Alemtuzumab or Tocilizumab in Combination with Etoposide and Dexamethasone for the Treatment of Adult Patients with Hemophagocytic Lymphohistiocytosis

PI: Naval Daver

MDACC IRB # 2014-0989
University of Texas

MD Anderson Cancer Center

Leukemia Department, Unit 428

1.0 OBJECTIVES

1.1 Primary Objectives

1. To determine the overall response rate (ORR) of alemtuzumab or tocilizumab in combination with etoposide and dexamethasone in patients with HLH.

1.2 Secondary Objectives:

1. To determine the safety of alemtuzumab or tocilizumab in combination with etoposide and dexamethasone in patients with HLH.
2. To determine the median time to response, duration of response, disease-free survival (DFS), and overall survival (OS) of alemtuzumab or tocilizumab in combination with etoposide and dexamethasone in patients with HLH.
3. To determine the rates of partial and complete response at 8 week.
4. To determine the incidence of serious infection and other adverse events by week 8 and prior to initiation of hematopoetic stem cell transplant (HSCT) preparative regimen (or week 24, if HSCT preparative regimen not yet begun).
5. To determine the overall survival to the initiation of HSCT preparative regimen or week 24, if HSCT preparative regimen not begun.
6. To determine the incidence and median time to reactivation prior to initiation of HSCT preparative regimen (or week 24, if HSCT preparative regimen not yet begun).
7. To determine overall survival to day +100 after HSCT, for patients who have undergone HSCT within 6 months of study entry.
8. To determine the induction mortality (mortality within first 4 weeks and 8 weeks of initiation of therapy) for patients with HLH treated with this combination.

2.0 BACKGROUND

2.1 Hemophagocytic lymphohistiocytosis:

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of severe immune activation and deregulation resulting in extreme and often life threatening inflammation[1, 2]. HLH occurs as either a primary (familial - FHL) or secondary (sporadic - sHLH) disorder[3, 4]. Both conditions manifest pathological immune activation and may be difficult to differentiate from each other.

Primary HLH is an autosomal recessive disease with an incidence of 1/50,000 live-born children. Patients often have a clear familial inheritance

or genetic mutation. Median survival is less than 2 months if untreated[5]. Disease symptoms usually appear during infancy or early childhood. Immunological triggers such as vaccinations and viral infections may trigger bouts of disease in these patients. However, in many circumstance no clear cut immune trigger is identifiable[6].

Secondary HLH includes adults and older children who lack family history or known genetic cause for HLH. Secondary HLH often occurs as a result of pathological immune activation in response to a trigger. Frequently noted triggers include malignancy (especially hematological malignancies including acute leukemia's, MDS and myelofibrosis), infections (especially EBV) and rheumatological disorders. The list of triggers associated with secondary HLH is extensive[7, 8]. Secondary HLH that develops in the setting of malignancy is also known as malignancy-associated hemophagocytic syndrome (MAHS). MAHS may manifest as the presenting feature of a yet undiagnosed malignancy or may manifest during the treatment of a known malignancy.

Categorizing patients into primary or secondary HLH is often difficult and may be of limited value. Treatment should be initiated expediently if the diagnosis of HLH is suspected.

2.2 Immune activation and HLH:

Patients with HLH have deregulation of the immune system including predisposing immunodeficiency, immune activation and immunopathology (Table 1) [1, 9]. Immune-activation and immune mediated pathology likely play a central role in the evolution of HLH. This included acute clinical signs and symptoms of immune activation including hepatomegaly, jaundice, adenopathy, rash, seizures, and focal neurologic deficits as well as laboratory measures such as strikingly high serum levels of numerous cytokines including interferon gamma (IFNγ), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), IL-10, and macrophage–colony-stimulating factor (M-CSF)[10-12]. Furthermore, biopsies of lymphoid tissues or histological examination of liver tissue from HLH patients reveal highly activated macrophages and lymphocytes, supporting striking activation of the immune system.

Table 1: Mechanisms of immune deregulation in HLH

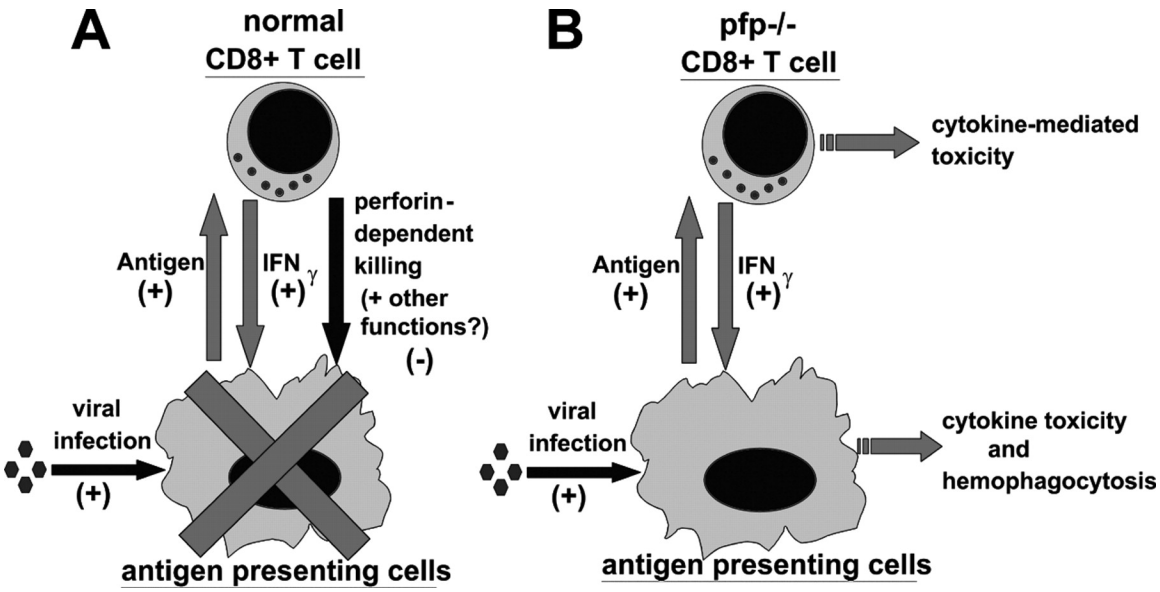
Category 1: predisposing immunodeficiency	Category 2: significant immune activation	Category 3: abnormal immunopat
Low or absent NK-cell function*	Fever*	Cytopenias*
Genetic defect of cytotoxicity*	Splenomegaly*/hepatomegaly	Decreased fibrinogen or increased trigl
Family history of HLH	Elevated ferritin* (> 3000 ng/mL)	Hemophagocytosis*
Prior episode(s) of HLH or unexplained cytopenias	Elevated sCD25*	Hepatitis
Markers of impaired cytotoxicity: decreased expression of perforin, SAP, XIAP, or mobilization of CD107a	Elevated sCD163[93]	CNS involvement

Adapted from Jordan M et al, Blood 2011[1]

2.3 Proposed immunological model of pathophysiology of HLH:

Jordan et al used perforin-deficient mice models to better define the immonobiology of HLH. Perforin deficient mice on exposure to lymphocytic choriomeningitic virus manifested most of the clinical features of HLH along with elevation of multiple serum cytokines and histological evidence of hemophagocytosis. They identified that CD8+ cells and IFN-gamma were essential to the development of HLH pathophysiology in response to LCMV infection (Figure 1).

Figure 1: Proposed model of pathophysiology of HLH:



(A) During a normal immune response, presentation of antigen promotes $CD8^+$ T-cell responses. In return, $CD8^+$ T cells secrete IFNγ, which activates antigen-presenting cells and further promotes antigen presentation. This positive feedback loop is restrained by perforin-dependent mechanisms. It remains unclear whether this occurs via specific destruction of professional antigen-presenting cells, general destruction of virally infected cells, or additional mechanisms.

(B) In LCMV-infected pfp$^{-/-}$ mice, the failure of perforin-dependent mechanisms allows for spiralling immune activation and excessive secretion of IFNγ. This leads to the development of an HLH-like syndrome.

