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Randomized Trial of Cladribine (CdA) with Simultaneous or Delayed Rituximab to Eliminate Hairy Cell Leukemia Minimal Residual Disease

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Commercial Agents: Rituximab (Rituxan®) and cladribine (Leustatin®)

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PRÉCIS

Background:

- Hairy cell leukemia (HCL) is highly responsive to but not curable by cladribine (CdA). HCL responds to rituximab, which is not yet standard therapy for HCL.
- Patients with the CD25-negative variant (HCLv) respond poorly to initial cladribine but do respond to rituximab in anecdotal reports.
- Purine analogs cladribine and pentostatin have similar efficacy for HCL, both inhibiting DNA synthesis selectively in HCL cells. Cladribine is effective after just 1 cycle. Rituximab is an anti-CD20 monoclonal antibody which induces apoptosis and either complement or antibody dependent cytotoxicity (ADCC or CDC).
- Patients in complete remission (CR) to cladribine have minimal residual disease (MRD) by immunohistochemistry of the bone marrow biopsy (BMBx IHC), a risk for early relapse. Tests for HCL MRD in blood or marrow include flow cytometry (FACS) or PCR using consensus primers. The most sensitive HCL MRD test is real-time quantitative PCR using sequence-specific primers (RQ-PCR).
- In studies with limited follow-up, MRD detected by tests other than RQ-PCR can be eliminated by rituximab after cladribine in > 90% of patients, but MRD rates after purine analog alone are unknown. Simultaneous cladribine and rituximab might be superior or inferior to delaying rituximab until detection of MRD.
- Only 4 HCL-specific trials are listed on Cancer.gov: a phase II trial of cladribine followed 4 weeks later by 8 weekly doses of rituximab, and phase I-II trials of recombinant immunotoxins targeting CD22 (BL22, HA22) and CD25 (LMB-2).

Objective:

- To determine if HCL MRD differs at 6 months after cladribine with or without rituximab administered concurrently with cladribine.

Eligibility:

- HCL with 0-1 prior courses of cladribine or pentostatin and treatment indicated.

Design:

- Cladribine 0.15 mg/Kg/day x5 doses each by 2hr i.v. infusion (days 1-5)
- Rituximab 375 mg/m^2/week x8 weeks, randomize half to begin day 1, then repeat for all patients with blood-MRD relapse at least 6 months after cladribine. Also, may repeat for those with blood-MRD relapse at least 6 months after delayed rituximab.
- MRD tests used for the primary objective will be limited to BMBx IHC, blood FACS, and bone marrow aspirate FACS, all CLIA certified. Blood MRD relapse is defined as FACS positivity or low blood counts (ANC < 1500/ul, Plt < 100,000/ul, or Hgb < 11) attributed to HCL. Patients FACS-negative in both blood and bone marrow aspirate are considered MRD-negative complete response (CR) regardless of blood counts. Randomization: 68 HCL patients with 0 and 62 with 1 prior course of purine analog
- Statistics: 80% power to discriminate rates of MRD of 5 vs. 25%, or 10 vs. 35%

- Non-randomized HCLv arm: 20 patients with HCLv will begin rituximab with cladribine.
- Non-randomized HCL arm: 25 newly diagnosed patients will be enrolled to receive rituximab beginning day 1, but beginning before the 1st dose of cladribine, rather than after.
- Accrual ceiling: 175 evaluable patients (155 HCL and 20 HCLv)

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine if hairy cell leukemia (HCL) minimal residual disease (MRD) differs at 6 months after cladribine with or without rituximab administered concurrently with cladribine.

1.1.2 Secondary Objectives

- To compare cladribine + rituximab vs. cladribine alone in terms of 1) initial MRD-free survival and disease-free survival, 2) response to delayed rituximab for relapse, to determine if early rituximab compromises later response, and 3) feasibility of administration as an outpatient by simultaneous rituximab beginning before the cladribine in nonrandomized HCL patients.
- To determine if MRD levels and tumor markers (soluble CD25 and CD22) after cladribine and/or rituximab correlate with response and clinical endpoints.
- To compare blood MRD-free survival in patients who receive cladribine and up to 2 courses of rituximab, with respect to whether the 1st course of rituximab was used simultaneous with cladribine.
- To determine, using MRD and tumor marker data, when bone marrow biopsy (BMBx) can be avoided in managing HCL.
- To compare response and MRD after the 1st and 2nd courses of cladribine.
- To evaluate the effects of cladribine and rituximab on normal T- and B-cells.
- To enhance the study of HCL biology by cloning, sequencing and characterizing monoclonal immunoglobulin rearrangements.

- To determine overall survival, particularly in patients with poor-prognosis HCL like HCLv.
- To correlate bone marrow MRI signal with bone marrow biopsy

1.2 BACKGROUND

1.2.1 Cladribine for HCL

HCL, an indolent B-cell leukemia comprising 2% of all leukemias [1, 2], or approximately 900 of the 44,000 new cases of leukemia/year in the US [3], is markedly responsive to cladribine. Deoxycytidine kinase phosphorylates 2-Chloro-2'-deoxyadenosine (CdA, also called Cladribine) to CdATP, which incorporates into DNA, leading to DNA strand breaks and inhibition of DNA synthesis [4]. While purines like adenosine are regulated by adenosine deaminase (ADA), cladribine is resistant to ADA [5], which allows cladribine increased potency due to low catabolism. CdATP also inhibits ribonucleotide reductase, leading to decreased concentrations of deoxyribonucleotides and further inhibition of DNA synthesis [6]. Cladribine was first reported in 1990 by Piro et al. to induce durable complete remission (CR) in 11 of 12 patients treated [7]. From the largest single institution HCL trial, held at the Scripps clinic, Saven et al. reported 91% complete remissions (CRs) and 6% partial responses (PRs) out of 349 evaluable patients, with only 24% CRs relapsing at a median of 30 months [8]. A total of 205 of these patients plus 5 others were followed for at least 7 years, and 95% of 207 evaluable patients achieved a CR lasting 8-172 (median 99) months and 34% of CRs relapsed [9]. The Group C phase II study of cladribine in HCL involved 979 patients treated by local physicians, and of 861 evaluable for response, there were 50% CRs and 37% PRs. Response rates were lower in this trial because 13% of the PRs failed to obtain BMBx to document CR, and some of the cases may have been misdiagnosed as HCL [10]. Nevertheless, the relapse free survival of 84% at 4 years was similar to the 79% reported from the Scripps study. Toxicity included neutropenic fever (42%), infections (13%) [8], nausea and vomiting (19%), lung injury (6%), and neurotoxicity (25%) [10]. Determination of CR in HCL after cladribine should be done more than 2 months after cladribine, based on data from serial bone marrow biopsies [11]. Cladribine has most commonly been administered by 7-day continuous infusion [8, 10], but other schedules, including subcutaneous injection for 7 days [12, 13], or 2-hour i.v. infusion for 5-7 days [14, 15] showed no difference in efficacy.

1.2.2 Rituximab for HCL

Rituximab is a genetically engineered, chimeric, murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant pre-B and mature B cells. The antibody is an IgG1 κ immunoglobulin containing murine light-and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids (based on cDNA analysis) and has an approximate molecular mass of 145 kD. Rituximab has a binding affinity for the CD20 antigen of ~8.0 nM. Rituximab kills cells by inducing apoptosis and mediating either complement or antibody dependent cytotoxicity (ADCC or CDC) [16]. HCL cells are strongly CD20 positive [17]. Case reports [18-22] documented efficacy of rituximab in HCL and in 4 small studies a total of 18 CRs out of 60 patients (30%) were reported [23-26]. The trial with highest response rate used 8 weekly doses of 375 mg/m2 with 7 (47%) CRs out of 15 patients and one additional patient had CR after 12 doses [26]. Preexisting cytopenias were present in 5 of the 8 CRs and in all 7 patients not achieving CR, and may have been a poor prognostic factor in the earlier trials.

Toxicities of rituximab in NHL were infusion-related (hypotension, bronchospasm, rhinitis, pruritis, rash, urticaria, and tumor-pain) and decreased with repeated dosing [27].

1.2.3 Other treatments for HCL

The first 2 systemic therapies for HCL with significant efficacy were interferon and the purine analog pentostatin (DCF). A randomized trial, which was the first and only large randomized trial ever performed in HCL, showed that DCF had higher efficacy [28]. CR rates and disease-free survival rates of DCF are as high as those of cladribine [29-34], but require up to 6 months of treatment cycles spaced 2 weeks apart. For this reason, cladribine is used much more commonly than DCF as first-line therapy for HCL, and a randomized trial comparing the 2 would not be practical. Other agents with significant systemic efficacy in HCL include the recombinant immunotoxin LMB-2 targeting CD25 [35, 36], and BL22, targeting CD22 [37, 38]. LMB-2 and BL22 have only been tested in patients with inadequate response to prior purine analogs. In a recent phase II trial, BL22 induced CR in 25% patients after a single cycle, and in 47% of 36 patients after 1-10 cycles.

1.2.4 Minimal residual disease in HCL

The criteria for CR in HCL generally require absence of HCL cells in the blood and bone marrow by Wright and H/E stains, and also normalization of neutrophils, platelets and hemoglobin to 1500/mm^3, 100,000/mm^3, and 11-12 g/dl, respectively [8, 10]. In patients in CR, MRD in the BMBx by immunohistochemistry (IHC) has been defined as a ratio of CD20+ or DBA-44+ cells to T cells of at least 1, most of the CD20+ or DBA-44+ cells having morphology consistent with HCL, and HCL cells undetectable by non-immunologic methods (i.e. H/E staining) [39, 40]. Patients with MRD had shorter relapse free survivals compared to those in CR without MRD after either cladribine or pentostatin [39]. Tallman et al. reported that 13 and 26% of patients treated with cladribine and pentostatin, respectively, had MRD by this definition. The estimated 4-year relapse-free survival was 55% in patients with MRD compared to 88% in patients without MRD. The sensitivity of bone marrow immunohistochemistry (BMBx IHC) has not been published for HCL, but is considered to be 1% [41], and based on the definition above, would depend on the number of CD3+ normal T-cells [39]. Bastie et al. reported an association between > 5% DBA-44+ cells in the bone marrow 5 months after cladribine and relapse [11]. Ellison et al. reported that MRD, defined as > 5 CD20+ or DBA-44+ cells in the BMBx IHC, was as high as 50% after cladribine [42]. Early reports suggested that IHC of BMBx was more sensitive than flow cytometry of marrow aspirate and blood [43], but more recent data [44-46] with highly sensitive 4-color flow cytometry suggest the opposite. Flow cytometry was reported to be more sensitive for detecting MRD than conventional PCR using consensus primers to immunoglobulin heavy chain (IgH) rearrangements, which are monoclonal and unique for each patient [46]. While consensus PCR can detect 1 HCL cell in 10^3-10^4 normal cells [47], flow cytometry can detect 1 in 10^4-10^5 [46]. To improve the sensitivity of PCR detection, the IgH rearrangements were cloned and a sequence-specific probe and primer were used for real-time quantitative PCR (RQ-PCR). This method, able to detect 1 HCL cell in 10^6 normal cells [48], is the most sensitive method of MRD detection in HCL. Data quantifying the risk of clinical relapse after detection of MRD are lacking using methods other than BMBx IHC.

1.2.5 Combination of purine analog with rituximab for HCL

Rituximab combined with either fludarabine [49], or DCF [50] has been reported for CLL. Rituximab combined with cladribine has been reported effective in treating indolent non-Hodgkin's lymphoma (NHL) [51], mantle cell lymphoma [52], and CLL [53]. In a retrospective review, 3 HCL patients received cladribine plus rituximab after prior therapy with purine analogs [54]. All 3 patients achieved CR without MRD. A phase II trial is currently underway at MD Anderson where patients receive cladribine by 2 hour i.v. infusion for days 1-5, followed by 8 weekly doses of rituximab beginning on day ~28. After the 1st dose of cladribine, patients in that trial are allowed to continue treatment with cladribine and rituximab with their local physicians. The objectives of the MD Anderson trial are to examine the efficacy of cladribine followed by rituximab including disease-free and overall survival (DFS and OS), and examination of MRD using PCR with consensus primers [41]. This is the only HCL-specific trial listed on Cancer.gov besides trials testing BL22, HA22 and LMB-2. Unlike the study described in this protocol, the MD Anderson Phase II trial of cladribine and rituximab is not able to determine whether rituximab is of value when added to cladribine. The trial achieved > 50% of its accrual by November, 2007, and its accrual is expected to be completed before this trial opens.

