the completion of the study. However, it is possible that some participants will encounter adverse events during their participation in the trial necessitating study drug dosage adjustment. Decrement adjustments should be uniform across centers and participants. When appropriate, downward dose adjustments in one level reduction should be considered (see Appendix II Peginterferon alfa-2a dosing adjustments).

Suggested dose adjustments to peginterferon alfa-2a are guidelines to maintain consistency between centers. These guidelines are for neutropenia, thrombocytopenia, anemia, indirect hyperbilirubinemia, and elevated alanine aminotransferase (ALT) activity. When possible, abnormal lab results should be confirmed as soon as possible following notification. If laboratory abnormalities improve or resolve, the dose of peginterferon alfa-2a may be increased back to the original dose at the discretion of the investigator with continued close monitoring. If adverse events continue at the same intensity despite maximal dose reductions, peginterferon alfa-2a may require discontinuation at the discretion of investigator. It should be noted that certain toxicities carry different levels of significance for each participant and therefore, the investigator should use these as guidelines only, within the context of clinical judgment. Growth factors may not be used to maintain normal levels of hemoglobin, neutrophils or platelets.

5.3.3.1. Peginterferon alfa-2a and Pregnancy

Peginterferon alfa-2a is a Pregnancy Category C drug that has not been adequately evaluated in humans for its teratogenic effect. Standard interferon has been shown to increase the rate of abortion in Rhesus monkeys when given approximately 20 to 500 times the human weekly dose. Interferon, however, is highly species specific in its

effects and animal studies may not reliably reflect the potential of side effects in humans. There have been no adequate and well-controlled studies of peginterferon alfa-2a in pregnant females. Therefore, in this study, females of childbearing potential will be enrolled only if they agree to use effective contraception during therapy and pregnancy testing will be performed at regular intervals and pregnancy testing will occur at every visit during the treatment phase.

5.4. Administration and drug accountability

Staff at each site will be responsible for drug accountability. This will include documenting drugs received, drug dispensed to study participants, used and unused study drug returned, and an accounting of drug destroyed. An accurate and up-to-date accountability log will be maintained by each site.

5.5. Concomitant medications

All concomitant medications (prescription and non-prescription) being taken by each subject will be queried and recorded by the study coordinator at each visit.

5.6. Prohibited medications

Use of the following medications is prohibited while participants are on study drugs:

1. Antivirals with activity against HBV, including valacyclovir. The need for ongoing use of these antivirals would be exclusionary at study entry, but would be recorded as a concomitant medication if medically indicated and prescribed during the course of the study.
2. Nephrotoxic agents including aminoglycoside antibiotics, amphotericin, cidofovir, foscarnet, hepatotoxic agents and immunosuppressive agents.
3. Systemic chemotherapeutic agents (e.g. cancer treatment medications).
4. Systemic corticosteroids. The need for ongoing use of systemic corticosteroids (> 7days) would be exclusionary at study entry, but would be recorded as a concomitant medication if medically indicated and prescribed during the course of the study.
5. Any investigational agents.
6. Agents affecting renal excretion and hepatic metabolism.
7. Growth factors – GCSF or erythropoietin.

6. Study procedures

6.1. Study enrollment

At each institution participants thought to be eligible for participation will be invited to participate in the study. An informed consent approved by the IRB at the respective institution will be obtained. Patients who are not enrolled in the HBRN Cohort study may be enrolled concurrently with the screening visit for this trial, or if they choose not to enroll into the Cohort Study, the necessary components of the Cohort baseline evaluation will be completed prior to enrolling in this study at the screening or baseline visit.

Screening will begin within 6 weeks prior to the first dose of study drugs. An Eligibility Screening Form will be used as a checklist to document whether or not a participant fulfills the entry criteria. The Eligibility Screening Form will be signed and dated by the investigator for all participants considered for enrollment in the study.

6.2. Screening assessments

The following assessments must be obtained within 6 weeks (42 days) of the baseline visit (start of treatment).

Screening assessments*

Informed consent/assent
Complete medical history
Vital signs & physical exam
Adverse Events and Concomitant medicines
Depression Assessment
CBC with differential
Hepatic panel (AST, ALT, bilirubin)
Other Hepatic panel (albumin, ALKP)
Renal Panel (Creatinine and CrCl (calculated), PO4, serum lipase, glucose, creatinine phosphokinase (CK), serum electrolytes)
Other renal panel (Ca, BUN)
Thyroid Peroxidase antibody
Hemoglobin A1C
Other Chemistries (uric acid, triglycerides, cholesterol, total protein, TSH, Free T4)
Urinalysis
Pregnancy test for females of childbearing potential
PT/INR
AFP
Anti-HCV, Anti-HDV, Anti-HIV^
Serum Bank (repository)
HBeAg
HBsAg
Quant HBV DNA

* An extra screening visit may be necessary to complete the screening labs for small children.
^These tests do not need to be repeated if participants do not receive study drug following initial screening but are subsequently rescreened for this protocol, unless dictated by a change in the participant's medical condition.

6.3. Baseline visit

The following assessments must be obtained during the baseline visit (day 0) prior to the initiation of study drugs. At the end of the baseline visit, study drug (Entecavir) will be provided to the participant with instructions on its use.

Baseline assessments

Vital signs & symptom directed physical
Adverse Events and Concomitant medicines
Depression Assessment
Child Health Questionnaire (CHQ)

Health behavior, Symptom questionnaire, and Tanner Stage
Drug Dispensing
CBC with differential
Hepatic panel (AST, ALT, bilirubin)
Other Hepatic panel (albumin, ALKP)
Renal Panel (Creatinine and CrCl (calculated), PO4, serum lipase, glucose, creatinine phosphokinase (CK), serum electrolytes)
Other renal panel (Ca, BUN)
Urinalysis
Pregnancy test for females of childbearing potential
PT/INR[1]
HBeAg, anti-HBe,
Serum banking
HBsAg quant, HBeAg quant, anti-HBs, Quant HBV DNA
HBV genotype and subtype, HBV precore/BCP, Antiviral resistance testing

6.4. On-treatment assessments
After starting treatment, participants will be assessed at 2 – 4 week intervals for the first 16 weeks of treatment and then at 4 week intervals until completion of therapy as shown in the Schedule of Assessments (Appendix III Table 1). For participants who have side effects related to treatment or adherence issues, more frequent visits can be performed at the discretion of the investigator. These visits will be conducted as an "Unscheduled Visit". At weeks 4, 10, 14, 20, 28, 32, 40 and 44, a telephone assessment will be conducted instead of a participant's visit when only local tests are required without central tests scheduled in order to save participant's trip to the site because the assessment visits are very frequent (Appendix III). At each regular visit, participants will undergo a limited clinical evaluation and physical examination assessing any possible adverse event or evidence of progressive liver disease. In addition, laboratory tests will be obtained during the visits as shown in Appendix III Table 1. At telephone assessments, drug adherence will be assessed and laboratory studies can be obtained at local laboratories.

6.5. End of treatment assessment
At week 48, all participants will complete the treatment phase. Laboratory testing will be performed as outlined in the Schedule of assessments (Appendix III Table 1).

6.6. Post Treatment assessments
Participants will be seen at week 52, 56, 60, 72, 84 and 96. Laboratory testing will be performed as outlined in the Schedule of assessments (Appendix III Table 2).

6.7. Assessment of adherence
During each visit, study personnel will review the study drug dosing schedule with the participant and counseling on adherence will be provided. At each study visit while on therapy, participants will be asked to return entecavir bottles and used peginterferon alfa-2a vials to document use of study medications. Serum drug levels may be

measured on stored serum samples in retrospect in order to assess compliance of individual participants.

6.8. Stopping rules

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw participants from the study if it is in the best interest of the participant. An excessive rate of withdrawals can render the study uninterruptable; therefore, unnecessary withdrawal of participants should be avoided. Should a participant decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

The investigator should contact the legal guardian either by telephone or through a personal visit or a responsible relative must be contacted to determine as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the participant's withdrawal should be made with an explanation of why the participant is withdrawing from the study.

Stopping rules for peginterferon alfa-2a are as follows:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Platelet count decreases to levels below 25,000/mm^3
- Evidence of hepatic decompensation
- Autoimmune hepatitis
- Pregnancy
- Appearance or worsening of psoriatic lesion
- Thyroid abnormalities that cannot be adequately treated
- Hypoglycemia, hyperglycemia or diabetes mellitus that cannot be effectively controlled by medication
- Severe cases of depression, suicidal ideation and suicide attempt
- New or worsening ophthalmologic disorders such as decreased or loss of vision
- Hypersensitivity reactions (e.g., anaphylaxis, angioedema, bronchoconstriction)
- Persistent or unexplained pulmonary infiltrates or pulmonary function impairment
- Participant refusal to continue treatment

Stopping rules for Entecavir:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event (AE), laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- A subject develops inadequate renal function with creatinine clearance < 30 mL/min.
- Pregnancy

It should be noted that if it is necessary to stop one drug then both drugs should be stopped.

Participants who discontinue treatment due to any of the stopping rules for entecavir or peginterferon alfa-2a will continue to be followed until these events are resolved or stabilized, according to the post-treatment follow-up schedule.