Adapted from Jordan M et al, Blood 2004[9]

2.4 Diagnostic criteria for HLH:

The initial HLH guidelines in 1991, included 5 features: (1) fever, (2) splenomegaly, (3) cytopenis affecting at least two or three lineages in the peripheral blood, (4) hypertriglyceridemia and/or hypofibrinogenemia, and (5) hemophagocytosis in bone marrow, spleen or lymph nodes[13]. The 2004 guidelines (Table 2) included 3 additional criteria: (6) low or absent NK-cell activity, (7) hyperferritinemia and (8) high levels of s-IL2r. Five of eight criteria must be fulfilled to make a diagnosis of HLH[2]. However, patients with a molecular diagnosis of HLH do not need to fulfill the diagnostic criteria. These diagnostic criteria have been developed for pediatric HLH and have not been validated for adult HLH. These will serve as a background for evaluating HLH in adults but are not to be used as diagnostic criteria for adult HLH.

Table 2: Diagnostic criteria of HLH (Guidelines 2004)

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled

(1) A molecular diagnosis consistent with HLH

(2) Diagnostic criteria for HLH fulfilled (five out of the eight criteria below)

(A) Initial diagnostic criteria (*to be evaluated in all patients with HLH*)

- Fever
- Splenomegaly
- Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood):
  - Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L)
  - Platelets $<100 \times 10^9$/L
  - Neutrophils $<1.0 \times 10^9$/L
- Hypertriglyceridemia and/or hypofibrinogenemia:
  - Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dl)
  - Fibrinogen ≤1.5 g/L
- Hemophagocytosis in bone marrow or spleen or lymph nodes
- No evidence of malignancy

(B) New diagnostic criteria

- Low or absent NK-cell activity (according to local laboratory reference)
- Ferritin ≥500 μg/L
- Soluble CD25 (i.e., soluble IL-2 receptor) ≥2,400 U/ml

Comments:

(1) If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.

(2) The following findings may provide strong supportive evidence for the diagnosis: (a) spinal fluid pleocytosis (mononuclear cells) and/or elevated spinal fluid protein, (b) histological picture in the liver resembling chronic persistent hepatitis (biopsy).

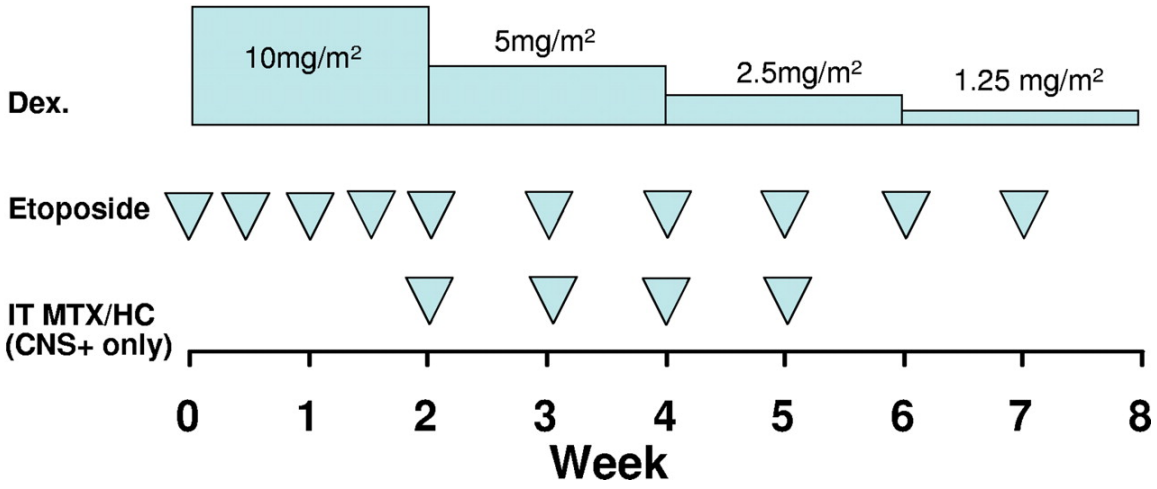
(3) Other abnormal clinical and laboratory findings consistent with the diagnosis are: cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash. Hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, VLDL ↑, HDL ↓.

2.5 Therapy of HLH:

The goal of initial therapy is to suppress the overactive immune system thus preventing immune-mediated organ damage. Induction therapy is often followed by allogeneic stem cell transplant if a suitable donor is available. If no suitable donor is identified, patients are followed closely for signs of relapse. The HLH-94 protocol proposed in 1997 included an 8-week regimen with etoposide, dexamethasone and intrathecal methotrexate (Figure 2) [14]. This systematic therapeutic approach significantly improved the outcomes and survival of patients with HLH.

The HLH-2004 protocol was opened in 2004. This protocol has completed accrual and is closed to new patient entry. The results of HLH-2004 are awaited. The major modifications from HLH-94 were to move cyclosporine dosing to the beginning of induction and add hydrocortisone to intrathecal therapy. Until the results of the HLH-2004 study are published, the current standard practice outside of a clinical protocol has been to treat patients who are not enrolled in a clinical study with a strategy based on HLH-94.

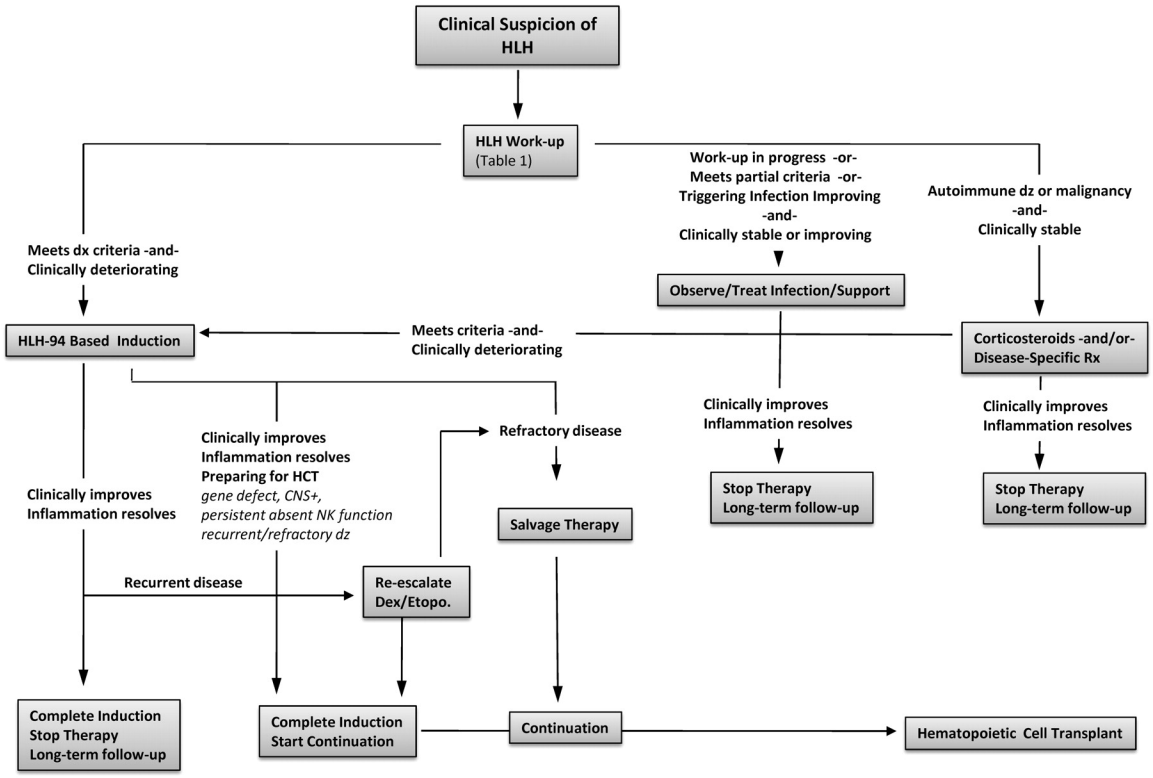
Figure 2: Induction therapy for HLH. Based on the HLH-94 study, this approach should be considered standard of care for all patients not enrolled in clinical trials, based on published evidence of efficacy. Etoposide is dosed as 150 mg/m2 per dose.



Induction therapy for HLH. Based on the HLH-94 study, this approach should be considered standard of care for all patients not enrolled in clinical trials, based on published evidence of efficacy. Etoposide is dosed as 150 mg/m^2 per dose. Alternatively, for patients weighing < 10 kg, consideration may be given to dosing etoposide as 5 mg/kg per dose. Dexamethasone (Dex.) is dosed as indicated and may be given orally or intravenously, although the latter is preferred at therapy initiation. Intrathecal methotrexate and hydrocortisone (IT MTX/HC) should be given to patients with evidence of CNS involvement, as early as LP may be safely performed (which may vary from the diagram) and dosed as follows: age < 1 year, 6/8 mg (MTX/HC); 1-2 years, 8/10 mg; 2-3 years, 10/12 mg; > 3 years, 12/15 mg. Weekly intrathecal therapy is generally continued until at least 1 week after resolution of CNS involvement (both clinical and CSF indices).