1.2.6 Safety profile of rituximab

No dose-limiting effects were observed in the Phase I/II studies. Reported adverse events including fever, chills, headache, nausea, vomiting, rhinitis, asthenia, and hypotension, occurred primarily during rituximab infusions and typically responded to an interruption of the infusion and resumption at a slower rate.

- **Fatal Infusion Reactions:** Severe and fatal cardiopulmonary events, including angioedema, hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, and cardiogenic shock, have been reported. These severe reactions typically occurred during the first infusion with time to onset of 30-120 minutes.
- **Cardiac Events:** Patients with preexisting cardiac conditions, including arrhythmia and angina, have had recurrences of these cardiac events during rituximab infusions.
- **Tumor Lysis Syndrome:** Tumor lysis syndrome has been reported and is characterized in patients with a high number of circulating malignant cells (≥25,000 ul) by rapid reduction in tumor volume, renal insufficiency, hyperkalemia, hypocalcemia, hyperuricemia, and hyperphosphatemia.
- **Renal Events:** Rituximab has been associated with severe renal toxicity including acute renal failure requiring dialysis, and in some cases has led to death. Renal toxicity has occurred in patients with high numbers of circulating malignant cells (≥25,000/mm^2) or high tumor burden who experience tumor lysis syndrome and in patients administered concomitant cisplatin.
- **Mucocutaneous Reactions:** Severe bullous skin reactions, including fatal cases of toxic epidermal necrolysis and paraneoplastic pemphigus, have been reported in patients treated with rituximab. The onset of reaction has varied from 1 to 13 weeks following rituximab exposure.

- **Hematologic Events:** In clinical trials, Grade 3 and 4 cytopenias were reported in 48% of patients treated with rituximab; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following Rituximab therapy were reported. In addition, there have been a limited number of postmarketing reports of prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia.
- **Infectious Events:** Rituxan induced B-cell depletion in 70% to 80% of patients with NHL and was associated with decreased serum immunoglobulins in a minority of patients; the lymphopenia lasted a median of 14 days (range, 1-588 days). Infectious events occurred in 31% of patients: 19% of patients had bacterial infections, 10% had viral infections, 1% had fungal infections, and 6% were unknown infections. Serious infectious events (Grade 3 or 4), including sepsis, occurred in 2% of patients.
- **Hepatitis B Reactivation:** Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some patients with hematologic malignancies treated with rituximab. The majority of patients received rituximab in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately four months after the initiation of rituximab and approximately one month after the last dose.
- **Other Serious Viral Infections:** The following additional serious viral infections, either new, reactivated or exacerbated, have been identified in clinical studies or postmarketing reports. The majority of patients received Rituxan in combination with chemotherapy or as part of a hematopoietic stem cell transplant. These viral infections included JC virus (progressive multifocal leukoencephalopathy [PML]), cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, and hepatitis C. In some cases, the viral infections occurred up to one year following discontinuation of Rituxan and have resulted in death.
- **Progressive multifocal leukoencephalopathy (PML).** PML is a rare and demyelinating disease of the brain caused by infection with the JC virus that usually leads to death or severe disability. JC virus infection resulting in PML and death has been reported rarely in patients with hematologic malignancies receiving rituximab. The majority of these patients had received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. Cases of PML resulting in death have also been reported in patients with systemic lupus erythematosus (SLE) treated with rituximab. These patients with SLE had longstanding disease, history of prior immunosuppressant therapy, and were diagnosed with PML within 12 months of their last infusion of rituximab. Physicians should consider PML in any patient presenting with new onset neurologic manifestations. Consultation with a neurologist, brain MRI, and lumbar puncture should be considered as clinically indicated. In

patients who develop PML, rituximab should be discontinued and reductions or discontinuation of any concomitant chemotherapy or immunosuppressive therapy should be considered.

- **Bowel Obstruction and Perforation:** Abdominal pain, bowel obstruction and perforation, in some cases leading to death, were observed in patients receiving Rituxan in combination with chemotherapy for DLBCL. In post-marketing reports, which include both patients with low-grade or follicular NHL and DLBCL, the mean time to onset of symptoms was 6 days (range 1−77) in patients with documented gastro-intestinal perforation. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.
- **Additional Safety Signals:** The following serious adverse events have been reported to occur in patients following completion of rituximab infusions: arthritis, disorders of blood vessels (vasculitis, serum sickness and lupus-like syndrome), eye disorders (uveitis and optic neuritis), lung disorders including pleuritis and scarring of the lung (bronchiolitis obliterans), that may result in fatal outcomes, and fatal cardiac failure.
- **Additional details:** The investigator brochure contains additional details regarding rituximab safety.

1.3 RATIONALE

1.3.1 Randomized trial of cladribine with simultaneous or delayed rituximab

Experts in the HCL field have agreed that now, 50 years after the discovery of the disease, and 20 years after its successful treatment with purine analogs, clinical progress has stalled because no treatment appears curative [55, 56]. There was no plateau on the time-to-treatment-failure curve even in 207 patients with at least 7 years of follow-up [9], making it unclear that any patients are cured. MRD prior to clinical relapse is detectable by several methods, and at least in the case of BMBx IHC predicts for earlier relapse [39]. Several recent studies have shown that MRD by BMBx IHC or by PCR or FACS of marrow or blood can be eliminated by rituximab without significant toxicity [41, 47]. We therefore believe it will become clinically accepted for patients with HCL MRD to be treated with rituximab even before clinically relapsing. We believe it will be impossible to randomize patients with MRD after CdA to either rituximab or observation, because patients and their physicians will not accept observing MRD after it is demonstrated. This is because MRD is a known risk factor for relapse and rituximab is well tolerated. Simultaneous treatment with cladribine and rituximab is well tolerated in small numbers of patients [53, 54]. Therefore, a more relevant testable question is whether MRD-free survival is enhanced when rituximab is used concurrently with cladribine, and whether delayed rituximab used for MRD-relapse induces a more prolonged response if rituximab was not used before. Although cladribine alone is not curative, strong CD20 expression on cladribine-resistant HCL cells makes it possible that rituximab could be a curative option for some patients in either situation.

1.3.2 Endpoints to test simultaneous vs. delayed rituximab

The primary endpoint is whether simultaneous cladribine and rituximab cause a lower rate of MRD at 6 months than cladribine alone. Based on studies of rituximab for MRD after CdA [41, 47], CR

without MRD should be achieved in > 90% at 6 months after cladribine + rituximab. CR without MRD should be about 40-60% after cladribine alone, depending on whether or not patients had prior cladribine [11, 39, 40, 42-46]. However, a significant difference at 6 months would not indicate superiority in the clinical outcome of simultaneous cladribine + rituximab over cladribine alone, since delayed rituximab after 6 months might work better if it had not been used previously. Therefore, the major secondary endpoint will be to assess blood MRD-free survival not only after cladribine, but also after up to 2 rituximab courses given if needed for MRD in blood. BMBx IHC, blood and bone marrow aspirate FACS will be used for detecting MRD for the primary endpoint. However, since non-compliance with BMBx is expected to increase with longer follow-up, MRD-relapse for the secondary endpoints for need for delayed rituximab will need to be determined by blood tests, either blood FACS positivity or presence of cytopenias (ANC < 1500/ul, Plt < 100,000/ul, or Hgb < 11 g/dl). Patients FACS-negative in both blood and bone marrow aspirate are considered in MRD-negative CR regardless of blood counts. FACS, consensus PCR and IHC are all CLIA certified tests. Patients with 'dry' bone marrow or bone marrow unable to be aspirated will be considered MRD+, since in MRD-free CR, the bone marrow aspirate should be liquid and able to be aspirated. RQ-PCR, which is the most sensitive test, will also be observed, but will not be used for clinical decisions or statistics because it is early in its development, particularly in following HCL after chemotherapy. If patients achieve significantly longer time MRD-free survival after cladribine + rituximab than after cladribine alone, but shorter MRD-free survival after delayed rituximab if rituximab was used previously with cladribine, this will argue against changing the standard treatment for HCL from cladribine alone. The delayed rituximab in the trial design also permits several secondary scientific objectives (see Section **1.1.2**) to be investigated for rituximab alone, including 1) determining if MRD levels and tumor markers (soluble CD25 and CD22) after rituximab correlate with response and tumor burden, 2) determining if BMBx can be avoided for judging response in HCL after rituximab, and 3) determining the effect of rituximab on normal B-cells without interference from simultaneous or recent cladribine.

1.3.3 Inability to assume that rituximab should be used with cladribine

Before a trial begins it is optimal for neither arm of a randomized trial to be obviously more advantageous than the other. There are potential advantages and disadvantages of simultaneous vs. delayed rituximab. It is possible, for example, that rituximab could have been more effective when begun at least 6 months after cladribine than concurrently, because by that time masses of tumor cells in the marrow, spleen and lymph nodes, which would present a tumor penetration problem for the rituximab Mab, might be at their minimum size. Secondly, if simultaneous rituximab were to fail to eliminate MRD, it could select for cells deficient in apoptosis and render patients unresponsive to rituximab and other agents when relapse occurs later in the disease course. Moreover, it is possible that the risk of infection could increase with combined use of rituximab and cladribine, since rituximab is known to cause rapid and prolonged (3-6 month) depletion of normal B-cells [57]. While this may not be a significant problem after single-agent short-course rituximab, it may be more problematic with the longer 8-cycle course, particularly after cladribine. Thus, a randomized trial was needed to determine if rituximab should be used with cladribine or delayed until after MRD appears. At this time, it is not considered standard to combine rituximab with cladribine for first-line or second-line treatment of HCL.

1.3.4 Need to assign patients with regard to prior purine analog

Based on CR rates of 91 and 62% for cladribine in patients with 0 and 1 prior courses of cladribine, respectively [8], response in this trial, including MRD endpoints, may likely be related to whether or not patients received prior cladribine or the other purine analog pentostatin. Therefore, patients need to be separated into two groups prior to randomization. This trial may result in a recommendation that cladribine + rituximab become the standard treatment only for newly diagnosed HCL, or only for HCL with 1 prior cladribine or pentostatin course, or for both groups, or for neither. Prospective randomization should facilitate the validity of such recommendations.

1.3.5 Rationale for accrual goals

Prior studies are inadequate to accurately predict what MRD rates will be after cladribine with or without rituximab using blood FACS/PCR and BMBx IHC, the methods used in this study. Prior studies have reported 15-50% MRD rates after cladribine alone with MRD studies [39, 58-60] which are less sensitive than those to be used in this study. Recent studies report < 10% MRD rates after rituximab for detectable MRD following cladribine, using MRD studies similar in sensitivity to those planned [41, 47]. For patients with no prior purine analog or rituximab our goal is to discriminate MRD rates of 25% after cladribine alone versus 5% after cladribine plus rituximab, a 20% difference. It will require 34 patients in each arm to provide an 80% power to detect this difference. For patients with 1 prior course of cladribine and no rituximab, our goal is to discriminate MRD rates of 10% after cladribine alone versus 35% after cladribine plus rituximab, a 25% difference. It will require 31 patients in each arm to provide an 80% power to detect this difference. Since adding rituximab to cladribine would not be expected to increase the rate of MRD, a one-tailed ($p = 0.1$) comparison is sufficient. Once randomization of the newly diagnosed HCL patients is complete, 25 non-randomized patients will be enrolled for simultaneous rituximab begun immediately before the 1st dose of cladribine, so that tolerance (particularly thrombocytopenia), feasibility of outpatient treatment, and MRD results can be historically compared to the 34 newly diagnosed HCL patients randomized to simultaneous cladribine plus rituximab.