6.9. Management of non-response

Definition: Primary non-response will be defined as a failure to achieve >1-$\log_{10}$ IU/mL decline in HBV DNA after the first 24 weeks of treatment by the quantitative HBV DNA assay, which will be reconfirmed on re-testing 4 weeks later. Compliance with study medications will be assessed and documented. Primary non-response is considered treatment failure. Actions to be taken include:

6.9.1. Reinforce compliance when non-response is initially suspected.
6.9.2. Treatment will be discontinued if non-response is reconfirmed on retesting 4 weeks later.
6.9.3. Participants will be assessed 4 weeks after treatment discontinuation and then 12, 24 and 48 weeks after treatment discontinuation .

6.10. Management of virological breakthrough
Definition: Virological breakthrough is defined as one of the following: (a) a >1-$\log_{10}$ IU/mL increase in HBV DNA level from nadir in a participant with an initial virological response, where initial virological response is defined as a > 1-$\log_{10}$ IU/mL decline in HBV DNA from baseline by the quantitative HBV DNA assay; or (b) redetection of HBV DNA after becoming undetectable.

These criteria will be confirmed on re-testing 4 weeks later. For the purposes of defining breakthrough, the HBV DNA levels in someone who previously had undetectable HBV DNA must be > 100 IU/mL at the confirmatory re-test.

- Virologic breakthrough is considered a treatment failure.

Management algorithm if virological breakthrough
6.10.1. Reinforce compliance. The importance of compliance with study medication will be reinforced with study participant at each visit.
6.10.2. Resistance testing will be performed but will not be used to guide decisions (as not available in real-time). Participants meeting the definition for virologic breakthrough on repeat testing will stop treatment.
6.10.3. Participants will be assessed at 4, 12, 24 and 48 weeks post-discontinuation of treatment .

6.11. Management algorithm for participants with ALT flare during the combination treatment phase 6.11.1. If ALT increases to >400 U/L for males and >350 U/L for females, retest the hepatic panel in 4 weeks. Continue testing of hepatic panel every 4 weeks as long as the ALT remains >400 U/L for males and >350 U/L for females.
6.11.2. If ALT decline below these levels, the frequency of testing the hepatic panel can return to the usual schedule.
6.11.3. Participants will remain in the study until week 48 unless they show signs of clinical decompensation or direct bilirubin ≥1.0 mg/dL or ALT >60 U/L in males and >45 U/L in females for ≥24 weeks.
6.11.4. Participants with clinical decompensation or direct bilirubin ≥1.0 mg/dL or persistent elevation of ALT as in 6.12.3. will continued to be followed in the study but any subsequent management will be at the discretion of the study physician.

6.12. Management algorithm for participants with ALT elevation during the follow-up phase (weeks 48-96)

6.12.1. If ALT increases to >400 U/L for males and >350 U/L for females, retest the hepatic panel in 4 weeks. Continue testing of hepatic panel every 4 weeks is indicated as long as the ALT remains >400 U/L for males and >350 IU/L for females.
6.12.2. If ALT decline below these levels, the frequency of testing the hepatic panel can return to the usual schedule.
6.12.3. If ALT is >60 U/L in males and >40 U/L in females for ≥24 weeks and HBV DNA ≥10,000 IU/mL, they will be observed without treatment for a full 48 weeks, unless there is evidence of clinical decompensation or direct bilirubin ≥1.0 mg/dL. After the 48 weeks of follow-up, participant will be managed per the judgment of the investigator.

6.13 Participants previously randomized to control group
Participants enrolled in this trial prior to version 3.1 of this protocol, who were randomized to the control (no treatment) group, will be offered treatment if they still meet the entry criteria for this trial (i.e. the most recent labs meet the inclusion and exclusion criteria listed in sections 4.1 and 4.2), and are willing to receive treatment. These participants will be re-consented prior to starting treatment, and will follow the visit schedule and assessment beginning with the baseline (Week 0) visit.

Participants who no longer meet entry criteria for this trial or are not interested in receiving treatment will complete a Termination Visit at the next scheduled visit for the control (no treatment) group. The procedures to be followed for this Termination Visit will be those specified for the Week 48 visit for the control group for the version 3.1 of the Pediatric Immune Tolerant Trial.

7. Adverse Events and toxicity management
7.1. Management of peginterferon alfa-2a side effects
Common side effects of peginterferon alfa-2a include influenza-like symptoms, particularly with the first few injections, fatigue, neuropsychiatric symptoms such as depression and reduction in white blood cell counts and platelet counts. Hyper- or hypothyroidism has been noted to occur in up to 5% of participants receiving peginterferon alfa-2a.

Some rare but potentially serious side effects include:
- neuropsychiatric complications, which may include suicide, suicidal ideation, acute psychosis and severe depression
- serious and severe bacterial infections; fatal infections have been reported during treatment with peginterferon alfa-2a
- pancreatitis
- interstitial pneumonitis, fatal cases have been reported with peginterferon alfa 2a

Other possible but uncommon adverse events associated with peginterferon alfa-2a include:
- development or exacerbation of existing autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, idiopathic thrombocytopenic purpura, hemolytic anemia, type 1 diabetes, psoriasis, etc
- pulmonary disorders including dyspnea, pulmonary infiltrates, and sarcoidosis
- exacerbations of inflammatory bowel disease
- eye disorders, including partial or total decrease or loss of vision, retinal hemorrhages
- hearing disorders, including loss of hearing and tinnitus

- hypersensitivity reactions including severe skin reactions in the spectrum of Stevens Johnson syndrome

7.1.1. Management of depression

Screening and management for depression

All children will be screened for depression using the Childhood Depression Inventory (CDI) at the screening visit (within 6 weeks of baseline visit), published by Multi-Health Systems, Inc [Kovacs, Maria. Children's Depression Inventory (CDI) Manual, New York: Multi-Health Systems Inc., 2001]. This is a 10 minute questionnaire that has been validated for children ages 7-17 years; guardians can complete the questionnaire for children <7 years. Children that are unable to read the questionnaires may have them read to them by the study coordinator. The instrument can be used for 17 year old adolescents. Analysis of the CDI data will be limited to children 7-17 years of age. In addition, each investigator will screen children at entry for a Major Depressive Episode using criteria from the American Psychiatric Association (See Table 1 Criteria for Major Depressive Episode).

Any child meeting the criteria for a major depressive episode at screening will not be enrolled in the study and will be referred to a mental health professional if the depression fits the criteria in Table 2A or to a psychiatrist if the depression fits the criteria in Table 2B (See Table 2 Indications for Mental Health Professional Care and Specialty Physician Care in Pediatric Participants with Depression).

Table 1. Criteria for major depressive episode*

A major depressive episode is indicated by the presence of five or more of the following symptoms nearly every day during the same two-week period, representing a change from the previous level of functioning:

- Depressed mood most of the day
- Markedly diminished interest or pleasure in all or almost all activities
- Clinically significant weight loss in the absence of dieting or weight gain (e.g., a change of more than 5 percent of body weight in a month) or a decrease in appetite †
- Insomnia or hypersomnia
- Observable psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness or excessive or inappropriate guilt
- Diminished ability to think or concentrate or indecisiveness
- Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

* Criteria are from the American Psychiatric Association. (Diagnostic and statistical manual of mental disorders, 4th ed.:DSM-IV. Washington, D.C.: American Psychiatric Association, 1994.) (Brent DA, Birmaher B. Adolescent Depression. N Engl J Med 2002:347:667-671.)
† In children, this criterion includes the failure to make the expected growth-related weight gains.

The CDI/CES-D will be used to screen children for depression at the screening and baseline visits and at 12, 24 weeks post-baseline, end of study drug, end of untreated follow up and at each of the annual visits. If the participant becomes 18 years of age or

older during the study, then the CES-D depression screening instrument will be used. If depression develops as defined by the CDI manual (score >19), or by the CES-D manual (score >15) (Radloff, L.S., The CES-D Scale: a self-report depression scale for research in the general population. "Applied Psychological Measurement", 1977;1:385-401.), then the investigator will perform a more thorough evaluation to determine the validity of the screening test result. If the participant meets the criteria listed above for a Major Depressive Episode and fits the criteria listed under A in Table 2, then he will be referred to a mental health professional and the peginterferon alfa-2a plus entecavir will be continued. If the management of the depression is not successful after eight weeks or if the participant develops criteria on Table 2B for referral to a specialty physician during that eight week period, the peginterferon alfa-2a plus entecavir will be stopped and the participant will be referred to a specialty physician. The participant will move to the first untreated follow-up visit.

Table 2. Indications for mental health professional care and specialty physician care in pediatric participants with depression*

2A. Indications for mental health professional care:

- Initial episode of depression
- Recent onset of depression
- Absence of coexisting conditions
- Ability to make no-suicide contract
- High level of family discord

Chronic, recurrent depression

2B. Indications for specialty physician care and immediate withdrawal of study drug(s):

- Lack of response to initial course of treatment**
- Coexisting substance abuse**
- Recent suicide attempt, current suicidal ideation with plan, or both**
- Psychosis**
- Bipolar disorder**
- Inability of family to monitor participant's safety**

7.2. Peginterferon alfa-2a dose reductions

Factors that will lead to a reduction in the dose of peginterferon alfa-2a include:

1. Disabling symptoms, which, in the opinion of the investigator, are related to peginterferon alfa-2a treatment and prevent the participant from performing his/her occupation or daily tasks.
2. A rash consistent with allergic reaction or vasculitis.
3. Reductions in the platelet count according to the guidelines in Appendix II
4. A reduction in neutrophil count according to the guidelines in Appendix II
5. Any adverse reaction, which, in the opinion of the investigator, places the participant at increased risk of a serious adverse effect.