The current standard of care consists of a decrescendo course of etoposide and dexamethasone, with or without intrathecal therapy (Figure 2). Ideally, critically ill patients should be treated at facilities familiar with care of cancer and bone marrow transplant patients. It is important to initiate therapy promptly, even in the face of unresolved infections, cytopenias, or organ dysfunction. Because etoposide is cleared by both renal and hepatic routes, we recommend dose reductions of 25% for creatinine clearance of 10-50 mL/minute, 50% for creatinine clearance of < 10 mL/minute, and 75% in case creatinine clearance is < 10 mL/minute and direct bilirubin is > 3 mg/dL. We do not dose reduce etoposide for isolated hyperbilirubinemia or neutropenia. HLA typing is sent at the start of induction therapy to avoid downstream delays in HSCT.

Figure 3 Treatment strategy for HLH. An algorithm for HLH treatment strategies in various clinical contexts (Jordan M B et al, Blood 2011)



3.0 RATIONALE FOR THIS STUDY:

The outcomes with the HLH-94 regimen remain less than ideal with only approximately half of patients experiencing complete resolution of disease. Furthermore, early mortality remains a significant problem. We have identified numerous cases of HLH in leukemia patients including frontline and salvage setting. Most frequently the patients have underlying myeloid disorders including myelodysplastic syndrome, acute myeloid leukemia or myelofibrosis. The mortality among the patients manifesting HLH-features has been high. There is no standard of care for HLH in adult patients. Thus, definitive measures to diagnose and treat HLH in our patient population are warranted.

Our collaborators (Dr Kenneth McClain and Dr Carl Allen) at Texas Childrens Hospital have developed the HIT-HLH, combining anti-thymocyte globulin with dexamethasone (20mg/m2/day) for the first week followed by weekly etoposide x 8 weeks with dexamethasone 10 mg/m2/day. They have discontinued cyclosporine as it was associated with increased incidence of neurotoxicity without significant therapeutic benefit in prior pediatric HLH studies. The current HIT-HLH regimen is similar to the HLH-94, except that patients will receive 2 fewer doses of etoposide. The data remain confidential as this is an ongoing

study, however the interim results do not appear to be compelling (personal communication; Kenneth McClain and Carl Allen, Texas Childrens Hospital).

Alemtuzumab is an effective salvage agent for refractory HLH, leading to improvement and survival to HSCT in many patients[15]. Alemtuzumab up-front in combination with dexamethasone (20mg/m2/day) and etoposide may afford the best combination to target HLH. Our collaborators at TCH believe this may provide the most effective regimen for HLH and are currently treating newly diagnosed pediatric HLH patients with a combination of alemtuzumab, etoposide, and dexamethasone. A pediatric protocol incorporating alemtuzumab, etoposide, and dexamethasone for the therapy of pediatric HLH is being developed. We propose a combination regimen of alemtuzumab with etoposide and dexamethasone for adult patients with HLH.

Tocilizumab is and anti-IL6 monoclonal antibody and has shown significant activity in cytokine release syndrome, a condition closely related to HLH with similar cytokine elevation profile. We have used tocilizumab in refractory cases of Adult M-HLH with successful and rapid improvement in levels of ferritin, LDH, coagulopathy, organomegaly, and fevers. Tocilizumab is also more tolerable with less risk of T-lymphodepletion associated secondary infections such as CMV and HSV. Based on our experience with tocilizumab in patients with CART associated CRS and in refractory HLH we propose the option to use tocilizumab in combination with etoposide and dexamethasone as an alternative to alemtuzumab with etoposide and dexamethasone in adults with HLH. The tocilizumab may be an especially attractive option in patients who are critically ill or already myelosuppressed from recent chemotherapy as it may have a lower risk of inducing further myelosuppression and secondary life-threatening infections.

For further details on etoposide and alemtuzumab please see the etoposide drug insert (Appendix D), alemtuzumab drug insert (Appendix E).

4.0 STUDY DESIGN

- This will be a phase II, single-arm open-label study. All patients will be registered through CORe. Up to 40 patients will be enrolled in the study.

- The study will consist of two phases:
  (1) Induction phase: Patients will receive treatment on one of two arms at the discretion of the treating physician and the PI:
  Arm 1: Alemtuzumab with etoposide and dexamethasone during the induction phase .

  OR
  Arm 2: Tocilizumab with etoposide and dexamethasone during the induction phase.

(2) Maintenance phase: The maintenance phase may last up to 16 weeks and will start after the induction phase. Patients who have evidence of budding relapse during the maintenance phase may revert back to receiving etoposide.

- Intrathecal methotrexate 12 mg and hydrocortisone 15 mg (IT MTX/HC) should be given to patients with evidence of CNS involvement, as early as LP may be safely performed. Weekly intrathecal therapy is generally continued for at least 5 consecutive weeks or until at least 1 week after resolution of CNS involvement (both clinical and CSF indices).

- Arm 1: Patients will initially receive alemtuzumab as an SQ or IV infusion. SQ administration will be preferred. It is suggested that alemtuzumab be administered daily for 4 days (recommended on Days 1-4) during the induction phase. The induction phase may last up to 8 weeks. During the maintenance phase it is suggested that patients receive alemtuzumab approximately once every 4 weeks for ~~the~~ up to 16 weeks. Patients may receive alemtuzumab more or less frequently during the induction and maintenance phase at the discretion of the treating physician only after discussion with the PI. Continuation of therapy after 24 weeks of therapy may be considered on a case-by-case basis. For further details please refer to the Treatment Plan (section 5.0).

  Arm 2: Patients will receive tocilizumab on Day 1 or 2 if they are thought to be better candidates for the Arm 2 based on review of the clinical parameters by the treating physician and the PI. The tocilizumab will be administered IV. The tocilizumab dosing may be repeated as needed during the initial 4 weeks of therapy to gain adequate disease control

4.0 PATIENT SELECTION:

Patient must meet all inclusion and exclusion criteria prior to being enrolled on study. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient, or if the patient is incapacitated at time of consent, the surrogate decision-maker, must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. Written consent will be obtained from the patient, or if incapacitated, will be obtained from the surrogate decision-maker, as per MDACC Institutional Policy.

The following criteria apply to all patients enrolled onto the study unless otherwise specified.

Inclusion Criteria

- Sign an IRB-approved informed consent document.

- Patients must be >/= 18 years of age.

- A documentation of diagnosis of hemophagocytic lymphocytosis, either newly diagnosed or relapsed/refractory by the treating physician and the PI in the patients chart.

  It must be noted that no diagnostic criteria have been established for diagnosis of HLH in adult patients as this was a hitherto poorly identified and considered to be a very rare disease in adults. We have seen an increasing number of cases of HLH at our institution over the last 2 years partly due to referrals and partly due to better understanding of the disease through discussions with our collaborators Dr Kenneth McClain and Dr Carl Allen at TCH (experts in pediatric HLH). Adult HLH seems to occur more frequently post malignancy and has a more fulminant course than pediatric HLH. The diagnostic criteria that have been traditionally used for children (HLH 1991 and HLH 2004) may not adequately diagnose HLH in adults. This is the first adult HLH protocol in the country. In the absence of standard diagnostic guidelines if the patient's symptoms are highly suspicious for HLH and after an adequate work-up to rule out alternate potential alternate etiologies is performed we will treat the patient for HLH as missing the diagnosis is associated with high mortality. These patients will be discussed with the PI (Dr Daver) prior to enrollment in all such cases.

- Organ function as defined below (unless due to the HLH process):
  Serum creatinine </= 3.0 mg/dL
  Total bilirubin </= 5.0 mg/dL

  If organ dysfunction is thought to be related to the HLH process this must be clearly documented in the chart and the patients may be enrolled on study irrespective of creatinine and bilirubin levels.

- Women of childbearing potential must practice contraception. Females of childbearing potential: Recommendation is for 2 effective contraceptive methods during the study. Adequate forms of contraception are double barrier methods (condoms with spermicidal jelly or foam and diaphragm with spermicidal jelly or foam), oral, depo provera, or injectable contraceptives, intrauterine devices, and tubal ligation. Male patients with female partners who are of childbearing potential: Recommendation is for male and partner to use at least 2 effective contraceptive methods, as described above, prior to study entry and for at least 3 months after the last dose of study drug.