1.3.6 Randomization ‘up front’ and assessment of MRD response at 6 months

The existing trial at MD Anderson, which employs rituximab 4 weeks after starting cladribine, allows assessment of MRD in all patients 1 month after cladribine alone, an important endpoint. However, beginning cladribine and rituximab together is more consistent with the literature regarding combined cladribine and rituximab in CLL and HCL [53, 54]. Combined treatment would also allow for synergism between the 2 agents. Also, it has been shown that while 2 or 3 months is insufficient, 6 months is enough time to judge bone marrow response to cladribine in HCL [11, 59, 61]. It would be very unlikely that patients receiving cladribine alone would need additional treatment before 6 months after cladribine. Thus, this protocol will allow 6 months to judge response after either cladribine or cladribine + rituximab, but, like the MD Anderson study, will also check for MRD by all tests at the 1 month time point.

1.3.7 Avoiding bone marrow biopsy (BMBx) in HCL

BMBx has always been the key test used to determine if HCL patients with hematologic recovery after treatment have achieved a CR. However, it is painful for patients and many physicians avoid BMBx in managing HCL. We found flow cytometry of the blood to be more sensitive than

immunohistochemistry of the BMBx in detecting HCL, since 5% vs. 11% of patients in CR after BL22 had MRD by these methods, respectively [38]. Secondly, success of treatment may be estimated by hematologic recovery and decrease in circulating HCL cells by flow cytometry, although without BMBx it cannot be determined whether patients had CR or PR. Finally, patients in CR or PR after treatment are generally followed without BMBx and not retreated until cytopenias or other indications for treatment (detailed in Section **2.1.1.3**) develop. We believe that even without BMBx, new tumor markers and flow cytometry techniques, together with CBC results, could be used with acceptable accuracy to predict whether patients have CR. More importantly, the status of tumor markers and MRD studies may be more important than BMBx in predicting how long a response will last. Finally, to correlate bone marrow MRI signal with bone marrow biopsy, patients will obtain cervical and thoracic spine MRI at baseline and at bone marrow restaging time points, when feasible. The MRI signal in HCL is abnormal at baseline, remains abnormal immediately after achievement of completely remission due to enhance production of normal blood cells, and eventually becomes more normal. A secondary research objective is to define this correlation in a large number of patients and time points, and to determine when after achievement of CR the MRI normalizes. If the correlation is strong, an MRI may be useful in the future to determine if patients might be relapsing or in long term CR. Thus, an important goal of this study is to prospectively correlate BMBx results to other less invasive tests of tumor burden and MRD; and to allow better management of HCL without BMBx.

1.3.8 Ability to recruit HCL patients for the trial

This trial requires recruiting samples from 155 HCL and 20 HCLv patients. The Clinical Immunotherapy Section of the Laboratory of Molecular Biology (LMB) has prescreened a total of 217 patients with HCL, 77 in the past 3 years. Although we did not request patients with 0-1 prior courses of cladribine, 5 (6%) patients had 0 and 15 (19%) had 1 prior courses of cladribine and likely would have been eligible. Patients eligible for this trial would not be eligible for HA22 or BL22, since those trials require at least 2 prior therapies. Those 2 therapies would either be 2 courses of purine analog or a course of purine analog and 1 of either rituximab or another purine analog. Thus, this trial will not conflict with other active NIH HCL trials 06-c-0150 using LMB-2 and 07-c-0130 using HA22. We believe it will be feasible to recruit all the patients using NIH as a single site. At a November 2007 international meeting of HCL experts, it was recognized that standard therapy for HCL has remained unchanged for nearly 20 years and that the optimal role of rituximab in HCL management needs to be determined with a randomized trial. We believe that these and other investigators will be able to contribute significantly to this trial by sending samples and/or treating patients.

1.3.9 Following tumor markers in HCL patients

We have developed several novel assays quantifying tumor burden in HCL, including soluble IRTA [62, 63] and soluble CD22 (sCD22). The sCD22 assay may be particularly appropriate for following HCL, especially in patients with limited expression of CD25 as in HCLv. While soluble CD20 (cCD20) has been described [64, 65], this marker would be difficult to assess because it would bind to rituximab which has a long half-life. sCD22 does not bind to rituximab and may be a sensitive indicator of extent of response and predicting early relapse.

1.3.10 Simultaneous rituximab and cladribine as an outpatient

An important secondary objective is to determine if combined cladribine and rituximab are feasible to administer as an outpatient. Enrollment of non-randomized patients to begin rituximab immediately prior to cladribine may facilitate outpatient administration by obviating the time needed for randomization. Also, changing the sequence of first doses of rituximab and cladribine to rituximab first may avoid rapid thrombocytopenia, which could in some cases requires inpatient treatment. A 25-patient cohort treated in nonrandomized fashion will permit >80% power to rule out a 12-16% decrease in MRD-free rate from that observed in the randomized newly diagnosed HCL patients receiving cladribine prior first dose of rituximab.

1.3.11 Relationship of this trial to current research performed in LMB

The Laboratory of Molecular Biology (LMB) is currently developing recombinant immunotoxins for treating several forms of cancer, and the most successful trials have been performed in patients with HCL [35-38]. A major and increasing focus of the lab is in studying MRD after treatment for HCL using the highly sensitive RQ-PCR assay, and this assay has already been used to study MRD after purine analog and rituximab [48]. The lab is also studying the biology of HCL through patient samples obtained and has the largest molecular database of HCL patients reported [66-68]. Patient recruitment for HCL trials has been very strong and the growing population of untreated patients seeking advice indicates that a trial earlier in the disease would also accrue well at NIH. In addition to the RQ-PCR assay in the LMB which needs to be tested after agents other than immunotoxins, the Laboratory of Pathology has excellent tools for studying HCL MRD, including flow cytometry, consensus PCR, and immunohistochemistry [46, 48]. Finally, this trial can benefit the LMB immunotoxin program by identifying a group of patients eligible in the near future for immunotoxin trials. Recombinant immunotoxin HA22 [69], an improved version of BL22, is the agent now being developed for treatment of cladribine-resistant HCL. The phase II trial of BL22 in patients with HCL showed that tumor burden affected response. For example, patients with spleens < 200 mm had a 95% overall response rate (ORR), compared to 20% ($p = 0.001$) in patients with larger spleens or 33% ($p = 0.0007$) in patients post splenectomy. These results argue that close follow-up of HCL patients after standard therapy and treatment with BL22 in early relapse may be beneficial. Thus an added benefit of this trial will be the ability to prospectively follow a large number of HCL patients after standard therapy, monitoring for early relapse. These patients may be eligible for a pivotal study of an immunotoxin for HCL.

1.3.12 Problem in randomizing patients with variant HCL (HCLv)

A CD25-negative variant of HCL has been recognized since 1980 in which patients have a poorer prognosis with standard therapy [70]. In contrast to the excellent results with initial cladribine in classic HCL, HCLv responds poorly. For example, only 4 PRs were reported in 8 with HCLv [71] , 2 PRs were reported out of 6 with HCLv [72], and 1 PR was reported in 3 Japanese HCLv patients [73]. Of these 17 HCLv patients from the literature, prior purine analog treatment included pentostatin in 4 cases, no purine analog in 8 cases, and in 5 cases was not reported. No progression free survival (PFS) information was given. We have screened 14 HCLv patients for LMB protocols for whom we have adequate prior information to judge response to initial cladribine, and in all cases cladribine was the 1st therapy. Of these 14 HCLv patients, there were 1 CR and 3 PRs. In these 14 patients, PFS was 1.7-96.3 (median 5.2) months, with PFS 26.3 months for CR and 8.3, 11.5 and 96.3 months for PR. None of these responses are ongoing. Thus, out of 31 total

HCLv, patients, there were a total of 1 (3%) CR and 11 total responses (ORR 35%). With respect to prior purine analog treatment, 3 (75%) of 4 with prior pentostatin responded, versus 7 (32%) of 22 with no prior purine analog (p=0.3). Thus, there is no suggestion that untreated HCLv patients have a better response to cladribine alone compared to HCLv patients with 1 prior purine analog, and thus both these groups of HCLv patients should be considered together without further stratification. Several anecdotal cases have been reported where patients with HCLv have responded to rituximab [74-77]. In the past few years we have prescreened over 20 HCLv patients, many of whom are or would have been eligible for this study. Because patients looking into our salvage immunotoxin trials are heavily skewed toward the HCLv phenotype, and because many of these patients have not yet had the 2 prior courses of purine analog needed for salvage protocols, we believe it will not be difficult to enroll 20 HCLv into this protocol.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Evidence of HCL by flow cytometry, reviewed by the Laboratory of Pathology, NCI, including positivity for CD19, CD22, CD20, and CD11c.

2.1.1.2 BMBx consistent with HCL, reviewed by Laboratory of Pathology, NCI. BMBx may be negative in HCLv in patients with increasing peripheral blood HCLv cells and spleen size.

2.1.1.3 Treatment indicated based on demonstration of at least one of the following no more than 4 weeks from the time of enrollment, and no less than 6 months after prior purine analog and no less than 4 weeks after other prior treatment, if applicable.

- Neutropenia (ANC < 1000 cells/µl).
- Anemia (Hgb < 10g/dL).
- Thrombocytopenia (Plt < 100,000/µl).
- Absolute lymphocyte count (ALC) of >5,000 K/uL
- Symptomatic splenomegaly.
- Enlarging lymph nodes > 2cm.
- Repeated infections requiring oral or i.v. antibiotics.

Patients who have eligible blood counts within 4 weeks from enrollment will not be considered ineligible if subsequent blood counts prior to enrollment fluctuate and become ineligible up until the time of enrollment.

2.1.1.4 No prior purine analog therapy except up to 1 prior course of either cladribine or pentostatin.

2.1.1.5 No prior rituximab unless HCLv patient

2.1.1.6 ECOG performance status [78] of 0-3 (see **Appendix C**).

2.1.1.7 Patients must be able to understand and give informed consent.

2.1.1.8 Women of child-bearing age and all men must use birth control of any type until at least 12 months after the last dose of therapy.

2.1.1.9 Creatinine ≤ 1.5 or creatinine clearance ≥ 60 ml/ml.

2.1.1.10 Bilirubin ≤ 2 unless consistent with Gilbert's (total/direct > 5), ALT and AST ≤ 2.5 x upper limits of normal.

2.1.1.11 No other therapy (i.e. chemotherapy, interferon) for 4 weeks prior to study entry, or cladribine for 6 months prior to study entry.

2.1.1.12 Age at least 18

2.1.1.13 Men and women of reproductive potential must agree to use an acceptable method of birth control during treatment and for twelve months after completion of treatment.

2.1.1.14 Subject has provided written informed consent

2.1.1.15 Patients must be willing to co-enroll in the investigator's companion protocol 10-C-0066 titled "Collection of Human Samples to Study Hairy Cell and other Leukemias, and to Develop Recombinant Immunotoxins for Cancer Treatment".

2.1.2 Exclusion Criteria

2.1.2.1 Presence of active untreated infection

2.1.2.2 Uncontrolled coronary disease or NYHA class III-IV heart disease (see **Appendix D: New York Heart Association Classification**).

2.1.2.3 Known infection with HIV. Hepatitis B is allowed only if viral load is undetectable and if on anti-hepatitis B therapy like Entecavir. Hepatitis C is allowed only if viral load is undetectable, and if the patient has received curative therapy.

2.1.2.4 Patients with documented history of no response to cladribine, and without 50% improvement in platelets, hemoglobin or granulocytes. This exclusion does not apply to HCLv. These patients are eligible regardless of prior response to CDA.

2.1.2.5 Pregnant or lactating women.

2.1.2.6 Presence of active 2nd malignancy requiring treatment. 2nd malignancies with low activity which do not require treatment (i.e. low grade prostate cancer, basal cell or squamous cell skin cancer) do not constitute exclusions.

