CLINICAL RESEARCH PROTOCOL

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

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TITLE: Mechanisms Associated with Favorable Response to Peginterferon-Alpha Add-on Therapy Following Long-term Nucleos(t)ide Analogue Treatment in Patients with Chronic Hepatitis B

SHORT TITLE: Add on PegIFN to NUC Therapy for CHB

IDENTIFYING WORDS: Peginterferon alfa-2a Nucleos(t)ide analogues, Chronic Hepatitis B, Liver Biopsy, HBeAg loss, HBsAg loss, Interferon stimulating gene.

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ESTIMATED DURATION OF STUDY: 3 Years
NUMBER AND TYPE OF PATIENTS: 60 patients with chronic hepatitis B, ages above 18 years, both male and female

SUBJECTS OF STUDY:
Number of patients: 60 Sex: Male & Female Age Range: Above 18 years
Volunteers: None

PROJECT USES IONIZING RADIATION: Yes, for medical indications only.

PROJECT USES "DURABLE POWER OF ATTORNEY": No

OFF-SITE PROJECT: No **MULTI-INSTITUTIONAL PROJECT**: No

Précis

Chronic hepatitis B virus (HBV) infection is a leading cause of liver associated morbidity and mortality. Currently available first-line therapies for treatment of chronic hepatitis B include pegylated interferon-alpha and the nucleos(t)ide analogues (NUCs) entecavir and tenofovir. These were shown to effectively suppress viral replication, but their ability to induce durable off-treatment response is limited to a small subset of patients. Combination treatment with peginterferon and NUCs has been attempted in several randomized controlled trials, with no apparent advantage over either agent given alone. In these studies however, treatment with peginterferon was initiated either simultaneously or shortly after NUCs administration. The efficacy of peginterferon following long-term viral suppression with NUCs was only tested in one small pilot study, nevertheless showing 60% HBsAg loss rate.

The underlying mechanisms responsible for improved efficacy of peginterferon in this setting are unknown and warrant further investigation. In this single arm study we propose to evaluate the efficacy and mechanisms associated with response to peginterferon add-on therapy following a minimum of 192 weeks of viral suppression induced by NUCs in a group of chronic HBV infected patients. Sixty patients with either HBeAg positive (n=30) or negative (n=30) chronic HBV infection will be enrolled to this study. After medical evaluation and pretreatment liver biopsy, treatment with subcutaneous injections of pegylated interferon alpha-2a 180 μg per week will be given for a total of 24 weeks, followed by an off-treatment evaluation period of 48 weeks. A second liver biopsy will be performed six hours following the first peginterferon injection. Primary end-point for this study will be the change in interferon-stimulated-genes response before and after first interferon injection in responders versus non-responders to treatment. The responsiveness to IFN-based therapy of treatment responders vs nonresponders will additionally be evaluated by studying intrahepatic and peripheral blood natural killer cells. The study will also assess HBeAg and HBsAg loss and seroconversion rates in comparison to historical controls treated with either peginterferon or NUCs monotherapy. Finally, we will assess whether treatment responders develop an HBV-specific T cell response similar in quantity and quality to that of patients who spontaneously resolve HBV infection.

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BACKGROUND

It is estimated that there are over 350 million persons with chronic hepatitis B virus (HBV) infection worldwide [1] Globally, chronic HBV infection is the leading cause of cirrhosis and hepatocellular carcinoma accounting for ~620,000 deaths annually [2] Epidemiological studies have demonstrated a strong association between high viral levels and worse outcome of chronic HBV infection [3, 4]. Thus, the goal of therapy is long-term, sustained suppression of HBV replication. Current first line recommendations for treatment of chronic HBV infection include entecavir, tenofovir (nucleos(t)ide analogues) or peginterferon-alpha [1, 5, 6].

NUCs with potent activity against the HBV reverse transcriptase and high barrier to resistance have been very effective at suppressing viral replication and were shown to be associated with improved clinical outcomes including reversal of cirrhosis [7, 8]. However, discontinuing therapy after one year is associated with high relapse rates and therefore most patients require long-term therapy to maintain suppression of viral replication [9-11]. Even with long-term use of NUCs, only about 50% of persons who were initially HBeAg positive undergo HBeAg seroconversion (the endpoint of therapy for HBeAg positive persons) [12, 13]and among those who were initially HBeAg negative, only about 1%-2% experience HBsAg seroconversion (the endpoint of therapy for HBeAg negative persons) [14, 15] Unfortunately, there are substantial costs associated with long-term therapy and there is the potential for serious side effects including renal toxicity and possibly loss of bone mass [16-20] Thus, new agents or approaches are needed to manage patients with chronic HBV infection. One approach that has been taken to improve HBsAg loss was to combine the two most potent NUCs. In one notable trial, the efficacy and safety of entecavir monotherapy was compared to the combination of entecavir and tenofovir in persons with HBeAg positive and negative chronic HBV infection. After 96 weeks of therapy, a comparable proportion of patients in each study arm achieved the primary end point of a level of HBV DNA<50 IU/mL (83.2% vs 76.4%, respectively), indicating no benefit of a combination NUC approach over potent monotherapy [21]. Another approach was to combine a nucleoside analogue with peginterferon. This strategy is theoretically appealing as the combined effect of peginterferon, an immunomodulator, and a nucleos(t)ide analogue, a potent inhibitor of viral replication, may result in a synergistic anti-viral effect. However, several trials comparing the efficacy of combination peginterferon-NUCs therapy to either agent alone failed to show a clear advantage of combination therapy over either agent alone. Four randomized controlled trials comparing peginterferon alfa and NUCs (either lamivudine or adefovir) to peginterferon alone or NUCs alone have been conducted in persons with HBeAg positive and negative chronic HBV infection. [22-25]. All

studies reported greater on-treatment viral suppression with the combination regimen compared to either agent alone, but higher rates of sustained off-treatment viral suppression with the combination regimen were only achieved in comparisoin to NUCs alone but not to peginterferon alone. Thus, there is really no advantage of combination peginterferon and lamivudine or adefovir to peginterferon alone, when treatment with the two agents is initiated simultaneously.

Studies assessing baseline predictors of response to peginterferon have shown that high viral levels are associated with a less favorable response in both HBeAg positive and negative HBV infected patients [26, 27]. In this context, it may be postulated that high viral titers may inhibit the action of peginterferon and that combination peginterferon plus a nucleos(t)ide analogue may be more effective if the peginterferon is preceded by a lead-in period of the nucleos(t)ide analogue. In one study testing this strategy, 185 persons with HBeAg positive chronic hepatitis B were randomized to receive either entecavir monotherapy for 48 weeks or a lead-in period of 24 weeks of entecavir followed by 24 weeks of combination entecavir plus peginterferon [28]. At end-of-treatment more patients in the combination arm, 19%, achieved the primary endpoint of HBeAg seroconversion compared to the entecavir monotherapy arm, 10%. This difference however was not statistically significant (p=0.095), and there was no peginterferon monotherapy arm. However, in a prespecified multivariate analysis adjusted for differences in HBV DNA at the start of the randomized therapy phase, add-on peginterferon was significantly associated with a 4.78-fold increase in response rate (P = .004). It is possible though that a longer period of viral suppression is required to improve the efficacy of peginterferon therapy in combination with a NUCs. Indeed in a small pilot study in which peginterferon was added after a minimum period of 3 years of entecavir therapy with complete viral suppression, showed that 60% of persons developed HBsAg loss [29]. These provocative results suggest that a peginterferon add-on strategy following a substantial period of nucleos(t)ide induced viral suppression, may result in high rates of HBsAg seroconversion. The underlying mechanisms responsible for improved efficacy of peginterferon in this setting, are unknown, or for that matter the mechanism of action of peginterferon in chronic HBV infection and warrant further investigation.

IFN-α is a naturally occurring cytokine with broad anti-viral activity. Its anti-viral effect is mediated through induction of a diverse set of target genes, referred to as IFN-stimulated genes (ISGs)[30]. This results in inhibition of HBV replication through a variety of mechanisms including a block of RNA-containing core particle formation, an accelerated decay of replication-competent core particles, and degradation of the pre-genomic RNA [31-33]. Recently, IFN-α was also shown to inhibit HBV replication by decreasing RNA transcription from cccDNA. This effect was mediated through epigenetic repression of cccDNA (via histone hypoacetylation), as well as by active recruitment to the cccDNA of transcriptional co-repressors [34].

In addition to its direct anti-viral activity, IFN-α is also a potent immunomodulator with robust effects on cells of the innate immune system, such as natural killer cells (NK). NK cells consistitute the largest lymphocyte population in the liver and can exert antiviral function via direct cytotoxic mechanisms and via indirect cytokine (e.g. IFN-gamma mediated) mechanisms. NK cells respond to IFN-a and we have previously shown that the response of peripheral blood NK cells to Peg-interferon correlates well with the overal response and clinical outcome of PegIFN-based regimens in chronic HCV infection [35, 36]. Interferon also modulates the adaptive, T cell-mediated response. T cells are typically impaired in their antiviral function in chronic HBV infection This impairment has been attributed to upregulation of inhibitory molecules such as PD-1, Tim-3, CTLA-4 and others due to chronic T cell receptor stimulation by HBV antigens resulting in downregulation of virus-specific T cell response of infected patients [37]. In this protocol we will determine whether a decrease in HBV titer by NUCs improves the innate and adaptive immune response to PegIFN.

We postulate that in patients with chronic HBV infection, who have long-term viral suppression due to NUCs therapy, the addition of peginterferon will lead to HBeAg or HBsAg seroconversion. We plan to explore the clinical response and possible mechanisms involved during therapy with peginterferon alfa when added to long-term nucleoside therapy in patients with chronic HBV infection (both HBeAg-positive and -negative). We hypothesize that the extended pretreatment with NUCs will modify the responsiveness to peginterferon. These effects will be studied by analyzing the expression pattern of ISGs, the response of NK cells prior to and after peginterferon injection resulting in improved transcriptional control of cccDNA , the induction of antibodies against HBe and HBsAg and T cell responses.