- Negative urine pregnancy test and/or serum pregnancy test within 7 days of initiation of therapy.

- Male patients with female partners who are of childbearing potential: Recommendation is for male and partner to use at least 2 effective contraceptive methods, as described above, prior to study entry and for at least 3 months after the last dose of study drug.

Exclusion Criteria

- Pregnant or breast feeding women

- Any serious/and or unstable pre-existing medical disorder (aside from malignancy exception above), psychiatric disorder, or other conditions that could interfere with subject's safety, obtaining informed consent or compliance to the study procedures.

- Patients unwilling or unable to comply with the protocol.

5.0 TREATMENT PLAN

5.1 General

All patients will be registered through CORe.

5.2 Schedule

- The current standard of care consists of a decrescendo course of etoposide and dexamethasone, with or without intrathecal therapy (HLH-2004). Unfortunately, the outcomes in adult patients remain dismal suggesting the need for improved regimens. In the more recent HIT-HLH protocol (appendix F) our pediatric collaborators used 5 days of ATG (or alemtuzumab in patients unable to tolerate ATG) with weekly etoposide and continuous dexamethasone. However, the outcomes with this combination have not been significantly better than the HLH-2004 (personal correspondence, Kenneth McClain and Carl Allen).

  Alemtuzumab as a single-agent has shown efficacy in relapsed HLH. Alemtuzumab has been well tolerated in pediatric patients with relapsed HLH with no unexpected toxicities[15]. A pediatric protocol combining alemtuzumab, etoposide, dexamethasone for the frontline treatment of HLH is currently being developed.
- Tocilizumab has shown to be very effective in postCART cell CRS. We have also used tocilizumab in adult highly refractory HLH

cases with success. Tocilizumab is less immunosuppressive than alemtuzumab and may be a good alternative especially in patients who are already immunosuppressed from recent Myeloablative therapies.

We will evaluate the addition of a biologic agent (either alemtuzumab or tocilizumab) to etoposide and dexamethasone for the treatment of adults with HLH in this phase II study.

5.2.1 Patients will be treated according to the following schedule:

Induction (Week 1-8)

- If patient receives etoposide, dexamethasone, alemtuzumab, tocilizumab, or intrathecal metroxate as standard of care prior to signing of consent, these medications will not need to be repeated upon signing of informed consent, as determined by the Principal Investigator.

- Patients will receive etoposide and dexamethasone at a fixed dose during the induction phase. This includes fixed dose of etoposide 150 mg/m2 once a week for up to 8 weeks, dexamethasone 20 mg/m2 daily for 1 week followed by dexamethasone 10 mg/m2 for 1 week followed by dexamethasone 5 mg/m2 for 2 weeks followed by dexamethasone 2.5 mg/m2 for 2 weeks followed by dexamethasone 1.25 mg/m2 for 2 weeks. The induction phase will last up to 8 weeks. The dexamethasone will be divided BID, given intravenously (IV) for at least 1 week before switching to oral formulation. Patients may receive etoposide and dexamethasone more or less frequently during the induction phase at the discretion of the treating physician after discussion with the PI. Dosages of etoposide and dexamethasone may also be adjusted at the discretion of the treating physician and after discussion with the PI. The etoposide and dexamethasone backbone will remain the same whether the patients are treated on Arm 1 (alemtuzumab combination) or arm 2 (tocilizumab combination).

- It is important to initiate therapy promptly, even in the face of unresolved infections, cytopenias, or organ dysfunction. Because etoposide is cleared by both renal and hepatic routes, we recommend dose reductions of 25% for creatinine clearance of 10-50 mL/minute, 50% for creatinine clearance of < 10 mL/minute, and 75% in case creatinine clearance is < 10 mL/minute and direct bilirubin is > 3 mg/dL. We do not recommend dose reduction of etoposide for isolated hyperbilirubinemia or neutropenia. HLA typing should be sent at the start of induction therapy to avoid

downstream delays in HSCT. Other dose modifications and dose-reductions will be according to institutional standards.

- Intrathecal methotrexate 12 mg and hydrocortisone 15 mg (IT MTX/HC) should be given to patients with evidence of CNS involvement (evidence of CNS involvement includes any of the following: elevated CSF protein or white count, seizures, focal or global neurologic deficit, MRI abnormalities consistent with CNS involvement by HLH) as early as lumbar puncture (LP) may be safely performed. Weekly intrathecal therapy is generally continued for a minimum of at least 5 consecutive doses. For patients with persistent CNS disease after 5 consecutive doses, additional weekly doses of IT therapy may be given at the discretion of the treating physician until at least 1 week after resolution of CNS involvement (both clinical and CSF indices). Lumbar puncture and IT therapy may be delayed if the treating physician deems that performing this procedure is excessively risky due to coagulopathy or thrombocytopenia.

- Arm 1: Alemtuzumab Arm: Alemtuzumab dosing will be similar to CLL with patients receiving an escalating dose of alemtuzumab titrated based on their tolerance of the alemtuzumab infusion. The maximum dose of alemtuzumab in this study (alemtuzumab 30 mg/day) is the same maximum dose used in the CLL Phase 3 studies [16]. This dosing of alemtuzumab has been well tolerated in pediatric population with no unexpected toxicities. A similar dosing schema of alemtuzumab, etoposide, and dexamethasone is used in a pediatric protocol that is being developed to treat HLH in children by our collaborators Dr Kenneth McClain and Carl Allen.

- Arm 2: Tocilizumab arm: The tocilizumab dosing will be similar to cytokine release syndrome. The recommended starting dose of tocilizumab is 4 to 8 mg/kg IV infusion. The tocilizumab may be given before or after or concomitantly with the etoposide. The start of tocilizumab administration may be delayed beyond day 1 if this is in the best interest of the patient and the reason for delay should be documented in the patient chart. Tocilizumab may be administered on the same days as etoposide or on other days during the induction. The tocilizumab dosing may be repeated as needed based on disease features and laboratory parameter changes. There is no required interval between tocilizumab doses although it is recommended to wait at least 24 hours before re-dosing. There is no maximum number or dose of tocilizumab that cannot be exceeded, although it is recommended that if the patients HLH is not responding after 4 consecutive tocilizumab infusions in combination with the etoposide and dexamthasone then alternate therapy for the HL be considered

- Patients will receive alemtuzumab as a SQ injection or IV infusion (IV infusion will be over approximately 2 hours). Patients may begin with SQ injection and switch to IV infusion or vice-versa. The goal is to administer a total alemtuzumab dose of 1.0 mg/kg over 4 days (days 1-4). The first injection/infusion will include alemtuzumab 0.1 mg/kg to be administered on day 1 of the induction. The start of alemtuzumab administration may be delayed beyond day 1 if this is in the best interest of the patient and the reason for delay should be documented in the patient chart. Alemtuzumab may be administered on the same days as etoposide or on other days during the induction. If administered on the same day, alemtuzumab should be administered approximately 1-2 hours after the etoposide infusion.

  A suggested alemtuzumab-dosing schema is as follows:
  Day 1 0.1 mg/kg
  Day 2 0.3 mg/kg
  Day 3 0.3 mg/kg
  Day 4 0.3 mg/kg

  Patients may receive alemtuzumab more or less frequently, at a lower dose or on a different dosing schedule during the induction phase at the discretion of the treating physician after discussion with the PI.

- All chemotherapeutic and biologic agents will be dosed, prepared, and administered according to MDACC institutional guidelines. BSA will be recalculated prior to each subsequent course.

- Alemtuzumab will be administered per local institutional guidelines, with dexamethasone (above) serving as the corticosteroid premedication, along with acetaminophen, and antihistamines, as deemed appropriate. If a patient is unable to tolerate alemtuzumab due to severe injection/infusion reactions, then site investigators must discuss alternative treatment (such as tocilizumab or ATG) with the principal investigator.

- Tocilizumab will be administered as per institutional guidelines, with premeds as deemed appropriate. If a patient is unable to tolerate tocilizumab due to severe injection/infusion reactions or concern for worsening CNS toxicity from the tocilizumab, then site investigators must discuss alternative treatment (such as alemtuzumab or ATG) with the principal investigator.