2.1.2.7 Inability to comply with study and/or follow-up procedures.

2.1.2.8 Presence of CNS disease, which is symptomatic.

2.1.2.9 At the Investigator's discretion, receipt of a live vaccine within 4 weeks prior to randomization. Efficacy and/or safety of immunization during periods of B-cell depletion have not been adequately studied. It is recommended that a patient's vaccination record and possible requirements be reviewed. Per the investigator's discretion, the patient may have any required vaccination/booster administered at least 4 weeks prior to the initiation of study treatment. Review of the patient's immunization status for the following vaccinations is recommended: tetanus; diphtheria; influenza; pneumococcal polysaccharide; *Varicella*; measles, mumps and rubella (MMR); and hepatitis B. Patients who are considered to be at high risk for hepatitis B virus (HBV) infection and for whom

the investigator has determined that immunization is indicated should complete the entire HBV vaccine series at least 4 weeks prior to participation in the study.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. Prior to distribution of any recruitment materials, such materials will be submitted to the IRB for review.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in Section **8.5.2**.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for this study for screening. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

2.2.2.1 Laboratory evaluation (needed for eligibility)

- Blood FACS
- CBC with differential
- Creatinine or 24 hour urine for creatinine clearance
- Direct bilirubin, total bilirubin, SGOT and SGPT
- Pregnancy test (urine or serum). Women of childbearing potential must have a negative pregnancy test within 7 days of enrollment.
- Hepatitis B (antigen), hepatitis C and HIV test
- Bone marrow biopsy with IHC for CD20 and CD3. This must have been done since last prior therapy and at least 6 months after prior cladribine.

2.2.2.2 Other evaluations

- EKG
- History & Physical with documented performance status

- CT neck-pelvis or abdominal MRI.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

2.3.2 Treatment Assignment and Randomization Procedures

Cohorts

Number	Name	Description
1	Cohort 1	Patients with HCL with (62 patients) and without (68 subjects) prior course of purine analog to be randomized between Arm 1 and Arm 2 (randomization stratified based upon prior purine analog [yes/no])
2	Cohort 2	Patients with HCL without prior course of purine analog to be directly assigned to Arm 3 (25 patients; after completion of the 68 patients without prior purine analog in Cohort 1)
3	Cohort 3	Patients with HCLv to be directly assigned to Arm 3 (20 patients)

Arms

Number	Name	Description
1	Arm 1	Cladribine + Rituximab
2	Arm 2	Cladribine (+ Rituximab after 6 months if MRD detected)
3	Arm 3	Cladribine + Rituximab (Rituximab will be administered just before rather than after the 1st of the 5 daily doses of cladribine on day 1)

Stratifications

Name	Distinct Options	Notes

Prior purine analog	Yes No	Applies to Cohort 1 patients randomized between Arm 1 and Arm 2 only (see below).

Randomization and Arm Assignment

At the time of registration, patients in Cohort 1 will be randomized between Arm 1 and Arm 2, and patients in Cohort 2 will be directly assigned to Arm 3. Patients with variant HCL (HCLv; Cohort 3) regardless of prior treatment with purine analog are assigned to Arm 3.

- Cohort 1: HCL Patients will be randomized between Arm 1 and 2. Randomization will be stratified on the basis of prior purine analog or not. There will be 68 patients randomized within the no prior purine analog stratum and 62 randomized within the 1+ prior purine analog stratum.
    - Note: The randomization for Cohort 1 will be performed by CRO personnel using randomization assignments generated by the study statistician. The Pharmacy Department will be notified of the treatment assignment once the patient is randomized.
- Cohort 2: 25 patients with no prior purine analog will be assigned directly to Arm 3 following completion of randomization of the 68 patients in the 'no prior purine analog' stratum in Cohort 1.
- Cohort 3: Patients with variant HCL (HCLv) regardless of prior treatment with purine analog will be enrolled in this cohort, will not be randomized and will be assigned to Arm 3.

2.4 BASELINE EVALUATION

2.4.1 Prior to treatment (any time)

- Research Blood (and bone marrow if available) for cloning/sequencing IgH rearrangements (multiple sets prior to treatment)
- HLA-A,B,C, DRB,DQB

2.4.2 Day 1 prior to treatment

- CBC with differential
- Needed for patients at NIH: Chemistries (albumin, alkaline phosphatase, ALT, AST, BUN, calcium, CK, chloride, CO2, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid), urinalysis

2.4.3 Within 4 weeks prior to treatment

- Serum for tumor markers, flow cytometry (FACS), PaxGene tube for RQ-PCR, and citrate tube for consensus PCR, ferritin, IgG, IgA, IgM, PT, PTT, fibrinogen, haptoglobin, amylase, lipase, GGT, free T3, free T4, quantification of T, B and NK cells (TBNK), C-reactive protein (CRP), thrombin time. Pregnancy test (if relevant) needed with 1 week prior to enrollment.

  Note: tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.
- Abdominal ultrasound (U/S) – may do spleen U/S instead per PI discretion.

2.4.4 Within 28 days from starting delayed rituximab

- HCL Imaging, EKG, amylase, lipase, GGT, lipid panel, TSH, free T3, free T4, ferritin, PCR, IgG, IgA, IgM, haptoglobin, CRP, urinalysis, and chemistries

2.4.5 Within 90 days prior to treatment

- CT neck-pelvis or abdominal MRI needed only before cladribine. If no nodes, all subsequent imaging may be done with spleen U/S (or abdominal U/S if no spleen), including baseline for delayed rituximab
- HBsAg, HBcAB, & HCV, HIV
- Echo, stress test, PFTs
- 24hr urine protein, creatinine clearance & UPEP
- SPEP/ immunoelectrophoresis, serum light chains, T3, T4

2.4.6 Within 360 days prior to treatment

- Cervical and Thoracic (C- and T-) Spine MRI to correlate the status of the BMBx with the vertebral BMA signal by MRI. May be cancelled at the discretion of the PI. Since the baseline MRI signal in a patient with marrow involvement of HCL is not expected to change significantly during the year prior to enrollment, it is not necessary to repeat the bone marrow biopsy because the MRI was obtained closer to the time of enrollment.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Overview

This is a randomized study of cladribine with or without rituximab in HCL, along with delayed rituximab for MRD relapse. The primary objective of the study is to determine if HCL MRD differs at 6 months after cladribine according to whether or not rituximab is administered concurrently with cladribine. MRD for the primary objective is defined as positive blood FACS, bone marrow aspirate FACS, or BMBX IHC.

Two groups, including 68 patients with HCL and no prior courses of purine analog and 62 with HCL and 1 prior course of purine analog (Cohort 1), will be randomized according to the following schema showing numbers of patients. Following enrollment of the first evaluable 68 patients with no prior purine analog, 25 additional patients will be enrolled into the Cohort 2 and they will all receive rituximab and cladribine without randomization. However, the first of the 8 weekly doses of rituximab will be administered just before rather than after the 1st of the 5 daily doses of cladribine on day 1. The goal of this 25-patient non-randomized group is 1) to enable combined treatment as an outpatient due to the time saved from not randomizing, and 2) to determine if rapid reversible thrombocytopenia can be avoided by beginning the rituximab immediately before the 1st dose of cladribine, rather than immediately afterward. If after 10 of these nonrandomized patients it is evident that toxicity is higher with rituximab begun before cladribine than afterward,

the remaining 15 nonrandomized patients will be enrolled, and treated as outpatients if possible, with the rituximab begun after the 1st dose of cladribine as in the randomized patients.

A third group will be enrolled and will contain 20 HCLv patients who will not be randomized and will receive rituximab concurrently with cladribine.

3.1.2 Schema (CdA = cladribine)

Enrollment	**Classic or typical (CD25+) HCL (68 + 25 patients) (0 prior courses CdA)**			**Classic or typical (CD25+) HCL (62 patients) (1 prior course CdA)**		**Variant (CD25-) HCL (20 patients)**
Randomize	**34**	**34**		**31**	**31**	
Non Randomize			**25**			**20**
Begin day 1	CdA+rituximab	CdA	CdA+rituximab*	CdA+rituximab	CdA	CdA+rituximab
When MRD+	rituximab	rituximab	rituximab	rituximab	rituximab	rituximab
If still MRD+		rituximab			rituximab	

***Rituximab beginning day 1 before dose 1 of cladribine (CdA). Otherwise CdA before rituximab on day 1.**

3.1.3 Definition of MRD positivity

To determine MRD rates for the primary endpoint, incidence of MRD 6 months after cladribine, the following 3 CLIA-certified MRD tests will be used:

- Blood FACS
- Bone marrow aspirate FACS
- BMBx IHC
- For the primary endpoint, patients will be considered MRD+ if any of these 3 tests are positive. MRD relapse is defined as FACS positivity or low blood counts (ANC < 1500/ul, Plt < 100,000/ul, or Hgb < 11). Patients FACS-negative in both blood and bone marrow aspirate are considered MRD-negative regardless of blood counts. MRD in the BMBx by IHC is defined as a ratio of CD20+ cells to T cells of at least 1, and most of the CD20+ cells having morphology consistent with HCL. FACS uses multicolor flow cytometry to specifically identify and quantify HCL cells based on expression of CD19, CD22, CD20, CD11c, CD103, and restricted (lambda or kappa) light chains. Blood consensus PCR is reported as clonal rearrangement present (positive) or polyclonal rearrangement pattern present (negative). While MRD generally refers to HCL detected while a patient is in CR, patients with more than minimal disease in the BMBx, who by definition are not in CR, will also be considered MRD+ by IHC. Patients not in CR due to positive BMBx may be MRD-free by blood tests. Thus, blood MRD-free survival is usually shorter than, but could be equal to or longer than disease-free survival.

3.1.4 Additional tests for MRD

- Although FACS/PCR of bone marrow aspirate is also CLIA certified, the sensitivity and specificity of these PCR are less than FACS. Therefore, PCR results

will be reported but not included in determination of MRD for the primary endpoint.

- RQ-PCR of blood, while extremely sensitive for MRD, will not be used for protocol decisions since it is not CLIA certified and its clinical relevance needs additional study.

3.2 DRUG ADMINISTRATION

3.2.1 Cladribine

Cladribine 0.15 mg/Kg/day by 2-hour i.v. infusion days 1-5. The infusion time may be changed to 1 hour at the discretion of the PI.

3.2.2 Rituximab

Rituximab 375 mg/m^2 i.v. infusion every week x8, begin day 1 in half of randomized patients and in all HCLv patients, and then again in all patients at least 6 months later when HCL is detected by blood FACS. Patients who first received rituximab at least 6 months after cladribine may receive a 2nd course of rituximab at the same dose and schedule at least 6 months after the beginning of the 1st course, if and when HCL is detected again by blood FACS. When rituximab is given on the same day as cladribine (day 1), it will be given at the end of the 2-hour infusion of cladribine. In the 25 nonrandomized newly-diagnosed HCL patients beginning rituximab prior to cladribine, the 2 hour infusion of cladribine will be begin at the end of the infusion of rituximab. Actual body weight measured within 4 weeks prior to initial treatment with rituximab will be used for calculations of body surface area.

It is possible, though uncommon for HCL and HCLv to change phenotype. This would be considered a second malignancy which would require patients to be taken off-treatment. However, we feel it is justified for these patients to remain on study and continue to receive treatment despite the development a new clone (second malignancy) because they are likely to receive on-going benefit from the treatment. Thus, at the discretion of the PI, these patients may continue with treatment including delayed Rituximab if appropriate.

3.3 TREATMENT MODIFICATIONS

3.3.1 Treatment Delay

- Dosing of cladribine may be delayed up to 2 weeks, rituximab up to 12 weeks for toxicity or logistical reasons, including inability of the patient to get to clinic. The one week interval between rituximab doses may also be shortened by one day for logistical reasons.
- Patients with excessive delay or dose-limiting toxicity (DLT) are off-treatment and may be followed but not retreated.
- Infusions should be discontinued in the event of serious or life-threatening cardiac arrhythmias. Subjects who develop clinically significant arrhythmias which are not dose-limiting should undergo cardiac monitoring during and after subsequent infusions.

3.3.2 Dose-reduction

Cladribine and rituximab may not be dose-reduced. Rituximab infusion should be interrupted for severe reactions, e.g., rapid tumor lysis. Treatment of infusion-related symptoms with diphenhydramine and acetaminophen is recommended. Additional treatment with bronchodilators or IV saline may be indicated. Epinephrine, antihistamines, and corticosteroids should be available for immediate use in the event of a hypersensitivity reaction to rituximab (e.g., anaphylaxis). In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100mg/hr to 50mg/hr) when symptoms and laboratory abnormalities have completely resolved. Patients with severe or life-threatening anaphylaxis or hypersensitivity reaction should be removed from the study.