7.2.1. Guidelines for subsequent peginterferon alfa-2a dose adjustments

Once a participant's dose has been decreased for laboratory abnormalities or adverse events, the investigator may attempt to increase the dose back to or toward the previous stable level only if the following conditions are satisfied:

1. The event or circumstance responsible for the dosage adjustment has resolved or improved;

2. The participant has been at the lower dose for ≤4 consecutive doses;
3. The participants had ≤ 6 total doses administered at the lower level during the entirety of the treatment period.

If four or more consecutive doses of peginterferon alfa-2a are held or otherwise not administered (i.e., the participant has not received test medication for more than 28 days), the participant will be considered intolerant of the test medication or non-compliant, whichever is more appropriate to the clinical situation. In such cases, the investigator will discontinue peg-interferon alfa-2a and entecavir.

7.3. Management of adverse effects of entecavir

7.3.1. Adverse effects of entecavir

- The most common adverse effects of entecavir have been headache, fatigue, dizziness, and nausea. Other adverse effects include diarrhea, dyspepsia, insomnia, somnolence, and vomiting.
- Lactic acidosis, usually associated with severe hepatomegaly and steatosis, has been associated with treatment with nucleoside analogues alone or with antiretrovirals. Symptoms of lactic acidosis include weakness/tiredness, muscle pain, trouble breathing, stomach pain with nausea and vomiting, feeling cold (particularly in extremities), dizziness/lightheadedness, and increased heart rate.
- Exacerbation of hepatitis B has been reported both during and after stopping treatment with entecavir. Hepatic function should be monitored closely while on treatment and for several months after treatment is stopped. Hepatitis “flares” in participants with chronic hepatitis B, liver enzyme changes are common.
- Laboratory studies have shown that entecavir does not interact with the enzymes in the liver that are responsible for most drug interactions. However, because the body gets rid of Entecavir in the urine, it is possible that drugs which affect kidney function could increase the levels of Entecavir when a participant uses both drugs. These types of drugs are not allowed in this study.

7.4. Pregnancy

Females of childbearing potential (FOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 6 weeks after the study in such a manner that the risk of pregnancy is minimized.

FOCBP includes any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea ≥ 12 consecutive months; or females on hormone replacement therapy [HRT] with documented serum follicle stimulating hormone [FSH] level >35 mIU/mL). Even females who are using oral, implanted or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential.

FOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG] within 72 hours prior to the start of the study medication.

7.4.1. Requirements for pregnancy testing

All FOCBP must have a negative pregnancy test within 72 hours as specified above prior to receiving the study drug. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive the study drug and must not be enrolled in the study.

Pregnancy testing must also be performed throughout the study as specified in Schedule of assessment in Appendix III (All female participant of child bearing potential will undergo urine pregnancy test on enrollment and prior to starting therapy and again at every visit) and the results of all pregnancy tests (positive or negative) recorded on the CRF or transferred electronically. In addition, all FOCBP should be instructed to contact investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during participation.

7.4.2. Reporting pregnancy

If, following initiation of the study drug, a participant is found to be pregnant, both peginterferon alfa-2a and entecavir therapy will be permanently discontinued. Exceptions to the study drug discontinuation may be considered for life-threatening conditions as specified in this protocol. The investigator must immediately (i.e., within 24 hours) notify Data Coordinating Center of this event, by recording pregnancy on the SAE form.

The study will collect data on the outcomes of any pregnancies that occur in females who conceived while taking study medication.

7.5. Adverse events- definitions and reporting

7.5.1. Definitions

Adverse event: An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can be any adverse change from the participant's baseline condition, including intercurrent illness which occurs during the course of the trial, after the consent form has been signed, whether the event is considered related to treatment or not.

The modified World Health Organization (WHO) grading system will be used for grading severity of AE. For AEs not outlined in the modified WHO grading system, study specific definitions will be identified in the Manual of Operation (MOP) the following definitions will be used

- Mild: discomfort noticed but no disruption of normal daily activity
- Moderate: discomfort sufficient to reduce or affect daily activity.
- Severe: inability to work or perform normal daily activity.
- Life threatening: represents an immediate threat to life.

A serious adverse event is an untoward medical occurrence that results in any of the following:

1. Death
2. Is life threatening (risk of death at the time of the event)
3. Requires in-patient hospitalization or prolongation of existing hospitalization

4. Results in persistent or significant disability/incapacity
5. Congenital abnormality or birth defect

A serious adverse event which is unexpected and is related will require expedited reporting to the DCC and the NIDDK and appropriate oversight committees or entities

Disease related outcomes, such as the following, will not be considered to be serious adverse events:

1. Development of HCC
2. Hepatic decompensation

Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs.

Unexpected:
An adverse event that is not listed in the Investigator's Brochure (or package insert) or is not listed at the severity that has been observed.

Suspected Adverse Reaction:
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event (i.e., there is evidence to suggest a causal relationship between the drug and the adverse event).

7.5.3 Reporting procedures
All serious adverse events that are unexpected and related to study drug(s) will be reported to the DCC within 24 hours of knowledge of the event, via the Serious Adverse Event form. This reporting includes serious adverse events that occur from the time the participant has signed the clinical trial consent.

The DCC will distribute the expedited report to the NIDDK, the appropriate oversight committees or entities, and clinical centers. Status reports on serious adverse events will be generated by the DCC and will include the relationship of the adverse event to trial medications, the severity of the event and if the event is resolved or ongoing.
All deaths will be reported to the DCC within 24 hours of knowledge of the death via the Serious Adverse Event form. This reporting begins at the time the participant has signed the informed consent up to the last scheduled participant visit. The report will include the relationship of the death to trial medications. A Clinical Outcome form will also be completed and sent to DCC for distribution. Deaths will be reported immediately to the NIDDK, the appropriate oversight committees or entities, and the clinical centers. A death will be reported as an expedited report only if it is unexpected and drug related. A death must also be reported in accordance with local law and regulations.

SAE reporting:

This trial will be conducted under U.S. IND. Therefore, the safety data required to meet IND regulatory requirements will be collected through adverse event reporting by the clinic investigators and will be provided by the Data Coordinating Center to the NIDDK for transmission to the FDA.

A serious adverse event which is unexpected and is related will require expedited reporting to the DCC and the NIDDK and appropriate oversight committees or entities.

Only serious and unexpected suspected adverse reactions with evidence of a causal relationship to the study and/or study drug will be reported to the FDA as an IND safety report according to 21 CFR 312.32.

Unexpected fatal or life-threatening suspected adverse reactions must be reported to FDA no later than 7 calendar days after the sponsor's initial receipt of the information.

All other unexpected serious suspected adverse reactions must be reported to FDA no later than 15 calendar days after the sponsor's initial receipt of the information.

A summary of adverse events will be reported to the FDA as part of the IND annual report

The HBRN will comply with Genentech/Roche and Bristol-Myers Squibb SAE reporting requirements (see Manual of Operations).

7.5.4 Data collection and reporting procedures for Adverse Events

Participants will be interviewed regarding medical conditions, medication changes, and symptoms that have occurred at each study visit. An Adverse Event form will be completed if any adverse event is reported. If the Study Coordinator or Principal Investigator learns of any hospitalizations or other adverse events between study visits, an Adverse Event form will be completed. All adverse events and Serious Adverse Events from time of study entry (consent) up to the end of follow-up (week 96) will be reported to the DCC.

A Serious Adverse Event form will be completed for all adverse events rated as serious.

Participants will be followed for all ongoing unresolved adverse events until they are either resolved, or in the opinion of the Principal Investigator, the participant is medically stable.

The investigator will assess the relationship of each adverse event to the use of study drug, based on available information, using guidelines outlined in the MOP.

8. Statistical considerations

8.1. Statistical analysis

Summary statistics will be generated to describe the participants at baseline. Descriptive statistics, including measures of central tendency (e.g. means, medians) and estimates of spread (e.g., standard deviations, quartiles) will be used for continuous variables such as age and baseline HBV DNA and HBsAg levels. Frequency distributions will be used for categorical variables such as HBV genotype and HBeAg status. Ninety-five percent confidence intervals will be calculated for all point estimates of continuous data. Graphical displays (e.g. histograms, box plots) will also be used to describe the data.

8.1.1. Analysis of primary endpoints

The primary endpoints include safety, measured by the rate of adverse and Serious Adverse Events, and efficacy, defined as HBeAg loss and HBV DNA levels ≤1,000 IU/mL at the time of last follow up 48 weeks after stopping therapy.