Maintenance (Week 9-24 or commencing upon completion of Induction Phase)

- Arm 1: The induction will be followed by a maintenance phase of for up to an additional 16 weeks. During the maintenance phase alemtuzumab will be administered at a dose of 0.2 mg/kg once every 4 weeks along with dexamethasone 1.25 mg/m2 three times a week by mouth. Patients who have evidence of refractory disease or budding relapse during the maintenance phase may revert back to receiving etoposide. Patients may receive alemtuzumab and/or dexamethasone more or less frequently, and dosages may also be adjusted, during the maintenance phase at the discretion of the treating physician after discussion with the PI. The maintenance duration may be reduced at the discretion of the treating physician in discussion with the PI and the reason for early discontinuation should be clearly documented. Continuation of therapy after 24 weeks of therapy may be considered on a case-by-case basis. Other agents such as ruxolitinib (or other drugs with proven benefit in pediatric or adult HLH) may be added during the maintenance phase of the protocol.

- Arm 2: Patients treated with tocilizumab in combination with etoposide and dexamethasone will not be required to receive maintenance with tocilizumab. These patients may be considered for alternate maintenance approaches such as maintenance with ruxolitinib or other anti-HLH medications.

- In general, every attempt should be made to diagnose and treat infections, which may be underlying or triggering HLH (regardless of whether one considers the patients to have ‘primary’ or ‘secondary’ HLH). If the patient is stable, consideration should be given to treating the infection before initiating therapy for HLH. Furthermore, if the patient is stable, initial treatment for HLH with corticosteroids alone may be considered. In such circumstances, the experience and judgment of the treating physician and discussion with the principal investigator is critical for making such assessments.

5.3 Dose Adjustments

Drug doses may be modified for drug-related > grade 2 nonhematologic toxicities. Dose interruptions/reductions of alemtuzumab and etoposide can also be made in other clinical situations where this step is considered to be in the best interest for the patient and after discussion with the principal investigator and clear documentation of the rationale for dose interruption/recommendation.

The following table is a suggestion for dose modifications of etoposide in subsequent treatment courses:

Table 3: Suggested dose modifications for non-hematologic toxicity

Dose level	Etoposide (mg/m2)
0	150 mg/m2
-1	100 mg/m2
-2	50 mg/m2
-3	50 mg/m2

Investigators should, whenever possible, determine which medication is causing the toxicity. Doses of each individual drug can be modified if toxicity is considered due a particular drug. The dexamethasone dose may be interrupted or reduced at the discretion of the treating physician.

Dose modifications of etoposide, alemtuzumab, and tocilizumab for organ dysfunction will generally be according to institutional standards. These must be discussed with the study PI and documented in the medical records.

Modifications of dose schedules other than the above will be allowed within the following guidelines:

- Further dose reductions can be made to keep clinically significant toxicities grade $\leq$ 2.

- Dose adjustments by more than 1 dose level (e.g. decrease in dose from dose level 0 to dose level -2 in table 3) at a time can be considered when judged in the best interest of the patient (e.g. sepsis, hemorrhage) when toxicity has resolved. The reason for this reduction will be discussed with the PI or Co-PI and documented in the medical record.

- A patient who has had a dose reduction because of any of the reasons mentioned above may have their dose escalated provided the patient has resolution (partial or complete) of the toxicity requiring dose adjustments as defined above for at least 2 weeks. Escalation will be made by 1 dose-level increment only, and not more frequent than every 2 weeks.

- Treatment interruptions and dose modifications other than the ones mentioned above can be considered after discussion with the PI and proper documentation of the rationale. Dose adjustment/delay of only one of the agents is permissible if the toxicity is most likely judged to be related to one of the agents by the investigator.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

1. Clinically significant progressive disease, or

2. Intercurrent illness that prevents further administration of treatment, or

3. Patient request, or

4. General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator, or

5. Unacceptable toxicity that in the opinion of the investigator makes it unsafe to continue therapy.

5.4.1 It is planned that up to a total of 24 weeks of therapy will be administered for patients deriving benefit from this regimen (Induction up to 8 weeks and maintenance up to 16 weeks). Continuation of therapy for patients completing 24 weeks of therapy may be considered on a case-by-case basis after discussion with the principal investigator.

5.4.2 A minimum of 2 weeks of therapy will be required for a patient to be considered as having received an adequate trial to evaluate efficacy. All patients receiving at least one dose of any of the two drugs will be considered evaluable for toxicity.

5.5 Supportive Care:

Supportive care measures including blood products, infection prophylaxis and growth factors will be administered according to institutional and Leukemia Department guidelines. As a number of patients with HLH have an underlying malignancy the concomitant use of chemotherapy (including but not limited to cytotoxic chemotherapy, biologic therapy, immunotherapy, molecularly targeted therapy, etc.) and the concurrent use of radiotherapy for the underlying tumor is allowed on the protocol. Furthermore, the addition of therapy with ruxolitinib as a maintenance or the addition of other maintenance therapies with a proven benefit in patients with adult or pediatric HLH will be permitted as long as the data is collected on the patient chart.

Infections secondary to myelosuppression are common in patients with HLH, and may be related to underlying disease, chemotherapy, or both. Therefore, the use of prophylactic

antibiotics, antifungal agents, and antiviral agents including anti-PCP (trimethoprim-sulfamethoxazole or pentamidine) is recommended according to institutional standards. Viral surveillance/ prophylaxis includes weekly blood PCR's for EBV, CMV, and other viruses the patient is known or suspected to harbor. Prompt treatment of EBV viremia with rituximab (375mg/m2, IV) weekly to help prevent EBV associated lymphoproliferative disorder is strongly suggested. CMV viremia should be treated with gancyclovir or foscarnet and cytogam, as appropriate. It is suggested that patients with serologic evidence or a history of varicella, CMV, or HSV should receive prophylaxis with acyclovir.

Immunoglobulin (IVIG) is suggested to be given to maintain serum IgG levels >1000 mg/dl or in the high end of the normal range. IVIG may need to be given relatively frequently in patients with HLH due to apparent consumption. IgG levels may need to be checked weekly or biweekly in such patients.

Concurrent therapy for CNS prophylaxis or continuation of therapy for controlled CNS disease is permitted.

6.0 PRETREATMENT AND POSTTREATMENT EVALUATION

Every effort will be made to adhere to the schedule of events and all protocol requirements. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. Such variations may include laboratory assessments completed outside of schedule, occasional missed required research samples such correlative assays.

6.1 Pretreatment evaluations

- A complete history and physical, documentation of all measurable disease, concomitant medications and performance status.

- CBC, platelet count, differential (differential can be omitted if WBC is ≤0.5 x10^9/L) within 3 days of initiation of therapy.

- Ferritin, soluble IL2R-alpha (sCD25), , NK function, , IgG/IgA/IgM, LDH, lymphocyte subsets, lymphocyte mitogens, perforin/granzyme protein levels, plasma cytokines (specifically IL-6, IL-10, , TNF-alpha), mobilization, uric acid, , , fibrinogen, D-dimer, PT, PTT, creatinine, total bilirubin, direct and indirect bilirubin, albumin, serum sodium level, GGT, ALT or AST, should be collected within 3 days of initiation of therapy. These studies are not mandatory but may facilitate the diagnosis of HLH and

should be ordered at the discretion of the treating physician.

- It is recommended that CSF studies (including but not limited to glucose, protein, LDH, cell count and differential, viral/bacterial/fungal cultures, EBV PCR, CMV PCR, HSV PCR, flow-cytometry) and MRI brain be performed within 7 days of initiation of therapy. MRI alone may be performed initially in coagulopathic patients. Lumbar puncture and IT therapy may be delayed if the treating physician deems that performing this procedure is excessively risky due to coagulopathy or thrombocytopenia. These studies are not mandatory but may facilitate diagnosis of HLH and should be ordered at the discretion of the treating physician.

- It is recommended that viral PCRs including EBV, CMV, HHV6, adenovirus and other relevant viruses; IgG and IgM for CMV and EBV; IgG for VZV and HSV; if Leishmania is a potential concern, then serum IgG and IgM titers and marrow PCR should be obtained blood and bone marrow cultures; bartonella, brucella, leishmania titers and PCR be collected within 7 days of initiation of therapy. It is required that Infectious disease be consulted at diagnosis to facilitate work-up. These studies are not mandatory but may facilitate diagnosis of HLH and should be ordered at the discretion of the treating physician.

- It is recommended that HLA typing be collected and Stem Cell transplant be consulted at the time of HLH diagnosis to avoid delays in identifying donors for HSCT. These studies are not mandatory but may facilitate diagnosis of HLH and should be ordered at the discretion of the treating physician.

- It is recommended that a CT abdomen or abdominal ultrasound be performed within 7 days of initiation of therapy. These studies are not mandatory but may facilitate diagnosis by documenting splenomegaly and/or hepatomegaly and should be ordered at the discretion of the treating physician.