3.3.3 DLT criteria

Grade III-IV cladribine or rituximab related toxicity except:

- Grade 3-4 hematologic toxicity resolving in 2 weeks is not DLT.
- Grade 3 fever is not DLT.
- Grade 3 gastrointestinal toxicity for < 5 days is not considered DLT.
- Severe or life-threatening anaphylaxis or hypersensitivity reaction

3.4 PROTOCOL EVALUATION

3.4.1 Pre-treatment evaluation (See Section **2.4**)

3.4.2 Evaluation during study (See Section **15.1**)

3.4.3 Post Treatment Evaluation (Follow-up)

- Patient follow-up at least semiannually will continue by clinic visit, phone, or secure email using NIH approved system to determine disease-free and overall survival. In patients who develop progressive disease, follow up will continue by annual phone call only, to determine overall survival.
- Patients will be closely followed to determine resolution of grade IV toxicity.

3.5 CONCURRENT THERAPIES

- Patients may not receive other treatments for HCL while on study
- This research study protocol allows the subject to receive up to 16 infusions of rituximab. Even if the treatment is shown to be of benefit, additional infusions of rituximab beyond that allowed in the protocol cannot be given to the subject while she/he is participating in this study.

3.6 RADIATION THERAPY GUIDELINES

Radiation therapy for HCL is not permitted while on protocol. Subjects requiring radiation therapy will be considered off treatment but not off study.

3.7 COST AND COMPENSATION

3.7.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center.

3.7.2 Compensation

Participants will not be compensated on this study.

3.7.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.8.1 Criteria for removal from protocol therapy

- Voluntary patient withdrawal
- Non-compliance with treatment and tests (discretion of PI)
- The patient has received 16 infusions of rituximab
- Progressive disease after the 2nd course of rituximab
- Progression prior to finishing 2 courses of rituximab which is considered by the PI to be too rapid to treat with rituximab alone.
- Dose-limiting toxicity
- Intercurrent illness or medical circumstances making it unsafe to remain on treatment
- Radiation therapy
- Positive pregnancy test

3.8.2 Off-Study Criteria

- Voluntary patient withdrawal
- Non-compliance at discretion of PI
- Death
- Screen failure
- Off-Study Procedure is in Section **2.3**. The reason for removing patients from study will be documented in the CRF and the medical record.

- Patients in long-term CR may become temporarily 'lost to follow-up' but may intend to eventually follow-up with restaging and taking them off study prematurely might greatly compromise the long-term objectives of the study. Therefore, patients may be reported as 'lost to follow-up' and unavailable for follow-up testing, but may continue to be followed once contact resumes. Regular communication with patients will be done to minimize this occurrence.

4 SUPPORTIVE CARE

4.1 INFECTIONS/FEVER AND NEUTROPENIA

Febrile neutropenia is a common side-effect of cladribine which require empiric antibiotics, either as an inpatient or outpatient. Hematopoietic growth factors may be used if clinically indicated. Fever is also common with rituximab and may be treated symptomatically and with interrupting or with decreasing the infusion rate, as detailed in Section **13.2.6**. Patients may receive prophylactic valacyclovir and less commonly prophylactic Bactrim if judged by the PI to be at significant risk of Herpes Zoster and Pneumocystis infections, respectively. Generally, prophylaxis will extend from the beginning of cladribine treatment until at least the 3-month time point when reassessments of CD4 and other blood counts are possible. If they have an allergy to Bactrim, Pentamidine or other medications/treatments may be ordered instead. Pneumocystis is extremely rare in HCL, particularly in newly diagnosed patients, allowing its prophylaxis to be reserved for patients with high risk, for example with very low CD4 counts or a prior history of infection.

4.2 BLOOD PRODUCT SUPPORT

Symptomatic anemia should be treated with appropriate red blood cell support. Transfusion is generally recommended if the hemoglobin falls below 8g/dL, but lower levels are acceptable particularly for younger patients. Recombinant erythropoietin may also be used. Platelets are generally given when the platelet count is $< 10{,}000/mm^3$ or when there is bleeding.

4.3 CYTOKINE SUPPORT

Prophylactic filgrastim (G-CSF) or sargramostim (GM-CSF) is not indicated for cladribine in HCL but may be used in special situations, for example, when patients have serious neutropenic infections and it is considered desirable to resolve the neutropenia as soon as possible.

4.4 TUMOR LYSIS SYNDROME

Although tumor lysis syndrome, with fever, hyperkalemia, hyperuricemia, hypocalcemia, hyperphosphatemia, and decreased renal function have been reported in CLL with rituximab, this syndrome has not been reported in HCL with either rituximab or cladribine. Patients with high baseline uric acid may be treated prophylactically with allopurinol but this protocol will not mandate prophylaxis for this syndrome. Patients should be followed closely particularly during the first week of treatment when the chance of tumor lysis syndrome would be highest.

4.5 INFUSION REACTIONS

Patients receiving rituximab, particularly those with high concentrations of circulating malignant cells, commonly have infusional toxicities including fever, chills, nausea and vomiting, dyspnea, hypotension, and palpitations. Infusional reactions with rituximab have been observed in HCL as well as other malignancies and are best managed by slowing or interrupting the rate of infusion.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

Patients treated both inside and outside of NIH will require source documentation regarding dosage and timing of drug administration. For patients treated outside NIH, results of lab, radiology and pathology tests should be faxed to the PI

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after the end of treatment. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

5.1.1 Protocol Specific Details to be Considered

- Best response to cladribine with or without immediate rituximab, and to each course of delayed rituximab
- Dates of achievement of CR (onset and relapse) and PR
- Dates of MRD negativity (onset and relapse) for flow cytometry of the blood and bone marrow, and immunohistochemistry of the bone marrow biopsy
- Dates of death for determination of overall survival
- A preexisting (baseline) laboratory abnormality will be considered the last one obtained prior to the first dose of drug (cladribine or delayed rituximab), unless the PI considers an abnormality of higher grade occurring within 100 days prior to the first dose to be a truer baseline
- Calcium values corrected for albumin will be used to report hypocalcemia

- Baseline signs and symptoms prior to enrollment cannot serve as the baseline for delayed rituximab courses, since baseline events likely resolve during the >6 months before delayed rituximab, and events occurring after delayed rituximab may no longer be due to disease

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

5.2 RESPONSE CRITERIA

5.2.1 Tests needed for response assessment

- Blood tests: CBC, flow cytometry
- CT scan or other imaging study if abnormal prior to cladribine
- BMBx with immunohistochemistry, and BMA

5.2.2 Time intervals for restaging

- See Section **15**, Study Calendar

5.2.3 Rules for response assessment

- No G-CSF or GM-CSF at least 4 weeks before major response.
- No transfusions at least 4 weeks before major response.
- Hgb requirement for major response may be dropped if iron stores are low (ferritin ≤ 20)
- Isolated low blood counts do not indicate relapse unless they are consistently low, i.e., at least 2 low counts > 1 week apart.
- A minimum duration of response is not required.

5.2.4 Complete remission (CR): All of the following:

- Absolute neutrophil count (ANC) ≥ 1500/ul
- Platelets ≥ 100,000/ul
- Hgb ≥ 11 g/dl
- Spleen non-palpable or not below costal margin on CT.
- Circulating HCL cells non-visible on Wright stain
- Lymph nodes ≤ 2 cm (short axis)
- Absence of hairy cells on BM aspirate smears and bone marrow biopsy by H&E and Wright stains.
- CR with MRD in the BMBx by IHC is defined as CR and a ratio of B cells to T cells of at least 1, and most of the B cells having characteristics consistent with HCL.

- CR with MRD in the peripheral blood is defined as CR and a positive peripheral blood FACS or PCR.
- Patients meeting all criteria for CR except minimum levels of ANC, platelets and Hgb will be considered CR if MRD is absent by BMBx IHC and by flow cytometry of blood and bone marrow.

5.2.5 Partial Response (PR): All of the following:

- Neutrophils ≥ 1,500/μL or 50% improvement over baseline.
- Platelets ≥100,000/μL or 50% improvement over baseline.
- Hgb ≥ 11.0 g/dL or 50% improvement over baseline. For patients who are transfusion-dependent at baseline, Hgb of ≥ 9.0 g/dL.
- ≥ 50% decrease in circulating malignant HCL count from the pretreatment baseline.
- ≥ 50% reduction in sum of products of perpendicular diameters or decrease to ≤ 2 cm (short axis) in evaluable (> 2cm) lymphadenopathy.
- ≥ 50% reduction in extent of spleen below costal margin by CT or physical exam, if abnormal at baseline.

5.2.6 Progressive disease (PD): Any of the following:

- ≥50% increase in sum of products of perpendicular diameters of evaluable (> 2cm) lymphadenopathy or appearance of new evaluable lymph nodes > 2 cm.
- ≥50% increase in extent of spleen below costal margin by CT or physical exam, if abnormal at baseline.
- ≥50% increase in the absolute number of circulating malignant lymphocytes.

5.2.7 Stable disease (SD): None of the above

5.3 TOXICITY CRITERIA

This study will utilize the CTCAE version 3.0 for all SAE reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). All appropriate treatment areas should have access to a copy of the CTC version 3.0.

6 STATISTICAL CONSIDERATIONS

6.1 PRIMARY ENDPOINT

The primary objective of this trial is to determine if the rate of minimal residual disease (MRD) identified in patients 6 months after beginning cladribine for treatment of HCL differs according to whether cladribine is combined with rituximab. Half of the randomized patients receive cladribine and rituximab concurrently, and all patients receive delayed rituximab at least 6 months after cladribine, if and when MRD is detected. Patients with blood-MRD at least 6 months after delayed rituximab may also receive a 2nd course of delayed rituximab if they did not receive rituximab simultaneous with cladribine. The most important secondary objectives are MRD-free

survival after cladribine, and MRD-free survival after delayed rituximab or after up to 2 courses of rituximab. MRD studies for the primary endpoint will be limited to 1) blood FACS, 2) bone marrow aspirate FACS and 3) BMBx IHC, so that patients will be considered MRD+ if any of these 3 tests is positive. Of these tests, only blood FACS will be used for triggering delayed rituximab, because it is the most sensitive test of MRD in blood and bone marrow results will be affected by compliance for the procedure. Blood and marrow RQ-PCR will also be studied but will not be used for the primary endpoint because RQ-PCR though extremely sensitive is too early in its development as an MRD study. All MRD studies except for RQ-PCR are performed in CLIA certified labs. If the patient has a non-HCL monoclonal population in the blood, including but not limited to monoclonal B-cell lymphocytosis (MBL) and monoclonal gammopathy of uncertain significance (MGUS), the blood or marrow PCR or RQ-PCR may be false-positive so these PCR tests are not evaluable for the primary endpoint.

Patients enrolled on this trial may have received 0 prior cycles of purine analog or 1 prior cycle of purine analog. Data from previous studies suggests that patients without prior purine analog exposure have a higher complete response rate when they receive cladribine (91%, with a median CR duration of 53 months) compared to those with 1 prior cycle of cladribine, who have a CR rate of 62% (along with 26% PRs) and an associated response duration of 23 months. As a result, since MRD and development of a complete response are inter-related, the levels of MRD found may quite possibly differ according to whether the patients have received previous cladribine or not. Patients will be assigned to cohorts (Cohorts 1 and 2) according to whether they have received prior purine analog or not. In addition, the study will be designed to have adequate numbers of subjects in each category (prior purine analog exposure or not) in order to ensure reasonable interpretation for each of the two strata.

The goal will be to randomize a total of 68 evaluable patients with 0 and 62 with 1 prior courses of purine analog at the time of starting cladribine on this trial. MRD evaluations will be performed every month for 3 months after treatment initiation, every 3 months for 2 years, and every 6 months thereafter. The evaluation made nearest to 6 months after cladribine will be considered the primary evaluation and may vary +/- 2 weeks (i.e. done at or 24-28 weeks) due to scheduling or drug administration. With 34 randomized patients in each arm of the group with no prior purine analog, there will be 80% power to detect a difference in MRD rates of 5% vs. 25% 6 months after cladribine with or without rituximab, respectively, a 20% difference, using a Fisher's exact test with a 0.1 one-sided significance level. With 31 randomized patients in each arm of the group with 1 prior course of purine analog, there will be 80% power to detect a difference in MRD rates of 10% vs. 35% 6 months after cladribine with or without rituximab, respectively, a 25% difference. Differences will also be explored using the other tests of MRD, including marrow and blood consensus PCR and RQ-PCR.