Primary endpoints of this trial will be the changes in expression levels of ISG's, intrahepatic total HBV DNA and cccDNA content , changes in NK cell effector cell function measured before and following a single dose of peginterferon stratified by treatment response and the rates of HBeAg and HBsAg loss and seroconversion in HBeAg positive patients, HBsAg loss and seroconversion rate in HBeAg negative patients. Secondary endpoints will be the induction of HBV-specific memory T cell responses. A post-hoc analysis will stratify reponse rates upon available viral genotype data and duration of NUC therapy prior to study enrollment. Other secondary end-points will include changes in quantitative HBeAg and HBsAg levels during therapy and safety of the combination regimen. Serological response rates, qHBsAg kinetics and safety end-points will be compared to historical controls of published multicenter randomized trials comparing the efficacy of peginterferon and NUCs in eAg positive and negative HBV carriers

HYPOTHESIS

In patients with chronic hepatitis B infection, under long-term viral suppression maintained by NUCs treatment, peginterferon add-on therapy will result in a greater proportion of subjects achieving serological response compared to before peginterferon was added. We hypothesize that response to treatment will be accompanied by measurable changes in host-response gene transcription profile, immune cell activation and function, and quantitative viral biomarker levels.

SPECIFIC AIMS

1. To assess the changes in ISG profile and NK cell function after a single dose of peginterferon in patients receiving long-term NUCs with maintained viral suppression
2. To determine rates of HBeAg and HBsAg loss with or without seroconversion and induction of HBV-specific memory T cells to peginterferon add-on treatment in eAg positive and negative HBV carriers on long-term NUC treatment.
3. To identify pre- and on-treatment predictors of serological response to peginterferon add-on/nucleos(t)ide combination therapy.
4. To determine if there are differences in epigenetic regulation of cccDNA between HBeAg positive and negative CHB at baseline and following a single dose of peginterferon treatment

PROTOCOL OVERVIEW

We intend to enroll a total of 60 patients with HBeAg positive (n=30) and HBeAg negative (n=30) chronic HBV infection, who have been on-treatment with one or more nucleos(t)ide analogues for at least 192 weeks prior to study enrollment with maintained viral suppression (HBV DNA level <100 IU/ml). After a thorough medical evaluation and liver biopsy, participants will receive therapy with subcutaneous injections of peginterferon-alpha 2a at a dose of 180mcg per week for a total of 24 weeks. A second liver biopsy will be performed 6 hours following the first peginterferon injection for the purpose of assessing ISGs, intrahepatic NK cell quantity and function and intrahepatic viral biomarker response patterns. Most interferon induced genes achieve maximal response in the liver and periphery within 4 hours, begin to decline by 8 hours, and return to baseline levels by 24 hours post-administration of interferon. Because peginterferon is slower to be absorbed we have selected the six hour rather that a four hour time point.[38]

Patients will be followed carefully on weeks 0, 2, 4, 8, 12 and 24 during treatment and at 12 week intervals thereafter until 48 weeks post-treatment.

During study visits patients will be assessed for compliance with study medications, as well as the development of any treatment related side effects and/or adverse reactions. In addition, blood will be drawn periodically for the assessment of routine blood tests, quantitative viral biomarker levels, serological response markers and immune cell functional status.

INCLUSION/EXCLUSION CRITERIA

Inclusion criteria: HBeAg positive group

1) Age >18 years and older, male or female.
2) Known serum HBsAg and HBeAg positivity at the time of screening.
3) Ongoing treatment with one or more NUCs for at least 192 weeks before study entry. Subjects may have a brief interruption of treatment for medical reasons (e.g. breast feeding) not to exceed 8 weeks and none within the 48 weeks before study entry.
4) HBV DNA levels <100 IU/mL, measured at least 12 months prior to, and upon enrollment to the study.
5) ALT level ≤2 ULN based on at least two determinations taken at least one month apart during the 24 weeks before study entry with the second being at time of screening
6) Written informed consent

Inclusion criteria: HBeAg negative group

1) Age >18 years and older, male or female.
2) Known serum HBsAg positivity and HBeAg negativity at the time of screening.
3) Ongoing treatment with one or more NUCs for at least 192 weeks before study entry. Subjects may have a brief interruption of treatment for medical reasons (e.g. breast feeding) not to exceed 8 weeks and none within the 48 weeks before study entry.
4) HBV DNA levels <100 IU/mL, measured at least 12 months prior to, and upon enrollment to the study
5) ALT level ≤2 ULN based on at least two determinations taken at least one month apart during the 24 weeks before study entry with the second being at time of screening
6) Written informed consent

Exclusion criteria (for both eAg positive and negative patients)

1) Co-infection with HDV as defined by the presence of anti-HDV in serum and/or HDV antigen in the liver.
2) Co-infection with HCV as defined by the presence of HCV RNA in serum.
3) Co-infection with HIV as defined by the presence of anti-HIV in serum.
4) Decompensated liver disease as defined by serum bilirubin >2.5 mg/dL (with direct bilirubin > 0.5 mg/dL), prothrombin time of greater than 2 seconds prolonged, a serum albumin of less than 3 g/dL, or a history of ascites, variceal bleeding or hepatic encephalopathy.
5) Presence of other causes of liver disease, (i.e. hemochromatosis, Wilson disease, alcoholic liver disease, severe nonalcoholic steatohepatitis defined as the presence of marked ballooning injury on liver biopsy, alpha-1-anti-trypsin deficiency).
6) A history of organ transplantation or in the absence of organ transplantation, any immunosuppressive therapy requiring the use of more than 5 mg of prednisone (or its equivalent) daily.
7) Significant systemic illness other than liver diseases including congestive heart failure, renal failure, chronic pancreatitis and diabetes mellitus with poor control, that in the opinion of the investigator may interfere with therapy.
8) Pregnancy or inability to practice contraception in patients capable of bearing or fathering children
9) Lactating women.
10) Hepatocellular carcinoma (HCC), or the presence of a mass on imaging studies of the liver that is suggestive of HCC, or an alpha-fetoprotein level of greater than 500 ng/mL.
11) eGFR < 50 ml/min, serum creatinine > 1.3 mg/dl or urine protein >1 gram/24-hours
12) History of hypersensitivity to pegylated interferon-alpha
13) Platelet count <70 mm^3/dL
14) Hgb <12 g/dL for males and <11 g/dL for females
15) Active ethanol/drug abuse/psychiatric problems such as major depression, schizophrenia, bipolar illness, obsessive-compulsive disorder, severe anxiety, or personality disorder that, in the investigator's opinion, might interfere with participation in the study.
16) History of malignancy or treatment for a malignancy within the past 3 years (except adequately treated carcinoma in situ or basal cell carcinoma of the skin).
17) Any medical condition requiring, or likely to require, chronic systemic administration of corticosteroids or other immunosuppressive medications during the course of this study.
18) History of immune-mediated disease, or cerebrovascular, chronic pulmonary or cardiac disease associated with functional limitation, retinopathy, uncontrolled thyroid disease,

poorly controlled diabetes or uncontrolled seizure disorder, as determined by a study physician.

19) Presence of conditions that, in the opinion of the investigators, would not allow the patient to be followed in the current study for at 1 year.
20) For subjects who interrupt therapy, documentation of a viral load >1,000 IU/ml while off therapy.

STUDY DESIGN AND PROCEDURES

Screening

Eligible patients will be seen in the outpatient liver clinic of the NIH Clinical Center. At the screening visit, potential benefits and risks associated with the study will be explained to the study participants by an investigator in laymen's language. Written informed consent will be obtained and documented in the participant's chart. Whenever possible, the informed consent form will be provided to the potential participants in advance. Only the principal and associate investigators from the Liver Disease Branch, NIDDK, will be eligible to obtain consent for this study.

The following screening procedures will be performed:

(1) Obtain signed informed consent
(2) History and physical examination
(3) Symptom questionnaire
(4) Fatigue questionnaire (Promise 7)
(5) Visual analogue scale
(6) Routine blood panel: Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count). Testing will also be done for anti-HAV, HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, anti-HCV, and stored sample for IL-28b testing. Baseline HBV DNA level by quantitative PCR (COBAS Ampliprep/COBAS Taqman HBV v2.0 Test: Roche Diagnostics, lower limit of quantification 116 copies /ml or 20 IU/ml, lower limit of detection 6 IU/ml)
(7) Extended blood panel: alpha fetoprotein, gGT, immunoglobulins, rheumatoid factor, ANUCs, SMA, lipid panel, thyroid panel, INR, reticulocyte count, ESR.

(8) Research blood- one 10 ml serum and one 10 ml plasma tube stored frozen in 0.5-1.0 mL aliquots). The stored serum and plasma will be used to measure selected cytokines, interferon-stimulated gene products [including IP10], quantitative HBsAg level (by using the Roche Elecsys HBsAg automated quant assay) and for future research.

(9) Fifty milliliters of whole blood (green top tubes) for baseline immunological studies: NK cell responses, CD4+ and CD8+ T cell HBV specific responses (peripheral blood mononuclear cell [PBMC] studies)

(10) Urine tests: routine urinalysis and a pregnancy test for females of childbearing potential

(11) Transient elastography

Admission for pretreatment liver biopsy

Once patients are deemed eligible to participate in the study based on results of the screeing labs, they will have 12 weeks to complete the liver biopsy and to begin add-on therapy with peginterferon. Eligible patients will be admitted to 5NW to undergo a percutaneous liver biopsy. The initial liver biopsy will be mostly for research purposes to measuring baseline levels of ISG expression, pregenomic and cccDNA concentration and frequency and activity of liver infiltrating HBV-specific NK cells but will also be used for the purpose of grading and staging of liver disease before treatment and will provide useful information to the clinician and patient on whether the liver disease is improving on current therapy. Grading and staging of the liver biopsy will performed by an expert hepatopathologist blinded to the clinical information using the modified Ishak scoring system. This includes the histological activity index (HAI) for inflammation and necrosis (scores ranging from 0 to 18). Fibrosis will be staged using the Ishak scoring system (0-6, 0=no fibrosis, 6=cirrhosis). Slides will also be immunostained for HBsAg, HBcAg, and HDV antigen by peroxidase-antiperoxidase techniques and scored as 0, 1, 2 or 3+ based upon the percent of cells reactive for each viral antigen (0=none, 1=10%, 2=11% to 50% and 3=≥51% of hepatocytes).

Liver-infiltrating lymphocytes will be assessed for frequency and function of NK cells. m-RNA levels of ISG's, immune cell markers and chemokines/cytokines will be quantitated by real-time PCR or nanostring. Intrahepatic pregenomic and cccDNA will also be quantitated by Southern blotting or real-time PCR.