That is, only participants achieving HBeAg loss and having HBV DNA ≤1,000 IU/mL at the end of follow-up will be considered as achieving the primary efficacy endpoint.
Safety: Summary statistics of adverse events (number of adverse events, number and percentage of participants with adverse events, rates per person-years) will be provided . Events will be summarized based on the date of onset for the event. A treatment emergent adverse event will be defined as an adverse event that begins on or after the date of first dose of study drug. Events that occur prior to the first dose of study medication or after the last dose of study medication will be summarized separately. Summary statistics of the following are planned:

- all adverse events recorded between screening and first dose of study medication,
- all treatment emergent adverse events,
- all emergent and related adverse events,
- all treatment emergent renal adverse events,
- all treatment emergent and related renal adverse events
- combined Grade 2, 3 and 4 treatment emergent adverse events,
- combined Grade 2, 3 and 4 related treatment and emergent adverse events,
- combined Grade 3 and 4 treatment emergent adverse events,
- combined Grade 3 and 4 related treatment and emergent adverse events,
- all adverse events that caused permanent discontinuation of study drug,
- all adverse events that caused temporary interruption of study drug,
- all serious adverse events, and
- all serious and related adverse events.

Efficacy: The percentage of participants achieving the primary efficacy endpoint will be summarized with the point estimate and 95% confidence interval.

8.1.2. Analysis of secondary endpoints

8.1.2.1. Analysis of secondary endpoint listed in 2.3.1(a): *Estimate the cumulative proportion of HBsAg loss over time during and after treatment*
Cumulative proportion of HBsAg loss over time will be calculated using the product limit (Kaplan-Meier) method and presented with point-wise 95% confidence interval.

8.1.2.2. Analyses of other secondary endpoints

For all of the secondary endpoints listed in sections 2.3.1. – 2.3.5, we will provide frequencies and percentages with corresponding 95% confidence intervals.

Time to such events may also be of interest (e.g. cumulative HBeAg loss). For time-to event analysis, Kaplan-Meier curves will be used to plot the cumulative proportion of events over time.

The second group of endpoints is continuous variables such as changes in ALT and HBV DNA levels from baseline at designated treatment or follow-up time points. Descriptive statistics, including measures of central tendency (e.g. means, medians) and estimates of spread (e.g. standard deviations, quartiles) will be used to characterize such changes.

8.2. Missing data

Primary analyses will be performed using all enrolled participants (intention-to-treat analysis). Participants who do not have primary endpoint data due to drop-out or other

reasons at the end of follow-up will be considered as treatment failures. Since this is a pilot study with a small sample size, formal procedures for handling missing data such as inverse-weighting and multiple imputation will not have enough power, nevertheless, we will apply the following methods to assess the sensitivity of our primary analysis results to different missing data assumptions:
1. Single LOCF Imputation: In this case the primary endpoint analysis will be repeated by replacing missing endpoint data at follow-up using data from prior visits.
2. Inverse-probability-weighting: This analysis will be carried out in two stages. In the first stage, for each subject we will estimate the probability of having complete data at week 96 based on the data collected at prior visits using logistic regression. In the second stage, the primary endpoint analyses will be repeated by weighting each subject with complete data by the inverse of the corresponding estimated probability from the first stage.
3. Multiple Imputations: The missing values at week 96 will be imputed by Markov-Chain Monte-Carlo algorithm, based on the data collected prior to week 96. Five such imputations will be used and the results of the primary endpoints analyses will be repeated on each of these imputed datasets and averaged.

8.3 Sample Size

In this study, we will enroll 60 participants into treatment. The sample size is considered to be achievable. Precision estimates, presented below, are based on various rates of primary endpoint of efficacy [loss of HBeAg and HBV DNA ≤1,000 IU/mL at the end of follow-up (week 96)]. Assumptions for the outcomes of selected endpoints are based on small pilot treatment protocols (14, 15, 19, 21, 22, 37-39).

To account for 12% attrition, precision estimates based on a sample size of 52, in addition to a sample size of 60, were calculated. For example, for a sample size of 52, if the expected proportion achieving the primary efficacy endpoint is 25%, then we would be able to estimate it with 95% Clopper-Pearson confidence that the expected width of the interval does not exceed 25% (or equivalently, the estimated 95% confidence limits will be within ± 12.5% of the estimate). Below are some further precision estimates:

Table: Precision (Width) Estimates for 95% Confidence Intervals under Different Primary Endpoint Proportions

Expected proportion with primary efficacy endpoint	N=60		N=52	
	(95% CI)	Width	(95% CI)	Width
0.15	(0.071, 0.266)	0.195	(0.066, 0.276)	0.210
0.20	(0.108, 0.323)	0.215	(0.102, 0.334)	0.232
0.25	(0.147, 0.379)	0.231	(0.140, 0.389)	0.249
0.30	(0.188, 0.432)	0.244	(0.181, 0.443)	0.262

9. Data and safety monitoring board

Data and safety will be monitored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in conjunction with an NIDDK-appointed Data and Safety

Monitoring Board (DSMB). This board serves in a consultative capacity to inform the NIDDK decisions regarding conduct of the study. The description of DSMB activities is included in the DSMB Charter.

9.1. Data and safety monitoring

The primary objective of this study is to examine the safety and efficacy of treatment with 8 weeks of entecavir followed by 40 weeks of both entecavir and peginterferon alfa-2a 48 weeks after cessation of therapy in children age 3 to <18 years who are in the immune tolerant phase of CHB infection. The risks to participants are outlined in sections 5.2.3 and 5.3.3. Section 7 of the protocol defines the toxicities associated with these drugs and outlines dose adjustment and discontinuation guidelines due to toxicity. The data and safety monitoring plan (DSMP) for this study focuses on close monitoring by the principal investigators (PI) and prompt reporting of excessive adverse events and all serious adverse events to the NIDDK, the Data and Safety Monitoring Board (DSMB) and to the participating centers' IRBs.

The Data Coordinating Center (DCC) will monitor clinical center performance (e.g., recruitment, retention, data completeness, timeliness of data collection and submission) and protocol compliance. These reports, with summaries of adverse event data, will be provided to the DSMB for their quarterly reports and biannual calls or meetings, and to the Steering Committee at its annual meeting.

The Data Coordinating Center will work with the safety officer and the Steering Committee to maintain a cumulative summary of adverse events (overall and stratified by serious/non-serious status) that will be forwarded to the DSMB every three months. Safety reports will also be sent to the Principal Investigators and the NIDDK Project Officers. The Project Coordinator will be responsible for distributing these reports and assuring that all parties obtain copies of these reports.

The frequency of data review for this study differs according to the type of data and can be summarized in the following table:

Data type	**Frequency of review**
Recruitment Retention Protocol adherence(e.g., meeting inclusion/exclusion criteria, treatment compliance) Pregnancy and outcomes in female participants	Quarterly reports for DSMB and NIDDK
Adverse Events and Serious Adverse Events (SAE) that do not meet expedited reporting criteria	Quarterly reports for DSMB and NIDDK
Serious Adverse Events that meet expedited reporting criteria)	As they occur for NIDDK, DSMB, appropriate industry partner(s), and cumulative reports quarterly
Laboratory data	Yearly for the DSMB

Definitions of adverse and serious adverse events and their management guidelines are provided in section 7.5 of this protocol along with the reporting procedures.

No interim analysis for efficacy is planned for this clinical trial.

9.2 Participant confidentiality

The central database of the study is on a server at the Epidemiology Data Center (EDC) in the Graduate School of Public Health at the University of Pittsburgh secured behind locked doors and an alarm with password access provided only to authorized personnel. Backups are performed daily to guard against data loss due to an equipment or power failure. Scheduled backups and archives at the EDC protect central and local information from hard disk failures. Tape backup volumes and CD-ROM copies of critical project files are located in a secured off-site storage area to prevent data loss due to catastrophic events. Routine virus detection is also enforced for all EDC computers involved in the study. All critical information regarding database transactions is logged and stored in journal files. In the event of accidental corruption of the project database, a previous database state may be restored from backup volumes or journal files. All servers used for this project are connected to uninterrupted power supplies to protect equipment against electrical surges and outages. A secured, raised-floor computer room in an area with a burglar alarm houses all project server equipment.

Participant confidentiality is preserved by encoding subject names into alphanumeric IDs at the clinical centers. Data sent to the DCC are identified by alphanumeric ID only. No reports of this study will use names or other identifying information such as social security numbers or addresses. Data, with alphanumeric ID only, will be stored at the DCC indefinitely. In addition, following the completion of the study data and information, with alphanumeric ID only, will be submitted in the NIDDK repository by a suitable date agreed upon by NIDDK and the steering committee.