Because progression of HCL following exposure to cladribine on this trial is likely to be very slow, it is extremely unlikely that any patients will have progressed within 6 months of randomization to the point of needing treatment; thus, the comparison between patients' MRD outcomes at 6 months with and without rituximab is expected to be done without any bias. In the unlikely event that a patient does receive rituximab within that time frame, or if a rare patient is given rituximab prior to demonstration of MRD, the main analysis will be done using that patient as randomized, despite receiving rituximab prior to the 6 month evaluation. This is a standard intent to treat analysis.

6.2 SECONDARY ENDPOINTS

In addition to the primary goal of determining differences in the MRD level according to rituximab administration, the following goals will also be explored in patients on this study.

The most important secondary endpoints are MRD-free survival after cladribine, and MRD-free survival after up to 2 courses of rituximab. For the secondary endpoints of this protocol, and for triggering delayed rituximab, patients with or without CR are considered MRD+ if the blood FACS is positive or if low blood counts become inconsistent with CR (ANC < 1500/ul, Plt < 100,000/ul, or Hgb < 11). Patients FACS-negative in both blood and bone marrow aspirate are considered MRD-negative regardless of blood counts. Failure of cladribine and/or rituximab to clear MRD by blood FACS requires that this test be positive at 6 months since MRD at 1-3 months may clear by 6 months. However, MRD relapse will be considered when first detected, even if before 6 months, if cladribine and/or rituximab fail to clear MRD at 6 months. If simultaneous cladribine and rituximab is superior to cladribine and delayed rituximab, MRD-free survival after cladribine and after delayed rituximab should together be longer if cladribine was combined with rituximab than if used alone. In order to compare the probability of development of MRD as a function of time according to whether rituximab is used immediately or not, Kaplan-Meier curves for this endpoint will be created, beginning at the date of randomization, and compared using a two-tailed log-rank test. Patients who develop MRD will be considered to have had an event for the actuarial curve, while those without MRD found in this manner will be considered censored and will remain in follow-up for this endpoint. With each stratum considered separately, or if both strata are combined, the power for this comparison will potentially be low, but the results may demonstrate a trend if one is present. Measurement of best response to cladribine vs. cladribine + rituximab will also be done at 1 and 6 months after cladribine. The CR and PR rates will be reported, as well as appropriate 95% exact confidence intervals.

Other secondary endpoints include determining disease-free and overall survival, MRD rates after cladribine and rituximab separately and combined, correlation of MRD rates and tumor markers with clinical endpoints and determining whether cladribine + rituximab can lead to long-term freedom from relapse of MRD or CR/PR, using MRD/tumor marker data to determine when bone marrow biopsy can be avoided in managing HCL, determining if rituximab benefit is different in patients receiving their 1st or 2nd course of purine analog, prospectively evaluating the effect of cladribine and rituximab on normal T- and B-lymphocytes, and studying HCL biology using monoclonal immunoglobulin sequences. When possible, blood will be drawn to obtain RNA for microarray analysis. This will be particularly important in contrasting patients with HCL and HCLv. Finally, to correlate bone marrow MRI signal with bone marrow biopsy, patients will obtain cervical and thoracic spine MRI at baseline and at bone marrow restaging time points, when feasible.

These secondary goals will be determined using appropriate actuarial methods as needed, along with descriptive analyses. Other than the comparisons identified as being of primary interest (comparison of MRD 6 months after starting trial), all of these evaluations will be done with exploratory intent and reported without formal adjustment.

As mentioned in Section **1.3.12** above, the CD25-variant of HCLv has a much poorer prognosis after cladribine than classic HCL and would create a bias if such patients are randomized. Therefore, patients will be initially grouped based on having HCLv vs. HCL, and the HCLv patients will receive concurrent cladribine and rituximab. The first 68 HCL patients with no prior

purine analog and the 62 HCL patients with 1 prior courses of purine analog will be randomized. Based on data in the literature and data from prescreening LMB patients, out of 31 HCLv patients a historical CR rate 3% (1/31) and ORR of 35% (11/31) may be assumed from cladribine alone. If 20 patients are enrolled, and if there are 3 or more CRs in the 20 patients, there is only 7.5% probability this would occur if the true complete response rate were 5%; there is a 90.9% probability that this would occur if the true complete response rate were 25%. Thus, observing 3 or more CRs in 20 patients would provide strong evidence that the true complete response rate was consistent with 25% and more than 5%. If 20 patients are enrolled, and if there are 11 or more responses in the 20 patients, there is only 5.3% probability this would occur if the true response rate were 35%; there is an 87.8% probability that this would occur if the true response rate were 65%. Thus, observing 11 or more responses in 20 patients would provide strong evidence that the true response rate was consistent with 65% and more than 35%.

6.3 ADDITIONAL CONSIDERATIONS

6.3.1 Additional non-randomized 25-patient cohort receiving rituximab plus cladribine

An important secondary objective is to determine if combined cladribine and rituximab are feasible to administer as an outpatient. Enrollment of non-randomized patients to begin rituximab immediately prior to cladribine may facilitate outpatient administration by obviating the time needed for randomization. Also, changing the sequence of first doses of rituximab and cladribine to rituximab first may avoid rapid thrombocytopenia, which could in some cases require inpatient treatment. It is important to verify, however, that the new sequence is non-inferior with respect to the primary endpoint, MRD at 6 mo after cladribine. If after enrolling 34 newly diagnosed HCL patients to immediate rituximab following cladribine, 1 has MRD (currently the number is 1 of 27), then the new 25-patient cohort receiving cladribine immediately after rituximab could be compared as follows: When the sample sizes in the groups are 34 and 25, a two-group test of proportions with a one-sided 0.050 significance level will have 84% power to reject the null hypothesis that the test and the standard are not equivalent (the difference in proportions, is 0.12or farther from zero in the same direction) in favor of the alternative hypothesis that the proportions in the two groups are equivalent, assuming that there is no expected difference in proportions is 0.000 and the proportion in the standard group is 0.97. If 2 of 34 patients have MRD (proportion = 0.94), there would be 81% power to rule out a 16% difference.

6.3.2 Expected accrual

It is expected that 2-3 patients per month may enroll on this trial to enroll a total of 130 patients who are randomized. A total of 20 HCLv and 25 classic HCL patients are allowed who will not be randomized, making a total of 175 evaluable patients enrolled for study intervention. An accrual period of 15 years is anticipated. The accrual ceiling will be 208 to account for inevaluable participants.

7 COLLABORATIVE AGREEMENTS

7.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

CRADA #02753 between Genentech and NCI was executed 11/8/2012 and amended 11/3/2017.

8 HUMAN SUBJECTS PROTECTIONS

8.1 RATIONALE FOR SUBJECT SELECTION

Both men and women from all races and ethnic groups are eligible for this trial.

8.2 PARTICIPATION OF CHILDREN:

HCL is a disease of adults, not children, and it is unlikely that any children will be diagnosed.

8.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **8.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see Section **8.5.1** for consent procedure.

8.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Rituximab may offer improved clinical endpoints when combined with cladribine, including improved response, disease-free and overall survival. Patients may benefit by close follow-up after treatment, particularly if treatment is needed for early relapse.

If rituximab fails to eliminate MRD, it could select for cells deficient in apoptosis and render patients unresponsive to rituximab and other agents when they relapse later in their disease course. Moreover, it is possible that the risk of infection may increase with use of rituximab with cladribine.

8.4.1 Risks from Study Procedures

8.4.1.1 Blood draws

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

8.4.1.2 Urine collection

There is no physical risk involved with urine collection.

8.4.1.1 Bone marrow biopsy

Bone marrow biopsy is minimally invasive and is typically a very safe procedure. Usually hipbone is numbed with anesthesia. Using a needle, the solid and liquid portion of bone marrow is taken out. This procedure causes some pain. Very rarely, infection or bleeding may occur at the needle site.

8.4.1.2 Local anesthesia

Bone marrow biopsy may be done under local anesthesia. Potential side effects of local anesthesia include drowsiness, headaches, blurred vision, twitching muscles or shivering, continuing numbness, weakness or pins and needles sensation.

8.4.1.3 Echocardiogram

There is no physical risk involved with echocardiogram. Side effects of an echocardiogram are discomfort from the transducer being firmly placed against the chest.

8.4.1.4 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

8.4.1.5 Stress test

A stress test is generally safe, and complications are rare. Risks of complication include hypotension, arrhythmias, or myocardial infarction.

8.4.1.6 Pulmonary function tests (PFTs)

PFTs are usually safe for most people. Risks of complication include dizziness, asthma attack, or collapsed lung.

8.4.1.7 Ultrasound

No physical risks are associated with ultrasound procedures.

8.4.1.8 Imaging

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

8.4.1.9 MR Imaging

The risks of MR imaging are relatively small.

Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage. MRIs include the additional risk of damage to hearing.

8.4.2 Risks from Radiation Exposure

On this study, patients will receive up to four CT scans (including screening). The total radiation dose for research purposes will be approximately 5.2 rem. The risk of getting cancer from the radiation exposure in this study is 0.5% and of getting a fatal cancer is 0.3%.

8.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825

Optional blood and bone marrow sample testing will be performed after consenting to the investigator's companion protocol, 10C0066. If the patient refuses to have samples saved for optional testing at that time, the refusal will be documented in the medical record and in the research record.

8.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **8.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **8.5.**

8.5.2 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in Section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies

and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

9 CORRELATIVE STUDIES FOR RESEARCH

9.1 BIOSPECIMEN COLLECTION

9.1.1 Description of data/specimens

9.1.1.1 Blood and bone marrow samples.

Note: Samples collected during the course of this study will be stored, tracked, and disposed of as specified in investigator's companion protocol, 10-C-0066, on which all subjects will be co-enrolled.

Samples collected from patients enrolled prior to Amendment W may be stored, tracked and disposed of as specified in investigator's companion protocol, 10-C-0066 if subject have been co-enrolled.

9.2 RESEARCH BEING CONDUCTED

Malignant cells may be stored to determine sensitivity to recombinant immunotoxins like BL22 or to related agents. Immunoglobulin expression is characterized to better understand origin of disease and prognostic factors. Genetic markers may be cloned to serve as sensitive indicators of minimal residual disease, and serum markers for disease may also be determined. Integrins and other factors on the malignant cells which are known to interact with the microenvironment in leukemias may be studied. PD1 expression by the malignant cells may also be studied, as well as other markers that may allow the malignant cells to suppress the immune system. ELISA assays for inflammatory cytokines, including interleukin 6 and interleukin-1 receptor antagonist, may be done on prospectively saved plasma samples.

9.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

Samples will be stored and cataloged longer than a year, in alarmed freezers at our Leidos Biomedical Research, Inc. contract lab in Frederick, MD, where neutralizing antibodies and PK samples are tested. After closure of the protocol, the samples will either be destroyed, or their storage and use will be governed by a subsequent protocol. Portions of samples which are stored at Frederick National Laboratory for Cancer Research may also be stored and tested in the LMB lab (Building 37) for longer than a year providing there is sample remaining after studies are done.

Samples sent to Amplimmune or MD Anderson will have identifiers removed and may be stored by these institutions for longer than a year before they are destroyed.

Samples at Leidos Biomedical Research, Inc. in Frederick, Maryland will be tracked in a secure electronic database and the PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **10.2**.