The following will be obtained on admission:

1) Brief clinical evaluation
2) Review patient's eligibility for study

3) Symptom questionnaire
4) Visual analogue scale
5) Fatigue questionnaire (Promise 7)
6) Abdominal ultrasound, CXR and 12 lead ECG (if not done within previous 6 months)
7) Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
8) HBV DNA viral load, qualitative and quantitative HBeAg and HBsAg measurement
9) 50 mL of blood in heparinized green top tubes for NK and T cell analysis.
10) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
11) Liver biopsy cut into four sections, one (at least 1.5 cm) placed in formalin for routine light microscopy and the other 3 (.3cm, .5cm and 1 cm) placed in RNA later and flash frozen in liquid nitrogen 24 hours later for RNA and protein analyses and flow cytometry. A liver biopsy fragment of > 5 mm will be used to assess the frequency and function of NK cells.
12) Discharge from hospital

Initiation of Treatment

Patients will be stratified on the basis of HBeAg status into 2 groups of 30 patients each, to receive peginterferon-alpha 2a (PEGASYS®, Roche), 180 mcg per week administered by subcutaneous injection for a total of 24 weeks. Patients will continue treatment with their previous nucleosi(t)ide analogue regimen during the treatment and follow-up phases of this study.
Patients will be seen in the outpatient clinic at weeks 2, 4, 8, 12, and 24, during treatment phase, at 12 week intervals thereafter up to 48 weeks post-therapy
Compliance will be monitored by medication diaries and counting of the residual vials returned at outpatient visits. The side effects will be monitored regularly at each visit during the treatment phase of the study by symptom questionnaires. Peginterferon alfa-2a will be dose reduced or stopped per the dose reduction and stopping rules for peginterferon alfa-2a. Peginterferon alfa-2a treatment can be restarted if the adverse event resolves and the patient is willing to re-start the medication.

On-treatment liver biopsy

At day 0, all patients will be readmitted to the NIH Clinical Center to start therapy with peginterferon alfa-2a and to undergo a repeat liver biopsy. Biopsy will be scheduled at 6 hours following the first injection of peginterferon, for the purpose of assessing ISG response pattern following the initial exposure to peginterferon. Liver tissue will also be subjected to routine histopathological grading and staging as well as for immune cell, quantitative viral kinetic and cccDNA epigenetic studies as previously described.

Since the hypothesis to be tested in this study is that response to treatment peginterferon (i.e. loss of HBeAg and HBsAg or both) is mediated by changes in hepatocyte gene transcription profile, it is necessary to obtain liver tissue at two time points-before and 6 hours after the first dose of interferon. Performing two liver biopsies within 12 weeks or even as short as 24 hours has been performed in other Liver Disease Branch studies and has not been associated with an increase in the complication rate or lower patient acceptance.

Monitoring

<u>Day 0</u>

1) Brief clinical evaluation
2) Review patient's eligibility for study
3) Symptom questionnaire
4) Visual analogue scale
5) Fatigue questionnaire (Promise 7)
6) Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
7) Females of child bearing potential: Pregnancy test
8) HBV DNA , qualitative and quantitative HBeAg, HBsAg, measurements
9) 30 mL of blood in heparinized green top tubes for NK and T cell analysis prior to PegIFN injection and 30 mL of blood in heparinized green top tubes for NK and T cell analysis 6h after PegIFN injection.
10) Blood sample for storage (10 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
11) Start peginterferon 180 ug SQ

12) Repeat liver biopsy 6 hours after first dose of peginterferon. Liver biopsy cut into four sections, one (at least 1.5 cm) placed in formalin for routine light microscopy and the other 3 (.3cm, .5cm and 1 cm) placed in RNAlater and flash frozen in liquid nitrogen 24 hours later for RNA and protein analyses and flow cytometry.

Day1

1) 24h after the first PegIFN injection: 50 mL of blood in heparinized green top tubes for NK. This is the time point where we expect the maximal response of NK cells to PegIFN based on our previous studies [35, 36]
2) Discharge from hospital

Week 2,4, 8 and 12 (± 3 days): Outpatient visit

Each outpatient visit will include assessment of compliance, adverse events reporting, vital signs, weight, symptoms evaluation, record of concomitant medications, drug dispensing

1) Symptom questionnaire
2) Visual analogue scale
3) Fatigue questionnaire (Promise 7) (week 2 only)
4) Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
5) HBV DNA viral load, HBeAg, anti HBe, HBsAg and anti HBs measurment
6) Blood sample for storage (10 mL of blood separated into serum and plasma stored frozen in 0.5-1.0 mL aliquots)
7) 50 mL of blood in heparinized green top tubes for NK and T cell analysis.
8) Females of child bearing potential: Pregnancy test
9) TSH and quantitative HBe and HBs antigen measurement at week 12.

End of Add-on Peginterferon Treatment (Week 24 ± 3 days)

At week 24, all subjects will discontinue peginterferon alfa-2a add-on therapy (but continue the NUCs and undergo the following evaluation:

1) Physical examination
2) Symptom questionnaire
3) Fatigue questionnaire (Promise 7)
4) Visual analogue scale
5) Assessment of adverse events, concomitant medications and adherence
6) Blood will be drawn for Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
7) 50 mL of blood in heparinized green top tubes for NK and T cell analysis.
8) Urine tests: routine urinalysis and creatinine, phosphate and beta-2-microglobulin
9) HBV DNA viral load, HBeAg, anti-HBe, HBsAg, anti-HBs and quantitative HBe and HBs measurement
10) Blood sample for storage (10 mL of blood separated into serum and plasma stored frozen in 0.5-1.0 mL aliquots)
11) Females of child bearing potential: Pregnancy test
12) TSH

Post-treatment weeks 12, 24, 36 (± 5 days)

1) Limited clinical evaluation
2) Symptom questionnaire
3) Visual analogue scale
4) Routine blood panel: Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
5) HBV DNA viral load, HBeAg, anti HBe, HBsAg and anti HBs levels
6) Blood sample for storage (10 mL of serum and 10 ml of plasma stored frozen in 0.5-1.0 mL aliquots)
7) 50 mL of blood in heparinized green top tubes for NK and T cell analysis.

Final study visit week 48 post-treatment (± 5 days)

1) Limited clinical evaluation
2) Symptom questionnaire

3) Fatigue questionnaire (Promise 7)
4) Visual analogue scale
5) Routine blood panel: Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
6) Extended blood panel: alpha fetoprotein, gGT, immunoglobulins, rheumatoid factor, ANA, SMA, lipid panel, INR, reticulocyte count, ESR.
7) 50 mL of blood in heparinized green top tubes for NK and T cell analysis.
8) HBV DNA viral load, HBeAg, anti HBe, HBsAg, and anti HBs levels and quantitative HBe and HBs measurement
9) Blood sample for storage (10 mL of serum and 10 ml of plasma stored frozen in 0.5-1.0 mL aliquots)
10) Fibroscan

Table 1: List of study related procedures

Procedure	Screen	Pre-Rx biopsy	Day 0	Wk 2, 4, 8	Wk 12	Week 24 End of Rx	Post-Rx wk 12, 24, 36	Post-Rx wk 48, End of Study
H&P/breif clinical evaluation, compliance, AE, concommit. drugs, etc	X	X	X	X	X	X	X	X
Symptom questionnaire	X	X	X	X	X	X	X	X
Visual Analog Scale	X	X	X	X	X	X	X	X
Promis 7	X	X	X	wk 2		X		X
Chem 20	X	X	X	X	X	X	X	X
CBC	X	X	X	X	X	X	X	X
Anti-HAV	X							
HBsAg	X	X	X	X	X	X	X	X
HBsAg quantitative			X		X	X		X
anti-HBs	X							
HBeAg	X	X	X	X	X	X	X	X
HBeAg quantitative			X		X	X		X
anti-HBe	X							
anti-HBc	X							

anti-HCV	X							
IL28B	X							
HBV DNA	X	X	X	X	X	X	X	X
AFP	X							X
gGT	X							X
Immunoglobulins	X							X
rheumatoid factor	X							X
ANA	X							X
SMA	X							X
Lipid panel	X							X
thyroid panel	X				TSH	TSH		
INR	X							X
reticulocyte	X							X
ESR	X							X
storage - 5mL serum and 5mL plasma	S&P	Serum	Serum	S&P	S&P	S&P	S&P	S&P
Immunology - 50mL	X	X	X	X	X	X		X
Urine pregnancy	X		X	X	X	X		
UA	X					X		
Urine creatinine, phosphate, beta-2-microglubulin						X		
Liver Biopsy		X	Timed					
Abdominal u/s		X						
CXR		X						
EKG		X						
Fibroscan	X							X

ASSESSMENT OF RESPONSE

Clinical Definitions

a) ***Loss of HBeAg (HBeAg-positive patients only)***: loss of detectable HBeAg with or without anti-HBe seroconversion confirmed on 2 consecutive visits at least 12 weeks apart.

b) ***Loss of HBsAg***: Loss of HBsAg, with or without anti HBs seroconversion confirmed on 2 consecutive visits at least 12 weeks apart.

c) ***Sustained response:*** virological, serological, biochemical, or combined response present at the time of last observation and at least six months after stopping therapy.

The primary goals of this study are centered on understanding the mechanisms, and identification of predictive factors associated with a favorable response to peginterferon therapy when added on to long-term NUCs treatment, in patients with HBeAg positive and negative chronic HBV infection.

Primary end-points

1) The primary end-point for this study will be the change in level of ISG expression and NK cell responses before and after 1st peginterferon injection in responders versus non-responders to peginterferon add-on therapy.
2) Proportion of eAg positive patients showing eAg loss with or without seroconversion at end of treatment and 24 and 48 weeks off therapy.
3) Proportion of eAg positive patients showing sAg loss with or without seroconversion at end of treatment and 24 and 48 weeks off therapy
4) Proportion of eAg negative patients showing sAg loss with or without seroconversion at at end of treatment and 24 and 48 weeks off therapy

Secondary endpoints

1. Level of qHBsAg decline at treatment week 12, at end of treatment and 24 and 48 weeks off therapy, compared to pretreatment levels in eAg positive and negative patients
2. Level of qHBeAg decline at treatment week 12, at end of treatment and 24 and 48 weeks off therapy, compared to pretreatment levels in eAg positive and negative patients
3. Correlation of qHBsAg levels at initial evaluation, week 12,24,36 and 48 with intrahepatic pregenomic and cccDNA concentration before and after initiation of peginterferon in responders versus non-responders to treatment.
4. Intrahepatic pregenomic and cccDNA concentration before and after starting treatment with peginterferon.
5. Epigenetic alterations in HBeAg positive and negative patients following first 1st peginterferon injection compared to pretreatment levels as measured by chromatin immunoprecipitation (ChIp) assays

6. Frequency and functional activity of circulating and liver infiltrating, HBV-specific T-cells, measured before and during treatment in responders versus non-responders to peginterferon add-on therapy.