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Appendix I: Entecavir study medication dosing table

Body weight (kg) [a]	**Body weight (lbs)**	**Dose final (mL, per 0.015 mg/kg) [b]**
10.0 - 10.8	22.1 - 23.8	3.0
10.9 - 12.4	24.0 - 27.3	3.5
12.5 - 14.1	27.6 - 31.1	4.0
14.2 - 15.8	31.3 - 34.8	4.5
15.9 - 17.4	35.1 - 38.4	5.0
17.5 - 19.1	38.6 - 42.1	5.5
19.2 - 20.8	42.3 - 45.9	6.0
20.9 - 22.5	46.1 - 49.6	6.5
22.6 - 24.1	49.8 - 53.1	7.0
24.2 - 25.8	53.4 - 56.9	7.5
25.9 - 27.5	57.1 - 60.6	8.0
27.6 - 29.1	60.9 - 64.2	8.5
29.2 - 30.8	64.4 - 67.9	9.0
30.9 - 32.5	68.1 - 71.7	9.5
32.6 - 33.0	71.9 - 72.8	10.0

a. If weight in pounds (lb) is not included in the table, use the closest higher body weight (lb.) in the table for study medication dose. The entecavir dose is calculated as follows:

$$\frac{\text{weight (kg)} \times 0.015 \text{ mg/Kg}}{0.05 \text{ mg/mL}} = \text{weight (Kg)} \times 0.3 \text{ mL/Kg} = \text{mL solution.}$$

Round to the nearest 0.5 mL.

b. Children with body weight > 32.6 kg (71.9 lb) should receive 10.0 mL of oral solution or the tablet formulation (0.5 mg) of the study medication.

Appendix II: Peginterferon alfa-2a dose adjustment guidelines

Specific dose adjustment guidelines for peginterferon alfa-2a are provided in the tables below for elevated serum ALT activities, neutropenia, and thrombocytopenia. For other adverse effects considered to be possibly related to peginterferon alfa-2a, including laboratory abnormalities, adverse events, and vital signs changes, investigators should utilize the table below labeled "a. Peginterferon alfa-2aDose Reduction Guidelines." When practicable, abnormal laboratory results should be confirmed as soon as possible following notification of the investigator. If appropriate, downward adjustments in one level increments should be considered. The lowest dose of peginterferon alfa-2a that should be administered is 45 mcg/1.73m^2 (or 26 mcg/m^2) weekly. It should be kept in mind that whereas these guidelines should be generally followed to promote consistency across centers, other responses by an investigator may be more appropriate in some circumstances. The participant will be instructed to inject the peginterferon alfa-2a at the same time on the same day each week. The dosing of peginterferon alfa-2a will be multiplied by the participant's body surface area (BSA): 180 µg/1.73 m^2 x BSA at each visit. The BSA will be computed by the Mosteller's formula:
BSA (m^2) = ([Height(cm) x Weight(kg)]/3600)$^{1/2}$.

Investigators will adjust the dose of peginterferon alfa-2a upward or downward to reflect the most current BSA if there is a significant increase or decrease (i.e. a change of greater than 10%) from the beginning of the study (baseline BSA).
Refer to the MOP for peginterferon alfa-2a dosing charts with calculated volume per injection by BSA.

a. Peginterferon alfa-2a dose reduction guidelines

Original Dose	One Level Adjustment	Two Level Adjustment	Three Level Adjustment
(180 mcg/1.73 m^2)* BSA	(135 mcg/1.73 m^2)* BSA	(90 mcg/1.73 m^2)* BSA	(45 mcg/1.73 m^2)* BSA
(104 mcg/m^2) * BSA	(78 mcg/m^2) * BSA	(52 mcg/m^2) * BSA	(26 mcg/m^2) * BSA

Number of Dose Reduction Levels					
Mild	Moderate Limited	Moderate Persistent	Severe Limited	Severe Persistent	Life-Threatening
0	0	0-1	0-1	1-3	Stop drug

b. Dose adjustments for low absolute neutrophil and platelet counts

Parameter	Response
ANC (cells/mm^3)	
≥1000	None
750-999	Week 1-2*: Immediate 1 Level Adjustment Week 3-40: None
500-749	Week 1-2: Delay or hold dose until ≥750 then resume dose with a 1 Level Adjustment Assess weekly x 3 to verify WBC's ≥750 Week 3-40: Immediate 1 Level Adjustment

250-499	Week 1-2: Delay or hold dose until ≥750 then resume dose with a 2 Level Adjustment Week 3-40: Delay or hold dose until ≥750 then resume dose with a 1 Level Adjustment
<250 or febrile neutropenia	STOP DRUG

*****in this table "Week" refers to weeks of peginterferon alfa-2a

c. Peginterferon alfa-2a dose adjustments for low platelet counts

Parameter	Response
Platelets (cells/mm^3)	
≥50,000	None
35,000-49,999	Delay or hold dose until 50,000 then resume dose with a Level 1 adjustment
25,000-34,999	Delay or hold dose until 50,000 then resume dose with a Level 2 adjustment
<25,000	STOP DRUG

Appendix III Table 1. Schedule of assessments for immune tolerant trial: treatment phase

Event	Screen (days)	Entecavir Alone (8 weeks)			Entecavir + Peginterferon alfa-2a (40 weeks)											
Weeks of Treatment	**-42 to -1**	**0[1]**	**4**	**8**	**10**	**12**	**14**	**16**	**20**	**24**	**28**	**32**	**36**	**40**	**44**	**48**
Informed consent	X															
Complete medical history	X															
Vital signs & physical exam	X															X
Vital signs & symptom directed physical		X		X		X		X		X			X			
Telephone Assessment			X		X		X		X		X	X		X	X	
Adverse Events and Concomitant medicines	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Child Health Questionnaire (CHQ)		X								X						X
Health Behavior		X														X
Depression Assessment	X	X				X				X			X			X
Tanner Stage		X														X
Symptom Questionnaire		X		X		X		X		X			X			X
Drug Dispensing		X		X		X		X		X			X			
Drug Adherence (Participant Diary Review)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with differential	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hepatic panel (AST, ALT, bilirubin)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Other Hepatic panel (albumin, ALKP)	X	X								X			X			X
Renal Panel (Creatinine and CrCl (calculated), PO4, serum lipase, glucose, creatinine phosphokinase (CK), serum electrolytes)	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Other renal panel (Ca, BUN)	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
Thyroid Peroxidase antibody	X															
Hemoglobin A1C	X															
Other Chemistries (uric acid, triglycerides, cholesterol, total protein, TSH and Free T4)	X					X				X			X			X
Urinalysis	X	X								X			X			X
Pregnancy test[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PT/INR	X	X								X			X			X
AFP	X															
Anti-HCV	X															
Anti-HDV	X															
Anti-HIV	X															
Serum banking	X	X		X		X		X		X			X			X
HBeAg	X	X		X		X				X			X			X
Anti-HBe		X														X
HBsAg	X															X
HBsAg quant		X		X		X		X		X			X			X
HBeAg quant		X		X		X		X		X			X			X
Anti-HBs		X														X
Quant HBV DNA	X	X		X		X		X		X			X			X
HBV genotype and subtype		X														

HBV precore/BCP		X														[1.]
Antiviral resistance testing		X													X	

[1] Day 0 is the Baseline visit and start of study drug.
[2] In childbearing potential females

Appendix III Table 2. Schedule of assessments for immune tolerant trial: follow-up after treatment discontinuation[1]

Weeks Post Treatment Discontinuation	**4**	**8**	**12**	**24**	**36**	**48**
Study Week	**52**	**56**	**60**	**72**	**84**	**96**
Vital signs & physical exam						X
Vital signs & symptom directed physical		X	X	X	X	
Adverse Events, Concomitant medicines	X	X	X	X	X	X
CHQ and health behavior						X
Depression Assessment			X		X	X
Tanner Stage				X		X
Symptom Questionnaire	X	X	X	X	X	X
CBC with differential	X		X	X		X
Hepatic panel (AST, ALT, bilirubin)	X	X	X	X	X	X
Creatinine	X	X	X	X	X	X
Urinalysis						X
PT/INR	X	X	X	X	X	X
TSH, Free T4, glucose, triglycerides, cholesterol, creatinine phosphokinase, serum electrolytes, total protein, albumin, calcium, BUN, alkaline phosphatase, uric acid				X		X
Serum lipase	X		X			X
HBeAg			X	X		X
Anti-HBe				X		X
Serum banking	X	X	X	X	X	X
HBsAg				X		X
HBsAg quant			X	X		X
HBeAg quant			X	X		X
Anti-HBs				X		X
Quant HBV DNA	X	X	X	X	X	X
HBV precore/BCP						X

[1]Schedule is used for participants:
(i) at end of treatment
(ii) stopping treatment due to an adverse event

Appendix IV: Research blood draw schedule

Visits	Scenario	Research lab and storage (ml)
Screening (for both groups)	12 kg-24 kg	2[1]
	24 kg-28 kg	2[1]
	>28 kg	4[1] (includes up to 2 for banking)
Baseline (for both groups)	12 kg-24 kg	5[2]
	24 kg-28 kg	5[2]
	>28 kg	7[2] (includes up to 2 for banking)
Follow-up visit	Telephone assessment	0
	Regular visit	7 (includes up to 2 for banking)
Off treatment visit		2-5[4] (includes up to 2 for banking)

[1] HBV DNA only
[2] HBV DNA, HBV genotype and subtype, HBV precore/BCP, antiviral resistance testing
[3] HBV DNA, HBV precore/BCP or antiviral resistance testing, banking
[4] HBV DNA, antiviral resistance testing, banking

Appendix V: Participating pediatric centers

1. University of Washington
2. University of California, San Francisco
3. University of Toronto
4. University of Texas Southwestern
5. University of Minnesota
6. Saint Louis University
7. Johns Hopkins University

Appendix VI: DAIDS Toxicity Grading Table

Division of AIDS table for grading the severity of
Adult and pediatric adverse events
Version 1.0, December, 2004; clarification August 2009

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ("DAIDS AE Grading Table") is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

This clarification of the DAIDS Table for Grading the Severity of Adult and Pediatric AE's provides additional explanation of the DAIDS AE Grading Table and clarifies some of the parameters.