10 NIH REQUIREMENTS/ DATA AND SAFETY MONITORING PLAN

10.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

10.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

10.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

10.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

10.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

10.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

10.4.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **10.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

11 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

11.1 ADVERSE EVENT REPORTING TO GENENTECH

- In the event of an adverse event, the first concern will be for the safety of the subject. Investigators are required to report to Genentech Drug Safety any **serious adverse event**, whether **expected** or **unexpected**, regardless of causality to rituximab.
- The Sponsor-Investigator further agrees to forward reports to Genentech of serious adverse events considered to be associated with the use of rituximab.
- All events meeting these criteria will be reported for the time period beginning with any amount of exposure to rituximab through the protocol-defined follow-up period. Serious criteria, definitions, and guidance for reporting follow.
- An **adverse event (AE)** is any untoward medical occurrence in a subject participating in an investigational trial or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.
- **Serious adverse events (SAE)** are adverse events occurring at any dose which meet one or more of the following **serious criteria**:
  - Results in **death** (i.e. the AE caused or lead to death)
  - Is **life-threatening** (i.e. the AE placed the subject at immediate risk of death; it does not apply to an AE which hypothetically might have caused the death if it were more severe)
  - Requires or prolongs inpatient **hospitalization** (i.e. the AE required at least a 24-hour inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion)
  - Is **disabling** (i.e. the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)
  - Is a **congenital anomaly/birth defect** (i.e., an adverse outcome in a child or fetus of a subject exposed to the trial drug prior to conception or during pregnancy)
  - It does not meet any of the above serious criteria but **may jeopardize the subject** and **may require medical or surgical intervention** to prevent one of the outcomes listed above
  - SAEs include any sign, symptom or medical condition that meets any of the above criteria and emerges during rituximab treatment or during a post-treatment follow-up period that (1) was not present at the start of treatment and is not a chronic condition that is part of the patient's medical history, OR (2) was present at the start of treatment or as part of the patient's medical history but worsened in severity and/or frequency during therapy.

- **Expected** adverse events are those adverse events that are **listed** or characterized in the current Investigator Brochure.
- **Unexpected** adverse events are those **not listed** in the current Investigator Brochure or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the Investigator Brochure. For example, under this definition, hepatic necrosis would be unexpected if the Investigator Brochure only referred to elevated hepatic enzymes or hepatitis.

11.1.1 Reporting of Serious Adverse Events Associated with Rituximab

All serious adverse events (SAEs) regardless of causality to rituximab (this applies to both expected and unexpected events) should be recorded on a MedWatch 3500A Form and faxed to:

Genentech Drug Safety

Tel: (888) 835-2555

Fax: (650) 225-4682 or (650) 225-4683

(Please use the safety reporting fax cover sheet attached to this document for your fax transmission.)

Forward all SAEs also to the IRB and PI as directed in Section **10.2**

11.1.2 Adverse Events of Special Interest (AESI)

Adverse events of special interest for this study include the following:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law:
  - Treatment-emergent ALT or AST > 3 ´ ULN in combination with total bilirubin > 2 ´ ULN
  - Treatment-emergent ALT or AST > 3 ´ ULN in combination with clinical jaundice
- Data related to a suspected transmission of an infectious agent by the study drug (STIAMP), as defined below:

Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

11.1.3 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (Section **5**) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)

- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

11.1.3.1 Follow-up information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including subject unique identifiers (i.e. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient unique identifiers are important so that the new information is added to the correct initial report)
- Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above.

11.1.3.2 Study Drug Relationship

The investigator will determine which events are associated with the use of the study drugs. For reporting purposes, an AE should be regarded as possibly related to the use of the investigational product if the investigator believes:

- There is a clinically plausible time sequence between onset of the AE and rituximab administration; and/or
- There is a biologically plausible mechanism for rituximab causing or contributing to the AE; and
- The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

11.2 PRODUCT COMPLAINT REPORTING

11.2.1 Product Complaint

Any written or oral information received from a complainant that alleges deficiencies related to identity, quality, safety, strength, purity, reliability, durability, effectiveness or performance of a product after it has been released and distributed to the commercial market or clinical trial.

11.2.2 Reporting of Product Complaint

For all Investigator Initiated Studies (interventional and non-interventional):

- Product Complaints **with** an AE (adverse event) should be reported via email/fax to:

usds_aereporting-d@gene.com OR 650-238-6067

- Product Complaints **without** an AE should call via:
  PC Hotline Number: (800) 334-0290 (M-F: 5 am to 5 pm PST)
- For Non-Interventional Investigator Initiated Studies:
  us-acmo-d@gene.com

All complaints must be filed within 1 business day for pre-approved products and 15 calendar days for approved products. Complaints can be reported using a Medwatch, CIOMS or any Genentech-approved reporting form (same as SAEs, AESI etc.).

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and the IRB.

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research

objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

13.1 CLADRIBINE (CHLORODEOXYADENOSINE, LEUSTATIN, CDA), FROM ORTHO BIOTECH

13.1.1 Mechanism of action

Deoxycytidine kinase phosphorylates cladribine to CdATP, which incorporates into DNA, leading to DNA strand breaks and inhibition of DNA synthesis.

13.1.2 Toxicities

13.1.2.1 Body as a Whole: fever (69%), fatigue (45%), chills (9%), asthenia (9%), diaphoresis (9%), malaise (7%), trunk pain (6%)

13.1.2.2 Gastrointestinal: nausea (28%), decreased appetite (17%), vomiting (13%), diarrhea (10%), constipation (9%), abdominal pain (6%)

13.1.2.3 Hematologic/Lymphatic: purpura (10%), petechiae (8%), epistaxis (5%), rare myelodysplasia and hemolytic anemia.

13.1.2.4 Hepatic: Reversible, generally mild increases in bilirubin and transaminases.

13.1.2.5 Nervous System: headache (22%), dizziness (9%), insomnia (7%)

13.1.2.6 Cardiovascular System: edema (6%), tachycardia (6%)

13.1.2.7 Respiratory System: abnormal breath sounds (11%), cough (10%), abnormal chest sounds (9%), shortness of breath (7%). Pulmonary infiltrates are usually due to infections.

13.1.2.8 Skin/Subcutaneous Tissue: rash (27%), injection site reactions (19%), pruritis (6%), pain (6%), erythema (6%), rare hypereosinophilia.

13.1.2.9 Musculoskeletal System: myalgia (7%), arthralgia (5%)

13.1.3 Formulation: Vials for i.v. infusion

LEUSTATIN Injection is supplied as a sterile, preservative-free, isotonic solution containing 10mg (1mg/mL) of cladribine as 10 mL filled into a single-use clear flint glass 20 mL vial. LEUSTATIN Injection is supplied in 10 mL (1 mg/mL) single-use vials (NDC 59676-201-01) available in a treatment set (case) of seven vials.

13.1.4 Preparation

Add the calculated dose (0.15 mg/kg) of LEUSTATIN Injection to an infusion bag containing 100 mL of 0.9% Sodium Chloride Injection, USP. Infuse over 2 hours. Repeat daily for a total of 5 consecutive days.

13.1.5 Stability and Storage:

When stored in refrigerated conditions between 2° to 8°C (36° to 46°F) protected from light, unopened vials of LEUSTATIN Injection are stable until the expiration date indicated on the package. Freezing does not adversely affect the solution. If freezing occurs, thaw naturally to room

temperature. DO NOT heat or microwave. Once thawed, the vial of LEUSTATIN Injection is stable until expiry if refrigerated. DO NOT refreeze. Once diluted, solutions containing LEUSTATIN Injection should be administered promptly or stored in the refrigerator (2° to 8°C) for no more than 8 hours prior to administration. Admixtures of LEUSTATIN Injection are chemically and physically stable for at least 24 hours at room temperature under normal room fluorescent light in Baxter Viaflex ® PVC infusion containers. Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised.

13.1.6 Administration Procedures:

Administer i.v. over 2 hours. The infusion time may be changed to 1 hour at the discretion of the PI.

13.1.7 Incompatibilities

The use of 5% dextrose as a diluent is not recommended because of increased degradation of cladribine.

13.1.8 Drug supply

Cladribine will be supplied by the NIH clinical center pharmacy and would not be considered off-label use for 1st and 2nd line treatment of HCL.

13.2 RITUXIMAB (RITUXAN) FROM GENENTECH

13.2.1 Mechanism of action

Rituximab is a highly purified, 1328-amino acid antibody with an approximate molecular mass of 145 kD. The chimeric mouse/human anti-CD20 antibody is a glycosylated IgG_1 κ immunoglobulin containing murine light and heavy chain variable regions and human γ_1 heavy chain and κ light chain constant regions. Rituximab induces apoptosis and either complement or antibody dependent cytotoxicity (ADCC or CDC).

13.2.2 Toxicities (% grade 3, grade 4) in non-Hodgkin's lymphoma

13.2.2.1 Body as a Whole: fever (53, 1%), chills (33, 3%), infection (31, 4%), asthenia (26, 1%), headache (19, 1%), abdominal Pain (14, 1%), pain (12, 1%), back Pain (10, 1%), throat Irritation (9, 0%), flushing (5, 0%)

13.2.2.2 Cardiovascular System: Hypotension (10, 1%), hypertension (6, 1%)

13.2.2.3 Digestive System: Nausea (23, 1%), diarrhea (10, 1%), vomiting (10, 1%)

13.2.2.4 Hematologic and Lymphatic: Lymphopenia (48, 40%), leukopenia (14, 4%), neutropenia (14, 6%), thrombocytopenia (12, 2%), anemia (8, 3%)

13.2.2.5 Metabolic and Nutritional: Angioedema (11, 1%), hyperglycemia (9, 1%), peripheral Edema (8, 0%), LDH Increase (7, 0%)

13.2.2.6 Musculoskeletal System: Myalgia (10, 1%), arthralgia (10, 1%)

13.2.2.7 Nervous System: Dizziness (10, 1%), anxiety (5, 1%)

13.2.2.8 Respiratory System: Increased Cough (13, 1%), rhinitis (12, 1%), bronchospasm (8, 1%), dyspnea (7, 1%), sinusitis (6, 0%)

13.2.2.9 Skin and Appendages: Night Sweats (15, 1%), rash (15, 1%), pruritis (14, 1%), urticaria (8, 1%)

13.2.2.10 Infusion reaction: In RITUXAN rheumatoid arthritis placebo-controlled studies, 32% of RITUXAN-treated patients experienced an adverse event during or within 24 hours following their first infusion, compared to 23% of placebo-treated patients receiving their first infusion. The incidence of adverse events during the 24-hour period following the second infusion, RITUXAN or placebo, decreased to 11% and 13%, respectively. Acute infusion reactions (manifested by fever, chills, rigors, pruritis, urticaria/rash, angioedema, sneezing, throat irritation, cough, and/or bronchospasm, with or without associated hypotension or hypertension) were experienced by 27% of RITUXAN-treated patients following their first infusion, compared to 19% of placebo-treated patients receiving their first placebo infusion. The incidence of these acute infusion reactions following the second infusion of RITUXAN or placebo decreased to 9% and 11%, respectively. Serious acute infusion reactions were experienced by <1% of patients in either treatment group. Acute infusion reactions required dose modification (stopping, slowing or interruption of the infusion) in 10% and 2% of patients receiving rituximab or placebo, respectively, after the first course. The proportion of patients experiencing acute infusion reactions decreased with subsequent courses of RITUXAN. The administration of IV glucocorticoids prior to RITUXAN infusions reduced the incidence and severity of such reactions, however, there was no clear benefit from the administration of oral glucocorticoids for the prevention of acute infusion reactions. Patients in clinical studies also received antihistamines and acetaminophen prior to RITUXAN infusions.

13.2.2.11 Hematologic Events: In clinical trials, Grade 3 and 4 cytopenias [79] were reported in 48% of patients treated with RITUXAN; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following RITUXAN therapy were reported. In addition, there have been a limited number of post-marketing reports of prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia (defined as occurring 40 days after the last dose of RITUXAN) in patients with hematologic malignancies. In reported cases of late onset neutropenia (NCI-CTC Grade 3 and 4), the median duration of neutropenia was 10 days (range 3 to 148 days). Documented resolution of the neutropenia was described in approximately one-half of the reported cases; of those with documented recovery, approximately half received growth factor support. In the remaining cases, information on resolution was not provided. More than half of the reported cases of delayed onset neutropenia occurred in patients who had undergone a prior autologous bone marrow transplantation. In an adequately designed, controlled, clinical trial, the reported incidence of NCI-CTC Grade 3 and 4 neutropenia was higher in patients receiving RITUXAN in combination with fludarabine as compared to those receiving fludarabine alone (76% {39/51} vs. 39% {21/53}) [80].

13.2.3 Formulation: Vials for i.v. infusion

Rituximab is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous (IV) administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for intravenous administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5.