DOSE CHANGES

Stopping peginterferon therapy:

Add-on peginterferon will be discontinued early for any of the following reasons:

a) ***Toxicity***- the dose of peginterferon will be modified or discontinued according to Table 1: Peg-Interferon Alfa-2a Dose Modification Guidelines (see below).

b) ***Withdrawal of informed consent*** (subject's decision to withdraw for any reason.

c) ***Any clinical adverse event, 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. If any of the following laboratory or clinical criteria is obtained for any subject, the result must be repeated/confirmed within 72 hours. If the results are confirmed, the subject must discontinue treatment. Clinical criteria must have Principal Investigator or Sub-Investigator assessment prior to permanent discontinuation.

– Evidence of confirmed hepatic decompensation (Child-Pugh Class B or C, Score > 6) (discontinue all treatment);

- Platelets < 25 x 10^9 cells/L;
- Hemoglobin <8.5 g/dL
- Absolute neutrophil count < 250 mm^3/ml

– Severe neuropsychiatric signs and symptoms (including depression) for both new and worsening events that are considered clinically significant by the investigator.

d) ***Pregnancy*** women of childbearing potential must contact the investigator or study staff immediately if they suspect they might be pregnant (eg, missed or late menstrual period) at any time during study participation. If subjects become pregnant the research staff will stop peginterferon, but subjects may continue taking their antiviral oral agent at the discretion of the study doctor. The investigator will discuss possible management options with the subject. Subjects who do become pregnant will continue to be followed for the outcome of the pregnancy. The NIH will not cover any aspects of obstetrical, child or related care.

e) ***Loss of ability to freely provide consent*** through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (eg, infectious disease) illness.

Stopping NUCs therapy

Treatment with NUCs will be stopped under the following conditions

a) HBeAg loss, with or without anti HBe seroconversion, in eAg positive patients, that is confirmed on 2 separate tests, at least 6 months apart.
b) HBsAg loss, with or without anti HBs seroconversion, that is confirmed in 2 separate tests, at least 6 months apart.

Peginterferon Dose Modifications

Dose modification of peginterferon **α**-2a should be managed according to Table 2. This table is based on recommendations from the peginterferon **α**-2a package insert, and have been modified as necessary for this study. As Tables 2 is a guideline, investigators may modify the dose based on clinical judgment.

Table 2: Peg-Interferon Alfa-2a Dose Modification Guidelines

Laboratory Value/Clinical Criteria:	Dose Modification:	Additional Instructions:
ANC:		
≥ 0.75 × 109 cells/L	Maintain at 180 **µ** g	
≥ 0.25 × 109 to < 0.75 × 109 cells/L	Reduce to 135 **µ** g	
< 0.25 × 109 cells/L	All study drugs should be interrupted until ANC values return to more than 1000/mm3	Reinstitute at 90 **µ** g and monitor ANC
Platelets:		

≥ 50 × 10^9 cells/L	Maintain at 180 µg	
≥ 25 × 10^9 to < 50 × 10^9 cells/L	Reduce to 90 µg	
< 25 × 10^9 cells/L	Discontinue all study drugs if value is confirmed	Treatment should not be reinstituted
ALT:		
ALT > 10 × ULN	Reduce to 135 µg or interrupt all study drugs if repeated ALT results are > 10 × ULN	If ALT increases are progressive or there is evidence of hepatic decompensation therapy should be interrupted
New Ocular symptom(s):		
New decrease or loss of vision or other clinical significant ocular sign or symptom	Interrupt all study treatment	Complete eye examination must be performed.
New or Worsening Neuropsychiatric Signs or Symptoms (Including Depression):	Mild No change Evaluate weekly by visit or phone until symptoms improve.	Evaluate weekly (with visit at least every other week). Consider psychiatric consultation if no mprovement. If improved and stable for 4 weeks, may resume normal dosing.
Moderate	Reduce dose to 135 µg	
Severe	Discontinue all study drug treatment	Psychiatric therapy necessary. Treatment should not be reinstituted.
Creatinine Clearance (CrCl):		
< 30 mL/min	Reduce to 135 µg	
Hemodialysis	Reduce to 135 µg	

Source: adapted from Pegasys® package insert.

STATISTICAL ANALYSIS

Power calculation

It is not possible to calculate power for the primary endpoints. We have calculated power for the secondary endpoints of the rates of HBeAg and HBsAg loss. Response rates in patients in both groups will be compared to historical controls of HBeAg positive and negative. It is assumed that if these patients were followed for an additional year without peginterferon, only 10% of the HBeAg positive patients would become HBeAg negative and 5% of the HBeAg negative patients would become HBsAg negative. A sample size of 29 HBeAg positive and 27 HBeAg negative patients will provide the study with a statistical power of 80% to detect a difference of 25% (35% versus 10% for HBeAg loss and 30% versus 5% for HBsAg loss). The sample size was increased to 30 patients in each arm to allow for withdrawals.

Variables to be analyzed in association with a serological response will include: baseline ALT and HBV DNA level, liver histology, HBV genotype, IL28B genotype, duration of NUC therapy, and ISG levels. Patients with missing data or who drop out of the study will be considered as treatment failures on an intention to treat basis. For comparison of means, Student's t-test will be used. All reported p-values will be 2-sided.

Demographics and Baseline Characteristics

The following will be summarized for treated subjects:

- Demographics: age, race, gender, geographic region, ethnicity;
- Disease characteristics at baseline: HBV DNA level, HBV genotype, IL28B SNP genotype and cirrhosis status, duration of NUC therapy;
- Physical measurements at baseline; height, weight, body mass index, hip and waist circumference;
- Laboratory tests at baseline;

Analysis plan

To address the primary hypothesis that ISG levels are related to virological response to peginterferon, the ISG analysis of the on treatment liver biopsy at 6 hours after the first dose of interferon will be correlated with off-peginterferon serological outcome (HBeAg loss/seroconversion and HBsAg loss). To address the hypothesis that ISG levels are related to HBsAg levels, the ISG analysis of the on treatment liver biopsy will be correlated with HBsAg levels at weeks 4, 12, 24.. The

analyses will be stratified by HBV genotype and duration of NUC therapy.

Analysis methodology

The analysis will be based on standard approaches to analysis of microarray data. Since the software and approaches change frequently in this field, we will provide a broad outline of the approach rather than detailed, specific steps.

a) We will perform quality control and background normalization of the gene array data.
b) Expression value estimates will be obtained and quantile normalization and median scaling of the genes will be performed.
c) A list of genes up- or down-regulated >2-fold with p>0.05 will be prepared using the following procedure:
    1. For each patient, genes up- or down-regulated >2-fold in the second biopsy compared to the first will be identified.
    2. Lists will be prepared of genes that are up- or down-regulated >2-fold for any of the subjects.
    3. A paired t-test will be used to identify genes from this list that have a p value <0.05 (Difference is not equal to zero).
    4. The subset of the genes in (3) that are up- or down-regulated in >50% of patients in groups defined relapse and/or presence of cirrhosis will be identified.
    5. This procedure will be repeated with $p < 0.01$, but the primary analysis will be based on the genes selected using $p < 0.05$.
d) The false discovery rate (FDR) of the t test will be estimated.

A more detailed statistical analysis plan will be developed prior to the start of data analysis.

HAZARDS AND DISCOMFORTS

The risks and discomforts of frequent phlebotomy.

To document stable levels of biochemical and serologic markers of chronic hepatitis and to monitor the effects and toxicities of the combination of peginterferon alfa-2a and the nucleos(t)ide analogue frequent blood sampling will be required. Patients will have between 11 and 12 venipunctures during the study. Each venipuncture will be for approximately 20 to 75 cc of blood. The total amount of blood drawn during an 8 week period will not exceed 10.5 ml/Kg or 550 mls, whichever is smaller.

The risks and discomforts of HIV testing.

Patients will have blood tested for anti-HIV at entry. Mention of anti-HIV is made in the consent form which includes the exact language used in the standard consent form used for anti-HIV testing at the Clinical Center. It is important to test for HIV infection as many of the drugs used to treat hepatitis B (tenofovir, tenofovir and emtricitabine, lamivudine and entecavir) have activity against HIV and a treatment regimen using only two drugs against HIV would not be considered standard of care.

The risks and discomfort of percutaneous liver biopsy.

Patients will undergo up to two liver biopsies in this protocol: one before therapy and a second six hours after the initial injection of peginterferon alfa-2a. Patients with inadequate platelet counts (<70,000/mm^3) or coagulation parameters (prothrombin time > 15 seconds, INR>1.7) will undergo transjugular liver biopsy after correction of the laboratory abnormalities as a part of this protocol. Patients requiring liver biopsy will be admitted to the Clinical Center for two days for this procedure and other blood testing. The major side effects of liver biopsy are pain, bacteremia, puncture of another organ and bleeding. Local pain and discomfort at the liver biopsy site occurs in about 20% of persons undergoing percutaneous liver biopsy. This is transient (lasting one to twelve hours) and is usually mild, rarely requiring analgesics. Bacteremia occurs in 1-2% of persons undergoing liver biopsy. In the absence of bile duct obstruction, this is almost always self-limited and is rarely symptomatic. Significant bleeding after liver biopsy is the most serious side effect of this procedure. In the absence of a blood coagulation defect or hepatic malignancy, significant bleeding is rare, occurring in less than one in a thousand cases of liver biopsy. Death due to bleeding after liver biopsy has been reported in less than 1/10,000 cases. At the NIH Clinical Center, the Liver Diseases Branch has performed approximately 150 liver biopsies each year for the last 20 years. During this time, only two patients died as a complication of biopsy. One of these patients had cirrhosis and advanced hepatocellular carcinoma and the second had severe coagulation disorders. Both bled from the liver biopsy site and died after surgical attempts to stop the bleeding were unsuccessful.

The risks and hazards of Peginterferon therapy

Local pain and erythema at the site of subcutaneous injection. This is usually mild and is no greater than the pain and irritation associated with other subcutaneous injections. Severe local reactions have been described but are rare

A syndrome of fever, malaise, headaches and muscle aches. These influenza- like symptoms occur typically after the first injection and decrease in severity with subsequent treatments. Fever can be as high as 40^{o}C. The fatigue often persists or reappears with prolonged therapy with interferon and is often the dose-limiting factor in therapy. These symptoms abate with discontinuation of treatment, but may last for up to 2 weeks after the final injection of interferon. There is considerable variation in the occurrence and severity of these symptoms.

Gastrointestinal symptoms of nausea, vomiting, diarrhea and anorexia are common with high doses

of interferon. These are rarely severe enough to warrant discontinuation or alteration in interferon therapy.