I. Instructions and Clarifications

Grading Adult and Pediatric AEs

The DAIDS AE Grading Table includes parameters for grading both Adult and Pediatric AEs. When a single set of parameters is not appropriate for grading specific types of AEs for both Adult and Pediatric populations, separate sets of parameters for Adult and/or Pediatric populations (with specified respective age ranges) are given in the Table. If there is no distinction in the Table between Adult and Pediatric values for a type of AE, then the single set of parameters listed is to be used for grading the severity of both Adult and Pediatric events of that type.

Note: In the classification of adverse events, the term "severe" is not the same as "serious." Severity is an indication of the intensity of a specific event (as in mild, moderate, or severe chest pain). The term "serious" relates to a participant/event outcome or action criteria, usually associated with events that pose a threat to a participant's life or functioning.

Addenda 1-3 Grading Tables for Microbicide Studies

For protocols involving topical application of products to the female genital tract, male genital area or rectum, strong consideration should be given to using Appendices I-III as the primary grading scales for these areas. The protocol would need to specifically state that one or more of the Appendices would be primary (and thus take precedence over the main Grading Table) for items that are listed in both the Appendix and the main Grading Table.

- 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

Grade 5

For any AE where the outcome is death, the severity of the AE is classified as Grade 5.

Estimating Severity Grade for Parameters Not Identified in the Table

In order to grade a clinical AE that is not identified in the DAIDS AE grading table, use the category "Estimating Severity Grade" located on Page 3.

Determining Severity Grade for Parameters "Between Grades"

If the severity of a clinical AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE. If a laboratory value that is graded as a multiple of the ULN or LLN falls between two grades, select the higher of the two grades for the AE. For example, Grade 1 is 2.5 x ULN and Grade 2 is 2.6 x ULN for a parameter. If the lab value is 2.53 x ULN (which is between the two grades), the severity of this AE would be Grade 2, the higher of the two grades.

Values Below Grade 1

Any laboratory value that is between either the LLN or ULN and Grade 1 should not be graded.

Determining Severity Grade when Local Laboratory Normal Values Overlap with Grade 1 Ranges

In these situations, the severity grading is based on the ranges in the DAIDS AE Grading Table, even when there is a reference to the local lab LLN.

For example: Phosphate, Serum, Low, Adult and Pediatric > 14 years (Page 20) Grade 1 range is 2.50 mg/dL - < LLN. A particular laboratory's normal range for Phosphate is 2.1 – 3.8 mg/dL. A participant's actual lab value is 2.5. In this case, the value of 2.5 exceeds the LLN for the local lab, but will be graded as Grade 1 per DAIDS AE Grading Table.

II. Definitions of terms used in the Table:

Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding. Young Children Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).
LLN	Lower limit of normal
Medical Intervention	Use of pharmacologic or biologic agent(s) for treatment of an AE.
NA	Not Applicable
Operative Intervention	Surgical OR other invasive mechanical procedures.
ULN	Upper limit of normal
Usual Social & Functional Activities	Adult Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc. Young Children Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ESTIMATING SEVERITY GRADE				
Clinical adverse event NOT identified elsewhere in this DAIDS AE Grading Table	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 Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYSTEMIC				
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions
Fever (nonaxillary)	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Pain (indicate body site) DO NOT use for pain due to injection (See Injection Site Reactions: Injection site pain) See also Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than emergency room visit) indicated

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
INFECTION				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (e.g., septic shock)
INJECTION SITE REACTIONS				
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Injection site reaction (localized)				
Adult > 15 years	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm^2 – 81cm^2)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
Pediatric ≤ 15 years	Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Pruritis associated with injection See also Skin: Pruritis (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA
SKIN – DERMATOLOGICAL				
Alopecia	Thinning detectable by study participant (or by caregiver for young children and disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	NA
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	NA	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
CARDIOVASCULAR				
Cardiac arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic, non-life-threatening AND Non-urgent medical intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Cardiac-ischemia/infarction	NA	NA	Symptomatic ischemia (stable angina) OR Testing consistent with ischemia	Unstable angina OR Acute myocardial infarction

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Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hemorrhage (significant acute blood loss)	NA	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≤ 2 units packed RBCs (for children ≤ 10 cc/kg) indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children > 10 cc/kg) indicated
Hypertension				
Adult > 17 years (with repeat testing at same visit)	140 – 159 mmHg systolic OR 90 – 99 mmHg diastolic	160 – 179 mmHg systolic OR 100 – 109 mmHg diastolic	**≥** 180 mmHg systolic OR **≥** 110 mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Correction: in Grade 2 to 160 - 179 from > 160-179 (systolic) and to ≥ 100 -109 from > 100-109 (diastolic) and in Grade 3 to ≥ 180 from > 180 (systolic) and to ≥ 110 from > 110 (diastolic).				
Pediatric ≤ 17 years (with repeat testing at same visit)	NA	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Pericardial effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no intervention	Effusion with non-life threatening physiologic consequences OR Effusion with non-urgent intervention indicated	Life-threatening consequences (e.g., tamponade) OR Urgent intervention indicated
Prolonged PR interval				
Adult > 16 years	PR interval 0.21 – 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec	Complete AV block
Pediatric ≤ 16 years	1st degree AV block (PR > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block	Complete AV block

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Prolonged QTc				
Adult > 16 years	Asymptomatic, QTc interval 0.45 – 0.47 sec OR Increase interval < 0.03 sec above baseline	Asymptomatic, QTc interval 0.48 – 0.49 sec OR Increase in interval 0.03 – 0.05 sec above baseline	Asymptomatic, QTc interval ≥ 0.50 sec OR Increase in interval ≥ 0.06 sec above baseline	Life-threatening consequences, e.g. Torsade de pointes or other associated serious ventricular dysrhythmia
Pediatric ≤ 16 years	Asymptomatic, QTc interval 0.450 – 0.464 sec	Asymptomatic, QTc interval 0.465 – 0.479 sec	Asymptomatic, QTc interval ≥ 0.480 sec	Life-threatening consequences, e.g. Torsade de pointes or other associated serious ventricular dysrhythmia
Thrombosis/embolism	NA	Deep vein thrombosis AND No intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Deep vein thrombosis AND Intervention indicated (e.g., anticoagulation, lysis filter, invasive procedure)	Embolic event (e.g., pulmonary embolism, life-threatening thrombus)
Vasovagal episode (associated with a procedure of any kind)	Present without loss of consciousness	Present with transient loss of consciousness	NA	NA
Ventricular dysfunction (congestive heart failure)	NA	Asymptomatic diagnostic finding AND intervention indicated	New onset with symptoms OR Worsening symptomatic congestive heart failure	Life-threatening congestive heart failure
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
Comment: Please note that, while the grading scale provided for Unintentional Weight Loss may be used as a guideline when grading anorexia, this is not a requirement and should not be used as a substitute for clinical judgment.				
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., diuretics or therapeutic paracentesis)	Symptomatic despite intervention	Life-threatening consequences
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis or perforation)

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Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
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				
Adult and Pediatric ≥ 1 year	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 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Pediatric < 1 year	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Liquid stools resulting in severe dehydration with aggressive rehydration indicated OR Hypotensive shock
Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated	Life-threatening reduction in oral intake
Mucositis/stomatitis (clinical exam) Indicate site (e.g., larynx, oral) See Genitourinary for Vulvovaginitis See also Dysphagia-Odynophagia and Proctitis	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences (e.g., aspiration, choking)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than emergency room visit)	Symptomatic AND Hospitalization indicated (other than emergency room visit)	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)

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Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Proctitis (functional-symptomatic) Also see Mucositis/stomatitis for clinical exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
NEUROLOGIC				
Alteration in personality-behavior or in mood (e.g., agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with usual social & functional activities	Alteration causing inability to perform usual social & functional activities	Behavior potentially harmful to self or others (e.g., suicidal and homicidal ideation or attempt, acute psychosis) OR Causing inability to perform basic self-care functions
Altered Mental Status For Dementia, see Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions
Cognitive and behavioral/attentional disturbance (including dementia and attention deficit disorder)	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
CNS ischemia (acute)	NA	NA	Transient ischemic attack	Cerebral vascular accident (CVA, stroke) with neurological deficit
Developmental delay – **Pediatric ≤ 16 years**	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Difficulty sleeping causing inability to perform usual social & functional activities	Disabling insomnia causing inability to perform basic self-care functions
Neuromuscular weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal interference with usual social & functional activities	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Seizure: (new onset) **– Adult ≥ 18 years** See also Seizure: (known pre-existing seizure disorder)	NA	1 seizure	2 **–** 4 seizures	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure: (known pre-existing seizure disorder) **– Adult ≥ 18 years** For worsening of existing epilepsy the grades should be based on an increase from previous level of control to any of these levels.	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR Infrequent break-through seizures while on stable medication in a previously controlled seizure disorder	Change in seizure character from baseline either in duration or quality (e.g., severity or focality)	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure **– Pediatric < 18 years**	Seizure, generalized onset with or without secondary generalization, lasting < 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5 – 20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Syncope (not associated with a procedure)	NA	Present	NA	NA
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
RESPIRATORY				
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 **–** 80%	FEV1 or peak flow 50 **–** 69%	FEV1 or peak flow 25 **–** 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress				
Adult ≥ 14 years	Dyspnea on exertion with no or minimal interference with usual social & functional activities	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities	Dyspnea at rest causing inability to perform usual social & functional activities	Respiratory failure with ventilatory support indicated