- It is recommended that an EKG and ECHO/MUGA scan be obtained within 7 days of initiation of therapy to establish baseline organ function and screen for coronary artery vasculopathy. These studies are not mandatory but may facilitate diagnosis and should be ordered at the discretion of the treating physician.

- Pregnancy test (urine or plasma) in females of childbearing potential should be performed within 7 days of initiation of therapy.

- Bone marrow aspirate during the last 28 days preceding study initiation. Cytogenetics will be obtained prior to therapy (results from prior analysis can be used for this purpose). For patients who already have an evidence of hemophagocytosis on tissue biopsy from another site, the bone marrow may be omitted after discussion and approval with the principal investigator.

6.2 Evaluations during study

- Physical exam at least once weekly for the first 4 weeks, at least once every 2 weeks for the next 4 weeks, at least once every 4 weeks for the next 16 weeks, and at least once every 8 weeks thereafter.

- CBC, platelet count, differential, ferritin, d-dimer, PT, PTT, LDH, fibrinogen, serum sodium, AST, ALT, total bilirubin, creatinine, uric acid at least twice weekly for the first 4 weeks, at least once every 2 weeks for the next 4 weeks, at least once every 4 weeks for the next 16 weeks, at least once every 8 weeks thereafter (differential can be omitted if WBC is $\leq 0.5 \times 10^9$/L).

- It is recommended that soluble IL2R-alpha, , IgG/IgA/IgM, lymphocyte subsets be obtained twice a week for the first 4 weeks, once every 2 weeks for the next 8 weeks, once every 4 weeks for the next 12 weeks. The IL2R-alpha evaluations may be omitted once these values are within normal range. These studies are not mandatory but may facilitate assessment of response and should be ordered at the discretion of the treating physician.

- Bone marrow aspiration in 4 weeks (+/- 7 days), then every 4-12 weeks. Bone marrow tests can be ordered more or less frequently if mandated by development of peripheral blood counts. No repeat bone marrow is necessary if nonresponse or progressive disease can be unequivocally diagnosed from peripheral blood tests or, in patients with a WBC < 0.3 if the bone marrow test is considered noncontributory by the investigator at any time point.

- For patients that remain on study with no significant toxicity for more than 24 weeks, subsequent evaluations during study may be modified after discussion with the principal investigator. These include a decrease in frequency of bone marrow aspirations to every 6-12 months (or as clinically indicated), physical examination to every 12-16 weeks, and other laboratory tests to once every 8-16 weeks.

- Patients with an objective response at completion of active study treatment will be followed for survival at MD Anderson Cancer Center (MDACC) every 3 to 6 months for up to 5 years after completion of active treatment. If the patient is unable to return to MDACC the follow-up visits may be conducted via telephone.

- The first 4 weeks treatment must be performed at MDACC (inpatient or outpatient). During the first 4 weeks of treatment all the laboratory evaluations will be done at MDACC. Subsequently, the patient may have the laboratory work done at a local clinic and the results reported to the research nurse for the study. The laboratory work done at the local clinic will be forwarded to the patient's attending physician at MDACC or PI of the study, who will sign off on the labs to verify that the results have been reviewed.

- **Outside Physician Participation During Treatment**

  1. MDACC Physician communication with the outside physician is required prior to the patient returning to the local physician. This will be documented in the patient record.

  2. A letter to the local physician outlining the patient's participation in a clinical trial will request local physician agreement to supervise the patient's care (Appendix G).

  3. Protocol required evaluations outside MDACC will be documented by telephone, fax or e-mail. Fax and/or e-mail will be dated and signed by the MDACC physician, indicating that they have reviewed it. Changes in drug dose and/or schedule must be discussed with and approved by the MDACC physician investigator, or their representative prior to initiation, and will be documented in the patient record.

  4. A copy of the informed consent, protocol abstract, treatment schema and evaluation during treatment will be provided to the local physician.

  5. Documentation to be provided by the local physician will include progress notes, reports of protocol required laboratory and diagnostic studies and documentation of any hospitalizations.

  6. The home physician will be requested to report to the MDACC physician investigator all life threatening events within 24 hours of documented occurrence.

7. All follow-up visits will be performed at MDACC. Patients will return to MDACC for physical examination as specified on the protocol evaluations.

- End of Treatment Visit to be completed 30 days (+/-7 days) after the last dose of study drug, when possible. Blood (about 2-3 teaspoons) will be drawn for CBC, platelet count, differential, creatinine, total bilirubin, ALT. No other procedures or labs will be needed.

- Patients with an objective response at completion of active study treatment will be followed for survival at MD Anderson Cancer Center (MDACC) every 3 to 6 months for up to 5 years after completion of active treatment and while still on study. If the patient is unable to return to MDACC the follow-up visits may be conducted via telephone.

Treatment may be discontinued for a variety of reasons, including patient withdrawal, investigator decision, and reasons specified by the protocol. Reasons for discontinuation of treatments are described below.

7.0 DISCONTINUATION OF TREATMENT:

7.1 Discontinuation Criteria for Individual Patients

7.1.1 Patient Withdrawal

Patients may voluntarily withdraw consent to participate in the clinical study at any time and without giving any reason. Their withdrawal will not jeopardize their relationship with their healthcare providers or affect their future care. Patients may also choose to withdraw from study treatment, but agree to remain in the study for follow-up procedures.

7.1.2 Investigator Discontinuation of Patient

The investigator may exercise medical judgment to discontinue study treatment if clinically significant changes in clinical status or laboratory values are noted.

7.1.3 Criteria for Protocol-Defined Required Discontinuation of Treatment

The protocol requires discontinuation of study treatment for the following reasons:

1. Patient requests discontinuation.
2. Unacceptable toxicity that in the opinion of the investigator makes it unsafe to continue therapy.
3. Clinically significant progressive disease.
4. Investigator discretion.
5. Patient death.

7.2 Follow-Up at Treatment Discontinuation or Early Withdrawal

Patients who discontinue treatment for any reason before 24 weeks should complete end-of-treatment procedures within 30 days (+/- 7 days) of the last dose of the study drug, when possible. End of treatment procedures will include a physical examination, CBC with differential and platelets, IgG/IgA/IgM, lymphocyte subsets and a limited chemistry profile (total bilirubin, serum creatinine, SGPT or SGOT). A bone marrow aspiration may be recommended only if non-response or progressive disease cannot be unequivocally diagnosed from peripheral blood. Although treatment will be discontinued at that time, all patients who do not withdraw consent for follow-up, die, or become lost to follow-up, will remain on study for follow-up evaluations as follows: subject will be followed for toxicity for at least 30 days after the last protocol treatment. The 30-day follow-up visit will be scheduled as a clinic visits for clinical evaluation and physical examinations, when possible. If the patient cannot make it to the MDACC clinic for this visit, the required follow up treatment procedures may be done with a local physician and the records forwarded to MDACC. The research nurse will contact the patient by telephone and get a verbal assessment of the patient's condition. The phone conversation will then be documented in the patient's charts.

7.3 Study Stopping Rules

The principal investigator has the right to terminate this clinical study at any time. The principal investigator and MDACC IRB office will be involved in any decisions regarding terminating the study, temporarily suspending enrollment, or stopping ongoing treatment with study treatment.
Reasons for terminating the clinical study include, but are not limited to, the following:

- The incidence or severity of an adverse reaction related to treatment in this study or other studies indicates a potential health hazard to patients
- Study site personnel are noncompliant with study procedures
- Pattern of noncompliance is observed

7.4 Protocol Violations and Deviations

Protocol violations are defined as significant departures from protocol-required processes or procedures that affect patient safety or benefit potential, or confound assessments of safety or clinical activity. A protocol deviation is a departure from the protocol that does not meet the above criteria. Protocol violations or deviations may be grouped into the following classes:

- Enrollment criteria
- Study activities (missed evaluations or visits) except for those allowed per protocol

- Noncompliance with dose or schedule, including dose calculation, administration, interruption, reduction, or delay; or discontinuation criteria
- Investigational product handling, including storage and accountability
- Informed consent and ethical issues

8 ADVERSE EVENT REPORTING

Please see appendix H for "Leukemia-specific Adverse Event Recording and Reporting Guidelines"

9.0 ALEMTUZUMAB ADMINISTRATON GUIDELINES

The following guidelines should be considered during the administration of alemtuzumab:

- Premedicate with diphenhydramine (50 mg) and acetaminophen (500 mg to 1000 mg) 30 minutes prior to first infusion and each dose escalation. Institute appropriate medical management (e.g., steroids, epinephrine, meperidine) for infusion reactions as needed.