Hair loss is noted by 20-25% of patients treated with interferon for more than two months. This is always temporary.

Bone marrow suppression occurs with the dose of interferon used to treat chronic hepatitis. Leukopenia and granulocytopenia are the most frequent findings and a 20-50% decrease in white blood cell counts is typical during therapy. This effect is always temporary and is dose related. Folliculitis and sinusitis, as well as more serious infections such as dissemination of cellulitis and bacterial peritonitis have occurred in patients receiving interferon. Indeed, among the more than 400 patients with chronic viral hepatitis treated with alpha interferon at the NIH, two died of infections (spontaneous bacterial peritonitis) during therapy. Both patients had hepatitis B and end-stage liver disease. Spontaneous bacterial peritonitis is a common complication of end-stage liver disease. Other instances of severe and rare forms of infection have been reported in patients receiving interferon. It appears that most of these severe infections are more related to the underlying condition than the leukopenia induced by interferon, but interferon may increase susceptibility to infections and severe outcomes. For these reasons, all patients will be cautioned, about infectious complications and advised to contact their physician or the investigators if they develop fever. Thrombocytopenia also occurs with interferon treatment, the platelet count falling by 13-52% of the pretreatment values. Serious bleeding episodes have not been reported with interferon therapy. Finally, mild anemia with a decrease in hematocrit and hemoglobin of 3-5% is also seen commonly with interferon therapy. The effects of interferon on the bone marrow have been temporary and rapidly reversible with discontinuation of therapy.

Significant cardiac side effects have included idiopathic and reversible heart failure. This is reversible with conventional management. This occurs with a frequency of <1%.

Pulmonary disorders: Dyspnea, pulmonary infiltrates, pneumonia, bronchiolitis obliterans, interstitial pneumonitis and sarcoidosis, some resulting in respiratory failure and/or patient deaths, may be induced or aggravated by interferon therapy. Pulmonary complications are rare and occur with a frequency of <1%.

Altered liver enzymes: Interferon can cause mild elevations in serum aminotransferases in patients who do not have liver disease. In patients with chronic viral hepatitis, these possible elevations in aminotransferases may be obscured by the pre-existing liver disease. Transient ALT elevations are common during hepatitis B therapy with interferon. Twenty-five percent and 27% of subjects

experienced elevations of 5 to 10 x ULN and 12% and 18% had elevations of greater than 10 x ULN during treatment of HBeAg negative and HBeAg positive disease, respectively. Flares have been accompanied by elevations of total bilirubin and alkaline phosphatase and less commonly with prolongation of PT and reduced albumin levels. Eleven percent of subjects had dose modifications due to ALT flares and less than 1% of subjects were withdrawn from treatment. Of note these data refer to patients not receiving concomitatnt oral antiviral agents. We believe the chance of significant ALT flares (>10x ULN) would be rare in the setting of combination therapy.

Renal effects: Interferon may have minor renal toxicities. A case of interstitial nephritis during alpha interferon therapy has been reported. We have had one patient develop acute renal failure while on alpha interferon. This was only partially reversible. We have treated many patients with renal disease using alpha interferon without any adverse effects on renal function. Renal complications are rare and occur with a frequency of <1%.

CNS Effects: Confusion has been noted with very high doses of interferon (>50 mu)69. At the doses used in our trials (180 mcg per injection) the only central nervous system side effects have been mild headaches, irritability, depression and psychological changes. Many patients complain of being short-tempered and irritable while on interferon. Approximately 18% of patients develop emotional lability and depression. These side effects are closely monitored and reverse with decrease in the interferon dosage. Another 5% of patients treated with interferon in our studies developed more severe psychiatric side effects including paranoia, delusional thinking and marked anxiety. We have found this side effect largely in patients with pre-existing psychiatric or neurological problems6. These side effects reverse within one to two weeks after discontinuing interferon therapy.

Seizures may occur in patients receiving alpha interferon. These have been described as grand mal seizures that were self-limited and stopped once interferon was discontinued. We have reported that there is a 1-2% incidence of seizures during interferon therapy. These seizures occurred near the end of therapy and did not recur. Several patients were hospitalized for the seizures and evaluated; no abnormalities were detected on neurological examination or imaging studies, and none are currently receiving anti-epileptic medication.

Neuropsychiatric: Life-threatening or fatal neuropsychiatric reactions may manifest in all patients receiving therapy with interferon and may include suicide, suicidal ideation, homicidal ideation, relapse of drug addiction, and drug overdose. These adverse effects occur in <1% of treated subjects. These reactions may occur in patients with and without previous psychiatric illness.

Autoimmune phenomena: Development or exacerbation of autoimmune disorders including myositis, hepatitis, thrombotic thrombocytopenic purpura, idiopathic thrombocytopenic purpura, psoriasis, rheumatoid arthritis, interstitial nephritis, thyroiditis, and systemic lupus erythematosus have been reported in patients receiving alpha interferon. These occur in less than 1% of patients.

RISK/DISCOMFORTS IN RELATION TO BENEFIT

Protection against risks

The risks of study medication regimen administration can be generalized into 3 areas, teratogenicity, changes in hematological parameters, and non-specific side effects. To protect subjects from teratogenicity, pregnancy tests will be performed every 2 weeks for the first 2 months, then every month thereafter. Additionally, women should practice 2 forms of contraception. CBC's will be performed every 2 weeks to monitor for hematologic changes, and symptoms or side effects will be monitored at every visit, which may result in a study medication regimen dose modification. Another major risk to subjects who agree to participate in this study is the risk of undergoing 2 liver biopsies (as outlined in Risks and hazards #3). Since a major endpoint of the study is to understand how peginterferon may lead to HBeAg or HBsAg loss, the timing of the biopsies were chosen to maximize our ability to demonstrate changes in interferon stimulated genes in the liver-up to 12 weeks before and 6 hours after the first dose of peginterferon. Knowledge gained from this analysis may inform on how best to use peginterferon in patients with chronic hepatitis B and identify new approaches to induce HBeAg and HBsAg loss-highly desirable endpoints that may permit cessation of treatment.

Potential benefits

For patients with chronic hepatitis B, the strategy of add-on peginterferon offers a potential benefit of loss of either HBeAg or HBsAg or both with the possibility of discontinuing all therapy and removal of the risks associated with long term nucleoside analogue use-predominantly renal toxicity and bone loss with tenofovir and cancer risk with entecavir. Thus, there is a prospect of overall clinical benefit from participation in this study.

DATA AND SAFETY MONITORING

The adequacy of data acquisition and storage of samples and the monitoring of safety in this trial will be performed by the principal investigator in collaboration with the associate investigators. Data and safety are reviewed weekly in clinical research rounds by the Liver Diseases Branch, NIDDK. These rounds are separate from regular clinical rounds and consist of review of all study patients including

flow sheets of major safety and efficacy measurements without knowledge of which therapy is used. Monitoring of this study will also be conducted by the statistical department of NIDDK who will have knowledge of the patient allocation. Reports of serious adverse events are made to the Clinical Director NIDDK, and the IRB as outlined below.

ADVERSE EVENT REPORTING

Definitions

Protocol Deviation: Any change, divergence, or departure from the IRB approved research protocol.
Unanticipated Problem: Any incident, experience or outcome that meets all of the following criteria:

1. Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied.
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized).

Expected Adverse Events become unanticipated problems when they occur at a greater frequency or severity than was previously known.

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events at least possibly related to the patient's participation in the research protocol, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event.

Non-serious protocol deviations will only be reported to the IRB (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from normal subject scheduling

variations or technical issues associated with sampling that do not impact the health of the subject or the interpretation of the study data will not be reported.

Adverse events that are expected as a part of treatment or procedures outlined in the protocol will not be reported unless they occur at a rate or severity greater than known to occur in patients undergoing the treatment or procedures. If AEs associated with the treatment and procedures in the study occur at a rate or severity greater than known to occur, they will be reclassified as a UP and reported as such. AEs with known relation to the natural history of chronic hepatitis B or to other pre-existing conditions will not be reported unless they occur at a rate or severity greater than known to occur in patients with hepatitis B or the subject's other pre-existing conditions. AEs that are unrelated to the research will not be reported. The PI is responsible for summarizing all reportable serious adverse events and adverse events at least possibly related at the time of Continuing Review. Deaths will be reported to the Clinical Director and IRB within 7 days after the PI first learns of the event.

PROTOCOL MONITORING PLAN

Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the Liver Diseases Branch quality assurance program plan. Audit and/or monitoring visits results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

ENROLLMENT OF CHILDREN, WOMEN AND MINORITY INDIVIDUALS

The current protocol excludes children (age limit > 18 years) as the Liver Diseases Branch does not currently treat children. The number of children on long-term nuclos(t)ide analogue therapy would expected to be small and the requirement for two liver biopsies may be prohibitive.

Chronic HBV infection is about twice as common among men than women. We expect to include women in the proportions that occur in this disease. Thus, we expect 30% to 40% of patients to be women. The exclusion of women interested in getting pregnant or who are not able to practice adequate birth control would be the only possible bias introduced in attaining adequate representation of women in this study.

The distribution of minority individuals in our ongoing hepatitis B study (07-DK-0207) are as follows: 73% of subjects were considered minorities, 82% of whom were Asian and 14% African-American based on self-reported race and ethnicity and NIH, HHS guidelines for defining minority race. Thus, we believe minority individuals will be adequately represented in this study.

Additional Potentially Vulnerable Subject Populations

This study does not plan to accrue subjects who are prisoners nor adults who are or may be unable to provide consent. These populations are excluded as this is a more than minimal risk study due to the requirement for two liver biopsies. NIH employees are not categorically recruited, but in the event that an eligible subject presents who happens to be an NIH employee, we will follow the guidelines outlined in the inclusion of employees in NIH intramural research studies.

RESEARCH USE, STORAGE AND DISPOSITION OF HUMAN SAMPLES AND DATA

Patients will have serum stored from selected time points during this study. These specimens will be used for repeat virological testing and special tests as needed (such as for viral levels, or measurement of serum levels of cytokines or interferon induced genes). Samples may be used to assess factors associated with response or non-response to antiviral therapy. Liver biopsy tissue may be stored if residual tissue is available after samples are taken for routine histological staining and evaluation, ISG determination and immunological testing. ISG and immunological determination will be evaluated in the Liver Diseases Branch and the routine histological evaluation by the surgical pathology services of the Clinical Center. Patient participation in this study will be kept confidential. No one other than study personnel at the clinic will be given the subject's name, address, and other personal identifying information. Some study-related forms such as the consent form will be kept in the medical record and have subject name and other personal identifying information on it. Other research forms such as study diaries and flowsheets will also contain subject name and personal identifying information. These records will be kept in locked files or computers that are password protected. Liver tissue samples will be stored in the pathology department and will have personal identifying information on it. However, other study material such as blood samples will be labeled with only an identifying number and code. Any study material that may be shared with investigators outside the NIH will not contain any personal identifying information. Only the study personnel at

the Liver Diseases Branch, Clinical Center can link your name or other personal identifiers to this code. We plan to collaborate with Dr. Gavin Cloherty, Abbott Diagnostics, to obtain the quantitative HBsAg testing, specified as a secondary aim of this study. Dr. Cloherty will receive ten to eleven 250 ul aliquots of sera from each subject for quantitative HBsAg testing. The results will be used for research and as part of a planned publication.