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Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Pediatric < 14 years	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 – 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilatory support indicated
MUSCULOSKELETAL				
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss				
Adult ≥ 21 years	BMD t-score -2.5 to -1.0	BMD t-score < -2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Pediatric < 21 years	BMD z-score -2.5 to -1.0	BMD z-score < -2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	Asymptomatic with radiographic findings AND No operative intervention indicated	Symptomatic bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
GENITOURINARY				
Cervicitis (symptoms) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	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

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Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

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Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Cervicitis (clinical exam) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Minimal cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption < 25% of total surface	Moderate cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption of 25 – 49% total surface	Severe cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption 50 – 75% total surface	Epithelial disruption > 75% total surface
Inter-menstrual bleeding (IMB)	Spotting observed by participant OR Minimal blood observed during clinical or colposcopic examination	Inter-menstrual bleeding not greater in duration or amount than usual menstrual cycle	Inter-menstrual bleeding greater in duration or amount than usual menstrual cycle	Hemorrhage with life-threatening hypotension OR Operative intervention indicated
Urinary tract obstruction (e.g., stone)	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences
Vulvovaginitis (symptoms) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	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
Vulvovaginitis (clinical exam) (Use in studies evaluating topical study agents) For other vulvovaginitis see Infection: Infection (any other than HIV infection)	Minimal vaginal abnormalities on examination OR Epithelial disruption < 25% of total surface	Moderate vaginal abnormalities on examination OR Epithelial disruption of 25 - 49% total surface	Severe vaginal abnormalities on examination OR Epithelial disruption 50 - 75% total surface	Vaginal perforation OR Epithelial disruption > 75% total surface
OCULAR/VISUAL				
Uveitis	Asymptomatic but detectable on exam	Symptomatic anterior uveitis OR Medical intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)

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Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

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PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Visual changes (from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
ENDOCRINE/METABOLIC				
Abnormal fat accumulation (e.g., back of neck, breasts, abdomen)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	NA
Diabetes mellitus	NA	New onset without need to initiate medication OR Modification of current medications to regain glucose control	New onset with initiation of medication indicated OR Diabetes uncontrolled despite treatment modification	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma)
Gynecomastia	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy (e.g., fat loss from the face, extremities, buttocks)	Detectable by study participant (or by caregiver for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY	***Standard International Units are listed in italics***			
Absolute CD4+ count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	300 – 400/mm^3 *300 – 400/µL*	200 – 299/mm^3 *200 – 299/µL*	100 – 199/mm^3 *100 – 199/µL*	< 100/mm^3 *< 100/µL*
Absolute lymphocyte count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	600 – 650/mm^3 *0.600 x 10^9 – 0.650 x 10^9/L*	500 – 599/mm^3 *0.500 x 10^9 – 0.599 x 10^9/L*	350 – 499/mm^3 *0.350 x 10^9 – 0.499 x 10^9/L*	< 350/mm^3 *< 0.350 x 10^9/L*
Comment: Values in children ≤ 13 years are not given for the two parameters above because the absolute counts are variable.				
Absolute neutrophil count (ANC)				
Adult and Pediatric, > 7 days	1,000 – 1,300/mm^3 *1.000 x 10^9 – 1.300 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	500 – 749/mm^3 *0.500 x 10^9 – 0.749 x 10^9/L*	< 500/mm^3 *< 0.500 x 10^9/L*
Infant[♦†], **2 – ≤ 7 days**	1,250 – 1,500/mm^3 *1.250 x 10^9 – 1.500 x 10^9/L*	1,000 – 1,249/mm^3 *1.000 x 10^9 – 1.249 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	< 750/mm^3 *< 0.750 x 10^9/L*
Infant[♦†], **≤1 day**	4,000 – 5,000/mm^3 *4.000 x 10^9 – 5.000 x 10^9/L*	3,000 – 3,999/mm^3 *3.000 x 10^9 – 3.999 x10^9/L*	1,500 – 2,999/mm^3 *1.500 x 10^9 – 2.999 x 10^9/L*	< 1,500/mm^3 *< 1.500 x 10^9/L*
Comment: Parameter changed from "Infant, < 1 day" to "Infant, **≤**1 day"				
Fibrinogen, decreased	100 – 200 mg/dL *1.00 – 2.00 g/L* OR 0.75 – 0.99 x LLN	75 – 99 mg/dL *0.75 – 0.99 g/L* OR 0.50 – 0.74 x LLN	50 – 74 mg/dL *0.50 – 0.74 g/L* OR 0.25 – 0.49 x LLN	< 50 mg/dL *< 0.50 g/L* OR < 0.25 x LLN OR Associated with gross bleeding

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Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Hemoglobin (Hgb)				
Comment: The Hgb values in mmol/L have changed because the conversion factor used to convert g/dL to mmol/L has been changed from 0.155 to 0.6206 (the most commonly used conversion factor). For grading Hgb 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 that lab.				
Adult and Pediatric ≥ 57 days (HIV POSITIVE ONLY)	8.5 – 10.0 g/dL *5.24 – 6.23 mmol/L*	7.5 – 8.4 g/dL *4.62–5.23 mmol/L*	6.50 – 7.4 g/dL *4.03–4.61 mmol/L*	< 6.5 g/dL *< 4.03 mmol/L*
Adult and Pediatric ≥ 57 days (HIV NEGATIVE ONLY)	10.0 – 10.9 g/dL *6.18* – 6.79 *mmol/L* OR Any decrease 2.5 – 3.4 g/dL *1.58 – 2.13 mmol/L*	9.0 – 9.9 g/dL *5.55 - 6.17 mmol/L* OR Any decrease 3.5 – 4.4 g/dL *2.14 – 2.78 mmol/L*	7.0 – 8.9 g/dL *4.34 - 5.54 mmol/L* OR Any decrease ≥ 4.5 g/dL *> 2.79 mmol/L*	< 7.0 g/dL *< 4.34 mmol/L*
Comment: The decrease is a decrease from baseline				
Infant*†, 36 – 56 days (HIV POSITIVE OR NEGATIVE)	8.5 – 9.4 g/dL *5.24 – 5.86 mmol/L*	7.0 – 8.4 g/dL *4.31 – 5.23 mmol/L*	6.0 – 6.9 g/dL *3.72 – 4.30 mmol/L*	< 6.00 g/dL *< 3.72 mmol/L*
Infant*†, 22 – 35 days (HIV POSITIVE OR NEGATIVE)	9.5 – 10.5 g/dL *5.87 - 6.54 mmol/L*	8.0 – 9.4 g/dL *4.93 – 5.86 mmol/L*	7.0 – 7.9 g/dL *4.34 – 4.92 mmol/L*	< 7.00 g/dL *< 4.34 mmol/L*
Infant*†, ≤ 21 days (HIV POSITIVE OR NEGATIVE)	12.0 – 13.0 g/dL *7.42 – 8.09 mmol/L*	10.0 – 11.9 g/dL *6.18 – 7.41 mmol/L*	9.0 – 9.9 g/dL *5.59- 6.17 mmol/L*	< 9.0 g/dL *< 5.59 mmol/L*
Correction: Parameter changed from "Infant < 21 days" to "Infant ≤ 21 days"				
International Normalized Ratio of prothrombin time (INR)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Methemoglobin	5.0 – 10.0%	10.1 – 15.0%	15.1 – 20.0%	> 20.0%
Prothrombin Time (PT)	1.1 – 1.25 x ULN	1.26 – 1.50 x ULN	1.51 – 3.00 x ULN	> 3.00 x ULN
Partial Thromboplastin Time (PTT)	1.1 – 1.66 x ULN	1.67 – 2.33 x ULN	2.34 – 3.00 x ULN	> 3.00 x ULN
Platelets, decreased	100,000 – 124,999/mm^3 *100.000 x 10^9 – 124.999 x 10^9/L*	50,000 – 99,999/mm^3 *50.000 x 10^9 – 99.999 x 10^9/L*	25,000 – 49,999/mm^3 *25.000 x 10^9 – 49.999 x 10^9/L*	< 25,000/mm^3 *< 25.000 x 10^9/L*
WBC, decreased	2,000 – 2,500/mm^3 *2.000 x 10^9 – 2.500 x 10^9/L*	1,500 – 1,999/mm^3 *1.500 x 10^9 – 1.999 x 10^9/L*	1,000 – 1,499/mm^3 *1.000 x 10^9 – 1.499 x 10^9/L*	< 1,000/mm^3 *< 1.000 x 10^9/L*