- Administer trimethoprim/sulfamethoxazole DS twice daily (BID) 3 times per week (or equivalent) as *Pneumocystis jiroveci* pneumonia (PCP) prophylaxis.

- Administer famciclovir 250 mg BID or equivalent as herpetic prophylaxis.

- Continue PCP and herpes viral prophylaxis for a minimum of 2 months after completion of alemtuzumab or until the CD4+ count is ≥200 cells/μL, whichever occurs later.

- Administer as an IV infusion over approximately 2 hours

- Do not administer as IV push or bolus

- Gradually escalate to the maximum recommended single dose of 30 mg. Escalation is required at initiation of dosing or if dosing is held ≥7 days during treatment.

- In clinical trials, the frequency of infusion reactions with alemtuzumab was highest in the first week of treatment. The following serious, including fatal, infusion reactions have been identified in post-marketing reports: syncope, pulmonary infiltrates, acute respiratory distress syndrome (ARDS), respiratory arrest, cardiac arrhythmias, myocardial infarction, acute cardiac insufficiency, cardiac arrest, angioedema, and anaphylactoid shock. Carefully monitor patients during infusions and withhold alemtuzumab for Grade 3 or 4 infusion reactions.

- Prolonged myelosuppression have been reported in patients receiving alemtuzumab. Alemtuzumab treatment results in severe and prolonged lymphopenia with a concomitant increased incidence of opportunistic infections. Assess CD4+ counts after treatment until recovery to ≥ 200 cells/μL. Consider obtaining complete blood counts (CBC) at weekly intervals during alemtuzumab therapy and more frequently if worsening anemia, neutropenia, or thrombocytopenia occurs. Consider withholding alemtuzumab for severe or life-threatening cytopenias (except lymphopenia). Consider discontinuing alemtuzumab for autoimmune cytopenias or recurrent/persistent severe cytopenias (except lymphopenia).

- Administer only irradiated blood products to avoid transfusion associated Graft versus Host Disease (TAGVHD), unless emergent circumstances dictate immediate transfusion.

- Routinely monitor patients for CMV infection during alemtuzumab treatment and for at least 2 months following completion of treatment. Consider withholding alemtuzumab for serious infections and during antiviral treatment for CMV infection or confirmed CMV viremia. Initiate therapeutic ganciclovir (or equivalent) for CMV infection or confirmed CMV viremia [17].

- It is recommended to not administer live viral vaccines to patients who have recently received alemtuzumab

10.0 RESPONSE EVALUATION

There are no standardized response criteria for HLH. Marsh et al proposed a response evaluation schema for patients with relapsed/refractory HLH treated with alemtuzumab. We will use this response schema for evaluation of response to alemtuzumab in combination with etoposide and dexamethasone. As outlined in section 6.2 (Evaluations during treatment) patients quantifiable symptoms and laboratory markers of HLH will be evaluated and recorded. Information to be recorded includes (but is not limited to) levels of soluble IL2R-alpha, ferritin, LDH, fibrinogen, and triglyceride; hemoglobin, neutrophil counts, platelet counts, AST, ALT, level of consciousness (documented in clinical exams in patients with CNS HLH), and presence of hemophagocytosis in pathology specimens.

Complete response (CR) is defined as normalization to within institutional normal limits of diagnostic clinical and laboratory abnormalities associated with HLH, including but not limited to: fever, cytopenias, splenomegaly, hepatomegaly or lymphadenopathy, decreased fibrinogen, increased triglyceride, increased VLDL, increased sCD25, (optionally),

elevated bilirubin, elevated liver enzymes, hyponatremia, hypoalbuminemia, elevated CNS proteins or mononuclear cells/lymphocytes and CNS symptoms. In the case of ferritin, normalization OR a 3 fold improvement in the pre-study level will be evidence of complete response, provided the post therapy level is also below 10000 ng/ml. It is also recognized that hemoglobin and platelet counts may be artificially increased with transfusion or may remain low in patients with underlying malignancy/recent myelotoxic chemotherapy and may not be immediately assessable.

Partial response (PR) is defined as sustained normalization of 3 or more of the diagnostic clinical and laboratory abnormalities noted above (or, with ferritin, improvement as described above) and no apparent progression of other aspects of disease pathology.

Parameter improvements (PI) is defined as at least a 25% improvement in two or more quantifiable symptoms and/or laboratory markers from those mentioned above within 8 weeks (+/- 7 days) of initiation of therapy with the following caveats: soluble IL-2 receptor response was defined as a greater than 1.5-fold decrease. For patients with initial platelet count <50,000 a response was defined as an increase in platelets of at least 100% AND a platelet count >50K in the absence of a transfusion within 7 days. For patients with initial platelet count >50,000 a response was defined as an increase in platelets of at least 100%. Responses based on hemoglobin were not used to determine response as these are often confounded by transfusions. For patients with an initial ANC of less than 500, a response was defined as an increase of ANC by at least 100% to greater than 500 cells/μl. For patients with an ANC 500–2,000 cells/μl, an increase by at least 100% to more than 5,000 cells/μl was considered a response. For patients with transaminitis with an ALT greater than 400, an ALT response was defined as a decrease of ALT of at least 50%. For patients with hemophagocytosis noted on a biopsy specimen within 28 days of therapy initiation, a response was defined as resolution of hemophagocytosis following alemtuzumab. For patients with refractory CNS HLH and altered level of consciousness, a response was defined as a normal level of consciousness following therapy. For patients who died or started preparative regimens for HSCT prior to completing 8 weeks on therapy, the last known values were used for evaluation of response.

Reactivation is defined as worsening of two or more HLH-defining clinical/laboratory parameters with the following specifications. Numerical laboratory values must become abnormal and worsen by >30%, on two sequential assessments abnormal and worse by >30% on 2 assessments performed on different days). Worsening in clinical criteria (fever, splenomegaly, etc.) may also be considered, provided that consistent observations of worsening are made over at least three days. Additionally, recurrent CNS symptoms felt to be due to HLH may count as a criteria for

reactivation. Patients (who previously responded) may also be considered to have reactivated if they die prior to a second sequential assessment of rising disease markers, and the proximal cause of death was deemed by the treating physician to be active HLH (on clinical or pathological grounds). Assessment of NK function will not be considered for determination of relapse because disease activity and NK function do not correlate in most patients and may be inversely correlated in some patients

Diagnostic criteria not utilized in assessment of response included fibrinogen, as this was not checked consistently in patients; fever, because information was often lacking or difficult to interpret; and liver and spleen size, as examinations and imaging results were not consistently documented. Cerebrospinal fluid (CSF) testing results were not analyzed in patients with CNS HLH because sampling was inconsistent.

11.0 STATISTICAL CONSIDERATIONS

This will be a single arm, single center, open label study of alemtuzumab or tocilizumab in combination with etoposide and dexamethasone in patients with HLH.

11.1 Sample Size

Up to a total of 40 patients will be recruited for this Phase II study to receive either alemtuzumab in combination with etoposide+dexamethasone or tocilizumab in combination with etoposide+dexamethasone. Patients will be allocated to receive the tocilizumab or the alemtuzumab arm based on the clinical presentation of the HLH, comorbid conditions, underlying organ function, and PI/treating physician decision. The patients may not be equally allotted to Arm 1 and 2. The goal of the study is not to compare between Arms 1 and 2 but to evaluate whether the addition of a biologic agent (alemtuzumab or tocilizumab) improves the response rate and outcomes in adult patients with HLH as compared to etoposide+steroid alone.

The primary objective of the phase II is to determine the efficacy of alemtuzumab/tocilizumab in combination with etoposide and dexamethasone in patients with HLH. The efficacy of the combination will be measured by the overall response rate (ORR), defined as CR and/or PR and/or PI within 8 weeks (+/- 7 days) of treatment initiation among patients with HLH. ORR and toxicity will be monitored simultaneously using the Bayesian approach of Thall, Simon, Estey (1995, 1996) and the extension by Thall and Sung (1998).