Research records and data as well as liver biopsy slides, biopsy reports, liver tissue and sera with the patient's name and a unique identifier will be stored indefinitely in our locked offices and freezers, the medical record department and the pathology department. These materials will be protected and tracked by standard operating procedures in the medical record and pathology departments as well as a compulsive filing system in our locked offices and freezers. There will be redundant storage of clinical information in the medical record department and our offices. Likewise, there will be redundant storage of biopsy information and materials in the pathology department and our offices. This should minimize the risk of loss or destruction of information and specimens. If that were to occur we would report it to the IRB. We do not plan to destroy this personal medical information or the liver biopsy specimens or research subject sera after completion of the study because it may be critically important for physicians (here or elsewhere) to have access them when caring for these patients in the future.

RECRUITMENT PLAN

Potentially eligible HBeAg positive and HBeAg negative subjects evaluated under protocol 91-DK-0214 and 07-DK-0207 will be informed of this trial and offered enrollment in this study. It is anticipated that the majority of subjects (~85%) will be recruited from within the Liver Diseases Branch Outpatient Clinic. For the remaining subjects, we will plan to send a recruitment letter to area Internists and Gastroenterologists to refer suitable candidates for the trial. Finally, we will advertise through the NIH patient recruitment office. All recruitment material will be submitted to the IRB review prior to dissemination.

PRIVACY AND CONFIDENTIALITY

Potentially eligible subjects will be identified through their enrolment in 91-DK-0214 and 07-DK-0207 and will be informed of this study at the time of a scheduled visit or by phone to notify them of their potential eligibility. Subjects will discuss protocol eligibility with the principal investigator or an associate investigator, and associate investigators will continue to be involved in research activities throughout subjects' participation in this study. Information collected about subjects will be obtained through routine clinical interactions including documentation in the subjects' medical

charts and through questionnaire completion, patient diaries and telephone calls. We will verbally verify with subjects their partner's compliance with subject reported reproductive contraception use, but this information will not be documented in the subjects' medical charts as these individuals are not considered subjects under this study. The information collected from subjects is primarily required to manage the subjects' clinical care under the current study. Data collected for research only is the minimal information required to complete analysis for primary and secondary aims.

CONSENT PROCESS

Potential study subjects will be informed of study rationale, design, participation burden, risks, benefits and side effects of therapy during routine LDB clinic visits and will have an opportunity to ask questions about the proposed study. They will be provided a copy of the study consent form to take home and review in more detail. . Consent will be obtained by the Principal Investigator, or an Associate Investigator following the subject's review of the provided consent documentation during a subsequent clinic visit. If a subject indicates a desire to participate in the research study, he/she will have all screening laboratories, radiologic imaging and if necessary, liver biopsy, performed through this protocol. The initial liver and second liver biopsy for participation in this study will be for research purposes. Patients who refuse either liver biopsy will be excluded from further participation in the study, since one of the primary endpoints of the trial is based on analysis of change in ISG levels comparing the second to the first liver biopsy. Potential study subjects who meet study inclusion and exclusion criteria will sign the consent form to participate in this study during a scheduled LDB clinic visit prior to screening.

We do not plan to enroll non-English speaking subjects; however they are not excluded from participation either. Should a non-English speaking subject be enrolled, IRB approval will be obtained to use the short form process in the absence of a fully translated consent document. Requests for IRB approval will be obtained prior to implementing the short form consent process. The short form consent process will be incompliance with SOP 12.9.1 and 45 CFR 46.117(b)(2).

COMPENSATION

Patients enrolled in this trial will not be offered compensation since they will be receiving study medications and all required monitoring at no personal cost. In addition patients stand to derive clinical benefit from participation in the study.

REFERENCES

1. Lok, A.S. and B.J. McMahon, *Chronic hepatitis B: update 2009.* Hepatology, 2009. **50**(3): p. 661-2.
2. Goldstein, S.T., et al., *A mathematical model to estimate global hepatitis B disease burden and vaccination impact.* Int J Epidemiol, 2005. **34**(6): p. 1329-39.
3. Chen, C.J., et al., *Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level.* JAMA, 2006. **295**(1): p. 65-73.
4. McMahon, B.J., *Natural history of chronic hepatitis B.* Clin Liver Dis, 2010. **14**(3): p. 381-96.
5. *EASL clinical practice guidelines: Management of chronic hepatitis B virus infection.* J Hepatol, 2012. **57**(1): p. 167-85.
6. Liaw, Y.F., et al., *Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2005 update.* Liver Int, 2005. **25**(3): p. 472-89.
7. Chang, T.T., et al., *Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B.* Hepatology, 2010. **52**(3): p. 886-93.
8. Marcellin, P., et al., *Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study.* Lancet, 2013. **381**(9865): p. 468-75.
9. Hadziyannis, S.J., et al., *Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B.* Hepatology, 2000. **32**(4 Pt 1): p. 847-51.
10. Hadziyannis, S.J., et al., *Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B.* N Engl J Med, 2005. **352**(26): p. 2673-81.
11. Shouval, D., et al., *Relapse of hepatitis B in HBeAg-negative chronic hepatitis B patients who discontinued successful entecavir treatment: the case for continuous antiviral therapy.* J Hepatol, 2009. **50**(2): p. 289-95.
12. Chang, T.T., et al., *Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B.* Hepatology, 2010. **51**(2): p. 422-30.
13. Marcellin, P., et al., *Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B.* Hepatology, 2008. **48**(3): p. 750-8.
14. Liaw, Y.F., et al., *2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B.* Gastroenterology, 2009. **136**(2): p. 486-95.

15. Marcellin, P., et al., *Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B.* N Engl J Med, 2008. **359**(23): p. 2442-55.
16. Fung, J., et al., *Extrahepatic effects of nucleoside and nucleotide analogues in chronic hepatitis B treatment.* J Gastroenterol Hepatol, 2014. **29**(3): p. 428-34.
17. Lu, J., et al., *Direct economic burden of hepatitis B virus related diseases: evidence from Shandong, China.* BMC Health Serv Res, 2013. **13**: p. 37.
18. Rifkin, B.S. and M.A. Perazella, *Tenofovir-associated nephrotoxicity: Fanconi syndrome and renal failure.* Am J Med, 2004. **117**(4): p. 282-4.
19. Toy, M., et al., *The cost-effectiveness of treating chronic hepatitis B patients in a median endemic and middle income country.* Eur J Health Econ, 2012. **13**(5): p. 663-76.
20. Tsai, N., et al., *Cost-effectiveness of entecavir versus adefovir for the treatment of chronic hepatitis B in patients with decompensated cirrhosis from a third-party US payer perspective.* Clinicoecon Outcomes Res, 2012. **4**: p. 227-35.
21. Lok, A.S., et al., *Efficacy of entecavir with or without tenofovir disoproxil fumarate for nucleos(t)ide-naive patients with chronic hepatitis B.* Gastroenterology, 2012. **143**(3): p. 619-28 e1.
22. Janssen, H.L., et al., *Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial.* Lancet, 2005. **365**(9454): p. 123-9.
23. Lau, G.K., et al., *Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B.* N Engl J Med, 2005. **352**(26): p. 2682-95.
24. Marcellin, P., et al., *Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B.* N Engl J Med, 2004. **351**(12): p. 1206-17.
25. Piccolo, P., et al., *A randomized controlled trial of pegylated interferon-alpha2a plus adefovir dipivoxil for hepatitis B e antigen-negative chronic hepatitis B.* Antivir Ther, 2009. **14**(8): p. 1165-74.
26. Bonino, F., et al., *Predicting response to peginterferon alpha-2a, lamivudine and the two combined for HBeAg-negative chronic hepatitis B.* Gut, 2007. **56**(5): p. 699-705.
27. Tseng, T.C., et al., *Effect of host and viral factors on hepatitis B e antigen-positive chronic hepatitis B patients receiving pegylated interferon-alpha-2a therapy.* Antivir Ther, 2011. **16**(5): p. 629-37.
28. al, S.M.J.X.Q.Z.N.P.e., *Adding peginterferon alfa-2a to entecavir increases HBsAg decline and HBeAg clearance - first results from a global randomized trial (ARES study).* Hepatology, 2012. **56**(S1): p. 199A.
29. Ouzan, D., et al., *Add-on peg-interferon leads to loss of HBsAg in patients with HBeAg-negative chronic hepatitis and HBV DNA fully suppressed by long-term nucleotide analogs.* J Clin Virol, 2013. **58**(4): p. 713-7.
30. Sadler, A.J. and B.R. Williams, *Interferon-inducible antiviral effectors.* Nat Rev Immunol, 2008. **8**(7): p. 559-68.
31. Li, J., et al., *Inhibition of hepatitis B virus replication by MyD88 involves accelerated degradation of pregenomic RNA and nuclear retention of pre-S/S RNAs.* J Virol, 2010. **84**(13): p. 6387-99.

32. Wieland, S.F., L.G. Guidotti, and F.V. Chisari, *Intrahepatic induction of alpha/beta interferon eliminates viral RNA-containing capsids in hepatitis B virus transgenic mice.* J Virol, 2000. **74**(9): p. 4165-73.
33. Xu, C., et al., *Interferons accelerate decay of replication-competent nucleocapsids of hepatitis B virus.* J Virol, 2010. **84**(18): p. 9332-40.
34. Belloni, L., et al., *IFN-alpha inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome.* J Clin Invest, 2012. **122**(2): p. 529-37.
35. Ahlenstiel, G., et al., *Early changes in natural killer cell function indicate virologic response to interferon therapy for hepatitis C.* Gastroenterology, 2011. **141**(4): p. 1231-9, 1239 e1-2.
36. Edlich, B., et al., *Early changes in interferon signaling define natural killer cell response and refractoriness to interferon-based therapy of hepatitis C patients.* Hepatology, 2012. **55**(1): p. 39-48.
37. Rehermann, B., *Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells.* Nat Med, 2013. **19**(7): p. 859-68.
38. Lanford, R.E., et al., *Genomic response to interferon-alpha in chimpanzees: implications of rapid downregulation for hepatitis C kinetics.* Hepatology, 2006. **43**(5): p. 961-72.