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

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Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

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LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
CHEMISTRIES	***Standard International Units are listed in italics***			
Acidosis	NA	pH < normal, but ≥ 7.3	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, serum, low	3.0 g/dL – < LLN *30 g/L – < LLN*	2.0 – 2.9 g/dL *20 – 29 g/L*	< 2.0 g/dL *< 20 g/L*	NA
Alkaline Phosphatase	1.25 – 2.5 x ULN[†]	2.6 – 5.0 x ULN[†]	5.1 – 10.0 x ULN[†]	> 10.0 x ULN[†]
Alkalosis	NA	pH > normal, but ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bicarbonate, serum, low	16.0 mEq/L – < LLN *16.0 mmol/L – < LLN*	11.0 – 15.9 mEq/L *11.0 – 15.9 mmol/L*	8.0 – 10.9 mEq/L *8.0 – 10.9 mmol/L*	< 8.0 mEq/L *< 8.0 mmol/L*
Comment: Some laboratories will report this value as Bicarbonate (HCO_3) and others as Total Carbon Dioxide (CO_2). These are the same tests; values should be graded according to the ranges for Bicarbonate as listed above.				
Bilirubin (Total)				
Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Infant[•†], ≤ 14 days (non-hemolytic)	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	25.1 – 30.0 mg/dL *429 – 513 µmol/L*	> 30.0 mg/dL *> 513.0 µmol/L*
Infant[•†], ≤ 14 days (hemolytic)	NA	NA	20.0 – 25.0 mg/dL *342 – 428 µmol/L*	> 25.0 mg/dL *> 428 µmol/L*
Calcium, serum, high				
Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL *2.65 – 2.88 mmol/L*	11.6 – 12.5 mg/dL *2.89 – 3.13 mmol/L*	12.6 – 13.5 mg/dL *3.14 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Infant[•†], < 7 days	11.5 – 12.4 mg/dL *2.88 – 3.10 mmol/L*	12.5 – 12.9 mg/dL *3.11 – 3.23 mmol/L*	13.0 – 13.5 mg/dL *3.245 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Calcium, serum, low				
Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL *1.95 – 2.10 mmol/L*	7.0 – 7.7 mg/dL *1.75 – 1.94 mmol/L*	6.1 – 6.9 mg/dL *1.53 – 1.74 mmol/L*	< 6.1 mg/dL *< 1.53 mmol/L*
Infant[•†], < 7 days	6.5 – 7.5 mg/dL *1.63 – 1.88 mmol/L*	6.0 – 6.4 mg/dL *1.50 – 1.62 mmol/L*	5.50 – 5.90 mg/dL *1.38 – 1.51 mmol/L*	< 5.50 mg/dL *< 1.38 mmol/L*
Comment: Do not adjust Calcium, serum, low or Calcium, serum, high for albumin				

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Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

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LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Cardiac troponin I (cTnI)	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cardiac troponin T (cTnT)	NA	NA	NA	≥ 0.20 ng/mL OR Levels consistent with myocardial infarction or unstable angina as defined by the manufacturer
Cholesterol (fasting)				
Adult ≥ 18 years	200 – 239 mg/dL *5.18 – 6.19 mmol/L*	240 – 300 mg/dL *6.20 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Pediatric < 18 years	170 – 199 mg/dL *4.40 – 5.15 mmol/L*	200 – 300 mg/dL *5.16 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Creatine Kinase	3.0 – 5.9 x ULN[†]	6.0 – 9.9 x ULN[†]	10.0 – 19.9 x ULN[†]	≥ 20.0 x ULN[†]
Creatinine	1.1 – 1.3 x ULN[†]	1.4 – 1.8 x ULN[†]	1.9 – 3.4 x ULN[†]	≥ 3.5 x ULN[†]

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Glucose, serum, high				
Nonfasting	116 – 160 mg/dL *6.44 – 8.88 mmol/L*	161 – 250 mg/dL *8.89 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Fasting	110 – 125 mg/dL *6.11 – 6.94 mmol/L*	126 – 250 mg/dL *6.95 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75 mmol/L*	> 500 mg/dL *> 27.75 mmol/L*
Glucose, serum, low				
Adult and Pediatric ≥ 1 month	55 – 64 mg/dL *3.05 – 3.55 mmol/L*	40 – 54 mg/dL *2.22 – 3.06 mmol/L*	30 – 39 mg/dL *1.67 – 2.23 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Infant[*†], < 1 month	50 – 54 mg/dL *2.78 – 3.00 mmol/L*	40 – 49 mg/dL *2.22 – 2.77 mmol/L*	30 – 39 mg/dL *1.67 – 2.21 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

Lactate		ULN - < 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
Comment: Added ULN to Grade 1 parameter					
LDL cholesterol (fasting)					
	Adult ≥ 18 years	130 – 159 mg/dL *3.37 – 4.12 mmol/L*	160 – 190 mg/dL *4.13 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
	Pediatric > 2 - < 18 years	110 – 129 mg/dL *2.85 – 3.34 mmol/L*	130 – 189 mg/dL *3.35 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Lipase		1.1 – 1.5 x ULN	1.6 – 3.0 x ULN	3.1 – 5.0 x ULN	> 5.0 x ULN
Magnesium, serum, low		1.2 – 1.4 mEq/L *0.60 – 0.70 mmol/L*	0.9 – 1.1 mEq/L *0.45 – 0.59 mmol/L*	0.6 – 0.8 mEq/L *0.30 – 0.44 mmol/L*	< 0.60 mEq/L *< 0.30 mmol/L*
Pancreatic amylase		1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Phosphate, serum, low					
	Adult and Pediatric > 14 years	2.5 mg/dL – < LLN *0.81 mmol/L – < LLN*	2.0 – 2.4 mg/dL *0.65 – 0.80 mmol/L*	1.0 – 1.9 mg/dL *0.32 – 0.64 mmol/L*	< 1.00 mg/dL *< 0.32 mmol/L*
	Pediatric 1 year – 14 years	3.0 – 3.5 mg/dL *0.97 – 1.13 mmol/L*	2.5 – 2.9 mg/dL *0.81 – 0.96 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
	Pediatric < 1 year	3.5 – 4.5 mg/dL *1.13 – 1.45 mmol/L*	2.5 – 3.4 mg/dL *0.81 – 1.12 mmol/L*	1.5 – 2.4 mg/dL *0.48 – 0.80 mmol/L*	< 1.50 mg/dL *< 0.48 mmol/L*
Potassium, serum, high		5.6 – 6.0 mEq/L *5.6 – 6.0 mmol/L*	6.1 – 6.5 mEq/L *6.1 – 6.5 mmol/L*	6.6 – 7.0 mEq/L *6.6 – 7.0 mmol/L*	> 7.0 mEq/L *> 7.0 mmol/L*
Potassium, serum, low		3.0 – 3.4 mEq/L *3.0 – 3.4 mmol/L*	2.5 – 2.9 mEq/L *2.5 – 2.9 mmol/L*	2.0 – 2.4 mEq/L *2.0 – 2.4 mmol/L*	< 2.0 mEq/L *< 2.0 mmol/L*
Sodium, serum, high		146 – 150 mEq/L *146 – 150 mmol/L*	151 – 154 mEq/L *151 – 154 mmol/L*	155 – 159 mEq/L *155 – 159 mmol/L*	≥ 160 mEq/L *≥ 160 mmol/L*
Sodium, serum, low		130 – 135 mEq/L *130 – 135 mmol/L*	125 – 129 mEq/L *125 – 129 mmol/L*	121 – 124 mEq/L *121 – 124 mmol/L*	≤ 120 mEq/L *≤ 120 mmol/L*
Triglycerides (fasting)		NA	500 – 750 mg/dL *5.65 – 8.48 mmol/L*	751 – 1,200 mg/dL *8.49 – 13.56 mmol/L*	> 1,200 mg/dL *> 13.56 mmol/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Uric acid	7.5 – 10.0 mg/dL *0.45 – 0.59 mmol/L*	10.1 – 12.0 mg/dL *0.60 – 0.71 mmol/L*	12.1 – 15.0 mg/dL *0.72 – 0.89 mmol/L*	> 15.0 mg/dL *> 0.89 mmol/L*

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Basic Self-care Functions – Young Children: Activities that are age and culturally appropriate (e.g., feeding self with culturally appropriate eating implement).

Usual Social & Functional Activities – Adult: Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

Usual Social & Functional Activities – Young Children: Activities that are age and culturally appropriate (e.g., social interactions, play activities, learning tasks, etc.).

URINALYSIS	***Standard International Units are listed in italics***			
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Proteinuria, 24 hour collection				
Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h *1.000 – 1.999 g/d*	2,000 – 3,500 mg/24 h *2.000 – 3.500 g/d*	> 3,500 mg/24 h *> 3.500 g/d*
Pediatric > 3 mo - < 10 years	201 – 499 mg/m^2/24 h *0.201 – 0.499 g/d*	500 – 799 mg/m^2/24 h *0.500 – 0.799 g/d*	800 – 1,000 mg/m^2/24 h *0.800 – 1.000 g/d*	> 1,000 mg/ m^2/24 h *> 1.000 g/d*

Basic Self-care Functions – Adult: Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.

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