11.2 Statistical Design

There is no standard therapy or historical data regarding response rates in adult patients with HLH. The target ORR with the experimental treatment in HLH is 20%. This regimen of the combination treatment will be considered worthy of further investigation if it elicits an increase in ORR to 30% with acceptable toxicity. A >30% drug-related grade 3/4 toxicity rate is considered unacceptable. Thus, interim monitoring rules, assuming the prior distributions below, were constructed that meet the following two conditions,

1) Stop if Prob{ p(ORR,E)<p(ORR,H) + 0.10 | data> 0.975, or

2) Stop if Prob{ p(TOX,E)>0.30| data}>0.975,

where P(ORR, E) and P(TOX, E) are the true ORR and toxicity rates for the combination treatment, and p(ORR, H) is the true ORR rate of the standard treatment. The first rule provides for stopping the study if the data suggest that it is unlikely (i.e., probability < 2.5%) that ORR rate of the combination treatment is greater than the ORR rate of standard treatment by 10.0%. The second condition will stop the study early if excessive therapy-related grade 3/4 toxicity (>30%) is highly probable (i.e., probability >97.5%) for the combination treatment. Monitoring for toxicity and futility will not begin until 5 patients have been evaluated, and cohort size for future evaluations is 5.

The monitoring rule for the toxicity rate, based on these assumptions and monitoring conditions above is found in Table 5. For example, accrual will cease if 4 or more patients experience toxicities among the first 5 patients.

Table 5. Stop accrual if the number of drug-related clinically significant non-hematological grade 3/4 toxicities (CTC version 4.3, appendix C) is greater than or equal to indicated (i.e., # patients with toxicities) among the number of patients evaluated

# patients evaluated	5	10	15	20	25	30	35	40
# patients with toxicities	4-5	7-10	9-15	11-20	13-25	15-30	17-35	Always stop with this many patients

Monitoring the ORR rate, based on the above assumptions and monitoring conditions is found in Table 6. For example, accrual will cease if less than 1 patients experience an overall response within 8 weeks (+/-7 days) of initiation of therapy in the first 20 patients treated.

Table 6. Stop accrual if the number with overall response is less than or equal to indicated (i.e., # patients with overall response) among the number of patients evaluated

# patients evaluated	5	10-15	20-25	30	35	40
# patients with overall response	Never stop with	0	0-1	0-2	0-3	Always stop with

	this many patients					this many patients

Multc Lean Desktop (version 2.1.0) was used to generate the toxicity and futility stopping boundaries and the OC table (Table 7). In order to utilize the software for the design, a constant rate of 10% and beta (0.2, 1.8) priors and delta of 10% were assumed for the standard treatment response distribution and experimental treatment response prior distribution, respectively. In addition, a 30% toxicity constant rate and beta (0.6, 1.4) priors were assumed for the standard treatment toxicity constant rate and experimental treatment toxicity prior distribution, respectively.

The probability of stopping the study early if the true ORR of the combination treatment was 20% and the true toxicity rate was 30% was 21.7%. Probabilities of stopping early for high true toxicity rates (i.e., 50%) were 76.6% when the true ORR was 20% and 73.0% when true ORR rate was 30%.

Table 7. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.10	0.10	0.6446
	0.15	0.3460
	0.20	0.1635
	0.25	0.0741
	0.30	0.0335
0.20	0.10	0.6473
	0.15	0.3510
	0.20	0.1698
	0.25	0.0811
	0.30	0.0408
0.30	0.10	0.6672
	0.15	0.3875
	0.20	**0.2166**
	0.25	0.1329
	0.30	0.0948
0.40	0.10	0.7542
	0.15	0.5476

Table 7. Operating characteristics for simultaneous monitoring response and toxicity rates for patients treated with combination treatment

True Toxicity Rate	True ORR	Prob(stop the trial early)
0.50	0.20	0.4213
	0.25	0.3595
	0.30	0.3314
	0.10	0.9008
	0.15	0.8174
	0.20	0.7664
	0.25	0.7414
	0.30	0.7301

Statistical Analysis Plan

All patients who received any dose of the study agent will be included in the analysis for efficacy and safety. Demographic/clinical characteristics (including duration of response) and safety data of the patients will be summarized using descriptive statistics such as mean, standard deviation, median and range. For the primary efficacy analysis, we will estimate the ORR for the combination treatment, along with the 95% credible interval. Patients who drop out of the study before completing the cycles within 8 weeks +/- 7 days will be treated as “failures” for the primary analysis if they do not have a response evaluation. The association between ORR and patient’s clinical characteristics will be examined by Wilcoxon’s rank sum test or Fisher’s exact test, as appropriate. Toxicity type, severity and attribution will be summarized for each patient using frequency tables.

The distribution of time-to-event endpoints (DFS and OS) including overall survival and progression free survival will be estimated using the method of Kaplan and Meier. Comparisons of time-to-event endpoints by important subgroups will be made using the log-rank tests. Paired t-tests will be used to determine the immunological and molecular changes in the peripheral blood and bone marrow from baseline to the time of response, and to the time of disease progression.

Statistical analysis of biomarker data: Descriptive statistics including plots, mean, median and standard deviations will be used to summarize data. For continuous outcomes, t-test and ANOVA will be used to compare outcome measures across patient characteristics. Dunnett’s and Tukey’s test that properly adjust for multiplicity in multiple tests will be implemented. Pair-wise comparisons will be performed using pre- and post-therapy samples from each patient. The chi-square test or Fisher’s exact test will be used to test the association between two categorical variables such as disease state and

performance status. Both univariate and multivariate logistic regressions will be performed to model prognostic factors.

12.0 PROTOCOL ADMINISTRATION

This study will be monitored by the MD Anderson Leukemia Department and a protocol-specific monitoring plan will be followed.

Protocol amendments

Changes to the protocol will be made only when protocol amendments have been signed by the principal investigator and approved by the sponsor and the IRB of the study center.

Archival of data

All patient data (including source data) generated in connection with this study will be kept in the archives of the MDACC for at least 15 years after the study has been completed. All data will be available for inspection by company representatives of the Medical Department

13.0 REFERENCES

1. Jordan MB, Allen CE, Weitzman S, et al. How I treat hemophagocytic lymphohistiocytosis. Blood 2011;118:4041-4052.
2. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatric blood & cancer 2007;48:124-131.
3. Henter JI, Arico M, Elinder G, et al. Familial hemophagocytic lymphohistiocytosis. Primary hemophagocytic lymphohistiocytosis. Hematology/oncology clinics of North America 1998;12:417-433.
4. Janka G, Imashuku S, Elinder G, et al. Infection- and malignancy-associated hemophagocytic syndromes. Secondary hemophagocytic lymphohistiocytosis. Hematology/oncology clinics of North America 1998;12:435-444.
5. Janka GE. Familial hemophagocytic lymphohistiocytosis. European journal of pediatrics 1983;140:221-230.
6. Henter JI, Ehrnst A, Andersson J, et al. Familial hemophagocytic lymphohistiocytosis and viral infections. Acta paediatrica 1993;82:369-372.
7. Fisman DN. Hemophagocytic syndromes and infection. Emerging infectious diseases 2000;6:601-608.
8. Henter JI, Arico M, Egeler RM, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH study Group of the Histiocyte Society. Medical and pediatric oncology 1997;28:342-347.
9. Jordan MB, Hildeman D, Kappler J, et al. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder. Blood 2004;104:735-743.

10. Ohga S, Matsuzaki A, Nishizaki M, et al. Inflammatory cytokines in virus-associated hemophagocytic syndrome. Interferon-gamma as a sensitive indicator of disease activity. The American journal of pediatric hematology/oncology 1993;15:291-298.
11. Akashi K, Hayashi S, Gondo H, et al. Involvement of interferon-gamma and macrophage colony-stimulating factor in pathogenesis of haemophagocytic lymphohistiocytosis in adults. British journal of haematology 1994;87:243-250.
12. Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in hemophagocytic syndrome. The American journal of pediatric hematology/oncology 1993;15:92-98.
13. Henter JI, Elinder G, Ost A. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. The FHL Study Group of the Histiocyte Society. Seminars in oncology 1991;18:29-33.
14. Henter JI, Samuelsson-Horne A, Arico M, et al. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. Blood 2002;100:2367-2373.
15. Marsh RA, Allen CE, McClain KL, et al. Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. Pediatric blood & cancer 2013;60:101-109.
16. Hillmen P, Skotnicki AB, Robak T, et al. Alemtuzumab compared with chlorambucil as first-line therapy for chronic lymphocytic leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25:5616-5623.
17. O'Brien SM, Keating MJ, Mocarski ES. Updated guidelines on the management of cytomegalovirus reactivation in patients with chronic lymphocytic leukemia treated with alemtuzumab. Clinical lymphoma & myeloma 2006;7:125-130.