1 Appendix 1. GSI Grading Scale for Severity of Adverse Events and Laboratory Abnormalities

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin HIV POSITIVE Adult and Pediatric ≥ 57 Days	8.5 to 10.0 g/dL 85 to 100 g/L	7.5 to < 8.5 g/dL 75 to < 85 g/L	6.5 to < 7.5 g/dL 65 to < 75 g/L	< 6.5 g/dL < 65 g/L
HIV NEGATIVE Adult and Pediatric ≥ 57 Days	10.0 to 10.9 g/dL 100 to 109 g/L OR Any decrease from Baseline 2.5 to < 3.5 g/dL 25 to < 35 g/L	9.0 to < 10.0 g/dL 90 to < 100 g/L OR Any decrease from Baseline 3.5 to < 4.5 g/dL 35 to < 45 g/L	7.0 to < 9.0 g/dL 70 to < 90 g/L OR Any decrease from Baseline ≥ 4.5 g/dL ≥ 45 g/L	< 7.0 g/dL < 70 g/L
Infant, 36–56 Days (HIV POSITIVE OR NEGATIVE) Infant, 22–35 Days (HIV POSITIVE OR NEGATIVE) Infant, 1–21 Days (HIV positive or negative)	8.5 to 9.4 g/dL 85 to 94 g/L 9.5 to 10.5 g/dL 95 to 105 g/L 12.0 to 13.0 g/dL 120 to 130 g/L	7.0 to < 8.5 g/dL 70 to < 85 g/L 8.0 to < 9.5 g/dL 80 to < 95 g/L 10.0 to < 12.0 g/dL 100 to < 120 g/L	6.0 to < 7.0 g/dL 60 to < 70 g/L 7.0 to < 8.0 g/dL 70 to < 80 g/L 9.0 to < 10.0 g/dL 90 to < 100 g/L	< 6.0 g/dL < 60 g/L < 7.0 g/dL < 70 g/L < 9.0 g/dL < 90 g/L
Absolute Neutrophil Count (ANC)	1000 to 1300/mm3	750 to < 1000/mm3	500 to < 750/mm3	< 500/mm3

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
Adult and Pediatric, > 7 Days	1.00 to 1.30 GI/L	0.75 to < 1.00 GI/L	0.50 to < 0.75 GI/L	< 0.50 GI/L
Infant, 2 – ≤ 7 Days	1250 to 1500/mm3 1.25 to 1.50 GI/L	1000 to < 1250/mm3 1.00 to < 1.25 GI/L	750 to < 1000/mm3 0.75 to < 1.00 GI/L	< 750/mm3 < 0.75 GI/L
Infant, 1 Day	4000 to 5000/mm3 4.00 to 5.00 GI/L	3000 to < 4000/mm3 3.00 to < 4.00 GI/L	1500 to < 3000/mm3 1.50 to < 3.00 GI/L	< 1500/mm3 < 1.50 GI/L
Absolute CD4+ Count HIV NEGATIVE ONLY Adult and Pediatric > 13 Years	300 to 400/mm3 300 to 400/µL	200 to < 300/mm3 200 to < 300/µL	100 to < 200/mm3 100 to < 200/µL	< 100/mm3 < 100/µL
Absolute Lymphocyte Count HIV NEGATIVE ONLY Adult and Pediatric > 13 Years	600 to 650/mm3 0.60 to 0.65 GI/L	500 to < 600/mm3 0.50 to < 0.60 GI/L	350 to < 500/mm3 0.35 to < 0.50 GI/L	< 350/mm3 < 0.35 GI/L
Platelets	100,000 to < 125,000/mm3 100 to < 125 GI/L	50,000 to < 100,000/mm3 50 to < 100 GI/L	25,000 to < 50,000/mm3 25 to < 50 GI/L	< 25,000/mm3 < 25 GI/L

HEMATOLOGY				
	Grade 1	Grade 2	Grade 3	Grade 4
WBCs	2000/mm3 to 2500/mm3 2.00 GI/L to 2.50 GI/L	1,500 to < 2,000/mm3 1.50 to < 2.00 GI/L	1000 to < 1,500/mm3 1.00 to < 1.50 GI/L	< 1000/mm3 < 1.00 GI/L
Hypofibrinogenemia	100 to 200 mg/dL 1.00 to 2.00 g/L	75 to < 100 mg/dL 0.75 to < 1.00 g/L	50 to < 75 mg/dL 0.50 to < 0.75 g/L	< 50 mg/dL < 0.50 g/L
Hyperfibrinogenemia	> ULN to 600 mg/dL > ULN to 6.0 g/L	> 600 mg/dL > 6.0 g/L	— —	— —
Fibrin Split Product	20 to 40 µg/mL 20 to 40 mg/L	> 40 to 50 µg/mL > 40 to 50 mg/L	> 50 to 60 µg/mL > 50 to 60 mg/L	> 60 µg/mL > 60 mg/L
Prothrombin Time (PT)	> 1.00 to 1.25 × ULN	> 1.25 to 1.50 × ULN	> 1.50 to 3.00 × ULN	> 3.00 × ULN
International Normalized Ratio of prothrombin time (INR)	1.1 to 1.5 x ULN	>1.5 to 2.0 x ULN	>2.0 to 3.0 x ULN	>3.0 x ULN
Activated Partial Thromboplastin Time (APTT)	> 1.00 to 1.66 × ULN	> 1.66 to 2.33 × ULN	> 2.33 to 3.00 × ULN	> 3.00 × ULN
Methemoglobin	5.0 to 10.0%	> 10.0 to 15.0%	> 15.0 to 20.0%	> 20.0%

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CHEMISTRY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hyponatremia	130 to <LLN mEq/L 130 to <LLN mmol/L	125 to < 130 mEq/L 125 to < 130 mmol/L	121 to < 125 mEq/L 121 to < 125 mmol/L	< 121 mEq/L < 121 mmol/L
Hypernatremia	146 to 150 mEq/L	> 150 to 154 mEq/L	> 154 to 159 mEq/L	> 159 mEq/L

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

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

CHEMISTRY				
	Grade 1	Grade 2	Grade 3	Grade 4
Hypomagnesemia	1.40 to <LLN mg/dL 1.2 to <LLN mEq/L 0.58 to <LLN mmol/L	1.04 to < 1.40 mg/dL 0.9 to < 1.2 mEq/L 0.43 to < 0.58 mmol/L	0.67 to < 1.04 mg/dL 0.6 to < 0.9 mEq/L 0.28 to < 0.43 mmol/L	< 0.67 mg/dL < 0.6 mEq/L < 0.28 mmol/L
Hypophosphatemia Adult and Pediatric > 14 Years Pediatric 1 Year–14 Years Pediatric < 1 Year	2.0 to < LLN mg/dL 0.63 to < LLN mmol/L 3.0 to 3.5 mg/dL 0.96 to 1.14 mmol/L 3.5 to 4.5 mg/dL 1.12 to 1.46 mmol/L	1.5 to < 2.0 mg/dL 0.47 to < 0.63 mmol/L 2.5 to < 3.0 mg/dL 0.80 to < 0.96 mmol/L 2.5 to < 3.5 mg/dL 0.80 to < 1.12 mmol/L	1.0 to < 1.5 mg/dL 0.31 to < 0.47 mmol/L 1.5 to < 2.5 mg/dL 0.47 to < 0.80 mmol/L 1.5 to < 2.5 mg/dL 0.47 to < 0.80 mmol/L	< 1.0 mg/dL < 0.31 mmol/L < 1.5 mg/dL < 0.47 mmol/L < 1.5 mg/dL < 0.47 mmol/L
Hyperbilirubinemia Adult and Pediatric > 14 Days Infant, ≤ 14 Days (non-hemolytic)	> 1.0 to 1.5 × ULN NUCs	> 1.5 to 2.5 × ULN 20.0 to 25.0 mg/dL 342 to 428 µmol/L	> 2.5 to 5.0 × ULN > 25.0 to 30.0 mg/dL > 428 to 513 µmol/L	> 5.0 × ULN > 30.0 mg/dL > 513 µmol/L

CHEMISTRY				
	Grade 1	Grade 2	Grade 3	Grade 4
Infant, ≤ 14 Days (hemolytic)	NUCs	NUCs	20.0 to 25.0 mg/dL 342 to 428 µmol/L	> 25.0 mg/dL > 428 µmol/L
Blood Urea Nitrogen	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
Hyperuricemia	>ULN to 10.0 mg/dL >ULN to 597 µmol/L	> 10.0 to 12.0 mg/dL > 597 to 716 µmol/L	> 12.0 to 15.0 mg/dL > 716 to 895 µmol/L	> 15.0 mg/dL > 895 µmol/L
Hypouricemia	1.5 mg/dL to < LLN 87 µmol/L to < LLN	1.0 to < 1.5 mg/dL 57 to < 87 µmol/L	0.5 to < 1.0 mg/dL 27 to < 57 µmol/L	< 0.5 mg/dL < 27 µmol/L
Creatinine	> 1.50 to 2.00 mg/dL > 133 to 177 µmol/L	> 2.00 to 3.00 mg/dL > 177 to 265 µmol/L	> 3.00 to 6.00 mg/dL > 265 to 530 µmol/L	> 6.00 mg/dL > 530 µmol/L
Bicarbonate	16.0 mEq/L to < LLN 16.0 mmol/L to < LLN	11.0 to < 16.0 mEq/L 11.0 to < 16.0 mmol/L	8.0 to < 11.0 mEq/L 8.0 to < 11.0 mmol/L	< 8.0 mEq/L < 8.0 mmol/L
Triglycerides (Fasting)	NUCs	500 to 750 mg/dL 5.64–8.47 mmol/L	> 750 to 1200 mg/dL > 8.47–13.55 mmol/L	> 1200 mg/dL > 13.55 mmol/L
LDL (Fasting) Pediatric >2 to <18 years	130 to 160 mg/dL 3.35 to 4.15 mmol/L 110 to 130 mg/dL	>160 to 190 mg/dL >4.15 to 4.92 mmol/L >130 to 190 mg/dL	> 190 mg/dL >4.92 mmol/L > 190 mg/dL	NUCs NUCs

CHEMISTRY				
	Grade 1	Grade 2	Grade 3	Grade 4
	2.84 to 3.37 mmol/L	>3.37 to 4.92 mmol/L	>4.92 mmol/L	
Hypercholesterolemia (Fasting)	200 to 239 mg/dL 5.16 to 6.19 mmol/L	> 239 to 300 mg/dL > 6.19 to 7.77 mmol/L	> 300 mg/dL > 7.77 mmol/L	NUCs
Pediatric < 18 Years	170 to 199 mg/dL 4.39 to 5.15 mmol/L	> 199 to 300 mg/dL > 5.15 to 7.77 mmol/L	> 300 mg/dL > 7.77 mmol/L	NUCs
Creatine Kinase	3.0 to < 6.0 × ULN	6.0 to < 10.0 × ULN	10.0 to < 20.0 × ULN	≥ 20.0 × ULN

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

ENZYMES				
	Grade 1	Grade 2	Grade 3	Grade 4
AST (SGOT)	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
ALT (SGPT)	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
GGT	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
Alkaline Phosphatase	1.25 to 2.50 × ULN	> 2.50 to 5.00 × ULN	> 5.00 to 10.00 × ULN	> 10.00 × ULN
Total Amylase	> 1.0 to 1.5 × ULN	> 1.5 to 2.0 × ULN	> 2.0 to 5.0 × ULN	> 5.0 × ULN
Pancreatic Amylase	> 1.0 to 1.5 × ULN	> 1.5 to 2.0 × ULN	> 2.0 to 5.0 × ULN	> 5.0 × ULN
Lipase	> 1.0 to 1.5 × ULN	> 1.5 to 3.0 × ULN	> 3.0 to 5.0 × ULN	> 5.0 × ULN

ENZYMES				
	Grade 1	Grade 2	Grade 3	Grade 4
Albumin	3.0 g/dL to < LLN 30 g/L to < LLN	2.0 to < 3.0 g/dL 20 to < 30 g/L	< 2.0 g/dL < 20 g/L	NUCs

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URINUCsLYSIS				
	Grade 1	Grade 2	Grade 3	Grade 4
Hematuria (Dipstick)	1+	2+	3-4+	NUCs
Hematuria (Quantitative) See Note below Females	>ULN - 10 RBC/HPF	> 10-75 RBC/HPF	> 75 RBC/HPF	NUCs
Males	6-10 RBC/HPF	> 10-75 RBC/HPF	> 75 RBC/HPF	NUCs
Proteinuria (Dipstick)	1+	2–3+	4+	NUCs
Proteinuria, 24 Hour Collection Adult and Pediatric ≥ 10 Years	200 to 999 mg/24 h	>999 to 1999 mg/24 h	>1999 to 3500 mg/24 h	> 3500 mg/24 h
Pediatric > 3 Mo to < 10 Years	201 to 499 mg/m2/24 h	>499 to 799 mg/m2/24 h	>799 to 1000 mg/m2/24 h	> 1000 mg/ m2/24 h
Glycosuria (Dipstick)	1+	2-3+	4+	NUCs

2 Notes:

3 Toxicity grades for Quantitative and Dipstick Hematuria will be assigned by Covance Laboratory,

4 however for other laboratories, toxicity grades will only be assigned to Dipstick Hematuria.

With the exception of lipid tests, any graded laboratory test with a result that is between the LLN and ULN should be assigned Grade 0.

If the severity of a clinical AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE.

CARDIOVASCULAR				
	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac Arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic, non-life-threatening AND Non-urgent medical intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Cardiac-ischemia/Infarction	NUCs	NUCs	Symptomatic ischemia (stable angina) OR Testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Hemorrhage (significant acute blood loss)	NUCs	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≤ 2 units packed RBCs (for children ≤ 10 cc/kg) indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs indicated (for children ≤ 10 cc/kg) indicated
Hypertension (with repeat testing at same visit)	140–159 mmHg systolic OR 90–99 mmHg diastolic	> 159–179 mmHg systolic OR > 99–109 mmHg diastolic	> 179 mmHg systolic OR > 109 mmHg diastolic	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization (other than ER visit) indicated

CARDIOVASCULAR				
	Grade 1	Grade 2	Grade 3	Grade 4
Pediatric ≤ 17 Years (with repeat testing at same visit)	NUCs	91st–94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Hypotension	NUCs	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Pericardial Effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no intervention	Effusion with non-life-threatening physiologic consequences OR Effusion with nonurgent intervention indicated	Life-threatening consequences (eg, tamponade) OR Urgent intervention indicated
Prolonged PR Interval	PR interval 0.21 to 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR Ventricular pause > 3.0 sec	Complete AV block

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

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

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

2

OCULAR/VISUAL				
	Grade 1	Grade 2	Grade 3	Grade 4

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

1

SKIN				
	Grade 1	Grade 2	Grade 3	Grade 4
Alopecia	Thinning detectable by study participant or caregiver (for disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	NA

Cutaneous Reaction – Rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	NA	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA

1

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [eg, tube feeding or total parenteral nutrition]
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (eg, diuretics or therapeutic paracentesis)	Symptomatic despite intervention	Life-threatening consequences
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (eg, sepsis or perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (eg, obstruction)

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Diarrhea Adult and Pediatric ≥ 1 Year	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline/24 hr	Persistent episodes of unformed to watery stools OR Increase of 4–6 stools over baseline per 24 hrs.	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (eg, hypotensive shock)
Pediatric < 1 Year	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Liquid stools resulting in severe dehydration with aggressive rehydration indicated OR Hypotensive shock
Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without medical intervention indicated	Symptoms causing severely altered dietary intake with medical intervention indicated	Life-threatening reduction in oral intake

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Mucositis/Stomatitis (clinical exam) See also Proctitis, Dysphagia-Odynophagia	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening consequences (eg, aspiration, choking)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24–48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (eg, IV fluids)	Life-threatening consequences (eg, hypotensive shock)
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other than ER visit)	Symptomatic AND Hospitalization indicated (other than ER visit)	Life-threatening consequences (eg, sepsis, circulatory failure, hemorrhage)

GASTROINTESTINAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Proctitis (functional-symptomatic) Also see Mucositis/ Stomatitis for Clinical Exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social/ functional activities OR Operative intervention indicated	Life-threatening consequences (eg, perforation)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated	Life-threatening consequences (eg, hypotensive shock)

1

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Alteration in Personality-Behavior or in Mood (eg, agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with usual social & functional activities	Alteration causing inability to perform usual social & functional activities	Behavior potentially harmful to self or others (eg, suicidal/homicidal ideation or attempt, acute psychosis) OR Causing inability to perform basic self-care functions

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Altered Mental Status For Dementia, see Cognitive and Behavioral/Attentiona l Disturbance (including dementia and ADD)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal interference with usual social & functional activities	Symptomatic ataxia causing greater than minimal interference with usual social & functional activities	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions
Cognitive and Behavioral/Attentiona l 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 Institutionalizatio n indicated

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
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 ER visit) OR Headache with significant impairment of alertness or other neurologic function

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
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 weak-ness 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

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Neurosensory Alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizure: (new onset)	NA	1 seizure	2–4 seizures	Seizures of any kind that are prolonged, repetitive (eg, status epilepticus), or difficult to control (eg, refractory epilepsy)

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Seizure: (pre-existing) For Worsening of Existing Epilepsy the Grades Should Be Based on an Increase from Previous Level of Control to Any of These Levels	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR infrequent breakthrough seizures while on stable meds in a previously controlled seizure disorder	Change in seizure character from baseline either in duration or quality (eg, severity or focality)	Seizures of any kind that are prolonged, repetitive (eg, status epilepticus), or difficult to control (eg, refractory epilepsy)
Seizure – Pediatric < 18 Years	Seizure, generalized onset with or without secondary generalization, lasting < 5 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting 5–20 minutes with < 24 hours post ictal state	Seizure, generalized onset with or without secondary generalization, lasting > 20 minutes	Seizure, generalized onset with or without secondary generalization, requiring intubation and sedation
Syncope (not associated with a procedure)	NA	Present	NA	NA

NEUROLOGICAL				
	Grade 1	Grade 2	Grade 3	Grade 4
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

1

MUSCULOSKELETAL				
	Grade 1	Grade 2	Grade 3	Grade 4
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss	BMD t-score or z-score –2.5 to –1.0	BMD t-score or z-score < –2.5	Pathological fracture (including loss of vertebral height)	Pathologic fracture causing life-threatening consequences

MUSCULOSKELETAL				
	Grade 1	Grade 2	Grade 3	Grade 4
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	NUCs	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

1

SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Acute Systemic Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema

SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions
Fever (nonaxillary)	37.7°C to 38.6°C 99.8°F to 101.5°F	38.7°C to 39.3°C 101.6°F to 102.8°F	39.4°C to 40.5°C 102.9°F to 104.9°F	> 40.5°C > 104.9°F
Pain- Indicate Body Site See also Injection Site Pain, Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than ER visit) indicated

SYSTEMIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Unintentional Weight Loss	NA	5% to 9% loss in body weight from baseline	10% to 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [eg, tube feeding or total parenteral nutrition]

1

INJECTION SITE REACTION				
	Grade 1	Grade 2	Grade 3	Grade 4
Injection Site Pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than ER visit) indicated for management of pain/tenderness
Injection Site Reaction (Localized), > 15 Years Pediatric ≤ 15 Years	Erythema OR Induration of 5 × 5 cm to 9 × 9 cm (or 25–81 × cm2) Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm2) Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity	Necrosis (involving dermis and deeper tissue) Necrosis (involving dermis and deeper tissue)

		extremity segment (eg, upper arm/thigh)	segment (eg, upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	
Pruritis Associated with Injection See also Skin: Pruritis (itching—no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 h treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 h treatment	Generalized itching causing inability to perform usual social & functional activities	NA

1

ENDOCRINE/METABOLIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Lipodystrophy (eg, back of neck, breasts, abdomen)	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	NA
Diabetes Mellitus	NA	New onset without need to initiate medication OR Modification of current meds to regain glucose control	New onset with initiation of indicated med OR Diabetes uncontrolled despite treatment modification	Life-threatening consequences (eg, ketoacidosis, hyperosmolar non-ketotic coma)

ENDOCRINE/METABOLIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Gynecomastia	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (eg, thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (eg, myxedema coma)

ENDOCRINE/METABOLIC				
	Grade 1	Grade 2	Grade 3	Grade 4
Lipoatrophy (eg, fat loss from the face, extremities, buttocks)	Detectable by study participant or caregiver (for young children and disabled adults)	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA

1

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

2

INFECTION				
	Grade 1	Grade 2	Grade 3	Grade 4

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

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

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