13.2.4 Preparation for Administration:

Use appropriate aseptic technique. Withdraw the necessary amount of rituximab and dilute to a final concentration of 1 to 4 mg/mL into an infusion bag containing either 0.9% Sodium Chloride USP or 5% Dextrose in Water USP. Gently invert the bag to mix the solution. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

13.2.5 Stability and Storage

Rituximab vials are stable at 2° to 8°C (36° to 46°F). Do not use beyond expiration date stamped on carton. Rituximab vials should be protected from direct sunlight. Rituximab solutions for infusion are stable at 2° to 8°C (36° to 46°F) for 24 hours and at room temperature for an additional 24 hours. However, since rituximab solutions do not contain a preservative, diluted solutions should be stored refrigerated (2° to 8°C). No incompatibilities between rituximab and polyvinylchloride or polyethylene bags have been observed.

13.2.6 Administration Procedures

- DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. Infusion and hypersensitivity reactions may occur. Premedication, consisting of acetaminophen and diphenhydramine, should be considered before each infusion of rituximab. Premedication may attenuate infusion-related events. Since transient hypotension may occur during rituximab infusion, consideration should be given to withholding anti-hypertensive medications 12 hours prior to rituximab infusion. See **Appendix E: Guidelines for Rituximab Preparation and Administration** for more information.
- First Infusion: The rituximab solution for infusion should be administered intravenously at an initial rate of 50 mg/hr. Rituximab should not be mixed or diluted with other drugs. If hypersensitivity or infusion-related events do not occur, escalate the infusion rate in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If needed the infusion may be slowed to 25 mg/hr or lower for prolonged periods.
- Rituximab infusion should be interrupted for severe reactions. In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100mg/hr to 50mg/hr) when symptoms have completely resolved. Most patients who have experienced non-life-threatening infusion-related reactions have been able to complete the full course of rituximab therapy
- Subsequent Infusions: If the subject tolerated the first infusion well, subsequent rituximab infusions can be administered at an initial rate of 100 mg/hr, and increased by 100 mg/hr increments at 30-minute intervals, to a maximum of 400 mg/hr as tolerated. If the first

infusion was not well tolerated, the guidelines for the first infusion should be followed for the subsequent infusions.

13.2.7 Incompatibilities: None

13.2.8 Rituximab Overdosage

There has been no experience with overdosage of rituximab in human clinical trials. Single doses higher than 500 mg/m^2 have not been tested in controlled studies.

13.2.9 Supply

Rituximab will be provided free of charge by Genentech and Biogen IDEC. The Sponsor/Investigator of the study will ensure maintenance of complete and accurate records of the receipt, dispensation, and disposal or return of all study drug in accordance with 21 Code of Federal Regulations (C.F.R.), Part 312.57 and 312.62 and Genentech requirements.

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15 APPENDICES

15.1 APPENDIX A: STUDY CALENDAR

For all calendars: Acceptable time deviations: +/- 4 days at week 5, +/- 7 days at week 9, +/- 2 weeks at 3 months, +/- 1 month from 0.5 to 1.5 years, and +/- 2 months thereafter.

15.1.1 Early Rituximab with Cladribine or Cladribine alone

Time point	Pre[1]	d1	d2	d3	d4	d5	w2	w3	w4	w5	w6	w7	w8
Treatment													
Cladribine		X	X	X	X	X							
Rituximab (if receiving)		X					X	X	X	X	X	X	X
Procedures													
Bone Marrow Biopsy[3], MRI[5]	X									X			
Echocardiogram, stress test, PFTs	X												
Abdominal/splenic ultrasound	X												
HCL Imaging[4]	X									X			
EKG	X									X			
History & Physical	X									X			
Laboratory Evaluation													
HBsAg, HBcAB, & HCV, HIV, SPEP/immunoelectrophoresis, serum	X												
24hr urine protein, creatinine clearance & UPEP	X												
FACS, amylase, CRP, lipase, lipid panel, GGT, TSH, free T3, free T4, TSH, ferritin, PCR consensus, IgG, IgA, IgM, D-Dimer, thrombin time, haptoglobin, fibrinogen	X									X			
PT, PTT	X												
Pregnancy test (if relevant)	X												
TBNK[2]	X					X[2]	X[2]	X[2]	X[2]	X	X[2]	X[2]	X[2]
CBC, diff	X	X[9]	X	X		X[11]	X[2]	X[2]	X[2]	X	X[2]	X[2]	X[2]
Urinalysis, chemistries[6]	X	X[2]	X	X		X				X			
Research Evaluations[8]													
Tumor Markers	X					X[2]	X[2]	X[2]	X[2]	X	X[2]	X[2]	X[2]
HLA-A,B,C, DRB,DQB, research blood (PBMCs)	X												
PaxGene	X									X			
Thrombomodulin, P-selectin, plasminogen activator inhibitor type 1, vWF activity, vWF antigen, d-dimer, FDP, fibrinogen, retic, LDH, haptoglobin		X[2,10,11]	X[2,11]	X[2,11]		X[2,11]							

Time point	Pre[1]	d1	d2	d3	d4	d5	w2	w3	w4	w5	w6	w7	w8
Cytokine Panel 13[7]		X[2, 9]	X[2]										

[1] Refer to Section **2.4** for timing of baseline studies. All patients will have baseline testing performed as outlined in this calendar.
[2] When patients are at NIH
[3] At NIH, if not already done within 6 months from starting delayed rituximab aspirate for FACS, PCR-molecular diagnostics, and cloning/sequencing IgH rearrangements.
[4] CT neck-pelvis or abdominal MRI needed only before cladribine. If no nodes, all subsequent imaging may be done with spleen U/S (or abdominal U/S if no spleen), including baseline for delayed rituximab
[5] MRI of C- and T-Spine (only at NIH) may be cancelled at discretion of PI. MRI may be performed on all randomized and HCLv patients. Non randomized HCL classic patients will not have research MRIs performed
[6] Chemistries=albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid
[7] It includes: Tumor Necrosis Factor-alpha, interleukin 2, interleukin 2 receptor soluble, interleukin 12, interferon gamma, interleukin 4, interleukin 5, interleukin 10, interleukin 13, interleukin 17, interleukin 1 beta, interleukin 6, and interleukin 8.
[8] Samples collected during the course of this study will be stored, tracked, and disposed of as specified in investigator's companion protocol, 10-C-0066, on which all subjects will be co-enrolled.
[9] Pre- and 6-8 hour post rituximab administration
[10] Repeat 6-8 hour post rituximab administration
[11] Before cladribine

15.1.2 Delayed Rituximab

Note that calendar resets for each course of delayed rituximab.

Time point	MRD+[1]	w1	w2	w3	w4	w5 +/- 4 days	w6	w7	w8
Treatment									
Rituximab		X	X	X	X	X	X	X	X
Procedures									
Bone Marrow Biopsy[2]	X	X							
HCL Imaging[3]	X								
EKG	X								
History & Physical	X					X			
Laboratory Evaluations									
Amylase, lipase, GGT, lipid panel, TSH, free T3, free T4, ferritin, PCR, IgG, IgA, IgM, haptoglobin, CRP, chemistries[12]	X	X							
FACS[5]	X					X			
Urinalysis	X	X							
CBC, Diff	X	X	X	X	X	X	X	X	X
Research Evaluations[7]									
PaxGene[5]	X					X			
Tumor markers	X	X[6]	X[6]	X[6]	X[6]	X	X[6]	X[6]	X[6]
TBNK	X[6]	X[6]	X[6]	X[6]	X[6]	X	X[6]	X[6]	X[6]

[1] MDR+ is the time point that MRD is detected after cladribine.
[2] New bone marrow biopsy is needed before delayed rituximab if none for >6 months
[3] CT neck-pelvis or abdominal MRI needed only before cladribine. If no nodes, all subsequent imaging may be done with spleen U/S (or abdominal U/S if no spleen), including baseline for delayed rituximab
[4] Chemistries=albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid
[5] Needed if >28 days from last FACS and PaxGene
[6] When patients are at NIH

[7] Samples collected during the course of this study will be stored, tracked, and disposed of as specified in investigator's companion protocol, 10-C-0066, on which all subjects will be co-enrolled

15.1.3 Follow up (Post-Treatment) - After Cladribine or Delayed Rituximab

Time point	**w9 +/- 7 days**	**3m +/- 2 wks**	**6m[10] +/- 1m**	**9m +/- 1m**	**1y +/- 1m**	**15m +/- 1m**	**1.5y +/- 1m**	**21m +/- 2m**	**2y +/- 2m**	**27m +/- 2m**	**2.5y +/- 2m**	**>2.5 y**
Procedures												
Bone Marrow Biopsy[1]			X				X				X	X[5]
MRI[2]			X				X				X	X[5]
HCL Imaging[3]			X				X				X	X[5]
EKG			X				X				X	X[5]
History & Physical			X				X				X	X[5]
Laboratory Evaluations												
GGT, amylase, lipase, lipid panel, TSH, free T3, free T4, TSH, ferritin, PCR consensus, IgG, IgA, IgM, D-Dimer, haptoglobin, CRP, fibrinogen, chemistries[4]			X				X				X	X[5]
Urinalysis			X				X				X	X[5]
CBC, Diff	X	X	X	X	X	X	X	X	X	X	X	X[6]
FACS, TBNK[8]		X	X	X	X		X		X		X	X[7]
Research Evaluations[9]												
PaxGene, Tumor markers		X	X	X	X		X		X	X	X	X[7]

[1] At NIH, aspirate for FACS, PCR-molecular diagnostics, and cloning/sequencing IgH rearrangements
[2] MRI of C- and T-Spine (only at NIH) may be cancelled at discretion of PI. MRI may be performed on all randomized and HCLv patients. Non randomized HCL classic patients will not have research MRIs performed
[3] CT neck-pelvis or abdominal MRI needed only before cladribine. If no nodes, all subsequent imaging may be done with spleen U/S (or abdominal U/S if no spleen), including baseline for delayed rituximab
[4] Chemistries=albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CK, chloride, CO2, creatinine, direct bilirubin, glucose, potassium, LDH, magnesium, sodium, phosphorus, total protein, uric acid
[5] Continue on an every 2 year schedule. Bone marrow biopsy may be skipped for patients no longer in CR.

[6] Continue on an every 6 month schedule while in CR, otherwise every 3 months.
[7] Continue on an annual schedule
[8] Only do TBNK while if patient is at NIH
[9] Samples collected during the course of this study will be stored, tracked, and disposed of as specified in investigator's companion protocol, 10-C-0066, on which all subjects will be co-enrolled
[10] At NIH, unless allowed at another site at the discretion of the PI

15.2 APPENDIX B: EXAMPLES OF DETERMINATION OF REMISSION DURATIONS

(MRD-free survival durations are presented in **Bold**).

A. Randomized to CdA + Rituximab, MRD negative after 2 months, no recurrence. Blood MRD-free survival: **> 23 months**.

↓ (Cladribine)

↓↓↓↓↓↓↓ (Rituximab qWk x8, begin C1D1)

(months from C1D1)

1	2	3			6			9			12			15			18			21			24	
+	-	-			-			-			-			-			-			-			-	

(MRD Status)

B. Randomized to CdA + Rituximab, MRD negative after 6 months, recurrence of MRD at 15 months, delayed rituximab at 16 months, MRD negative at 17 months, no recurrence.

Blood MRD-free survival: **>24 months**.

↓ (Cladribine)

↓↓↓↓↓↓↓ (Rituximab) ↓↓↓↓↓↓↓ (Rituximab)

1	2	3			6			9			12			15			18			21			24	
+	+	+			-			-			-			+	+	-	-	-			-			-

C. Randomized to CdA + Rituximab, MRD negative after 1 month, recurrence of MRD after 2 months, delayed rituximab at 6 months, MRD negative at 12 months, recurrence of MRD at 24 months.

Blood MRD-free survival: **24 months**.

↓ (Cladribine)

↓↓↓↓↓↓↓ ↓↓↓↓↓↓↓ (Rituximab)

1	2	3			6			9			12			15			18			21			24	
-	+	+			+	+	+	+			-			-			-			-			+	

D. Randomized to CdA alone, MRD positive at 3 and 6 months, rituximab given at 6 months, no recurrent MRD

Blood MRD-free survival: **>24 months**.

↓ (Cladribine)

↓↓↓↓↓↓↓ (Rituximab)

1	2	3			6			9			12			15			18			21			24	
+	+	+			+	+	+	-			-			-			-			-			-	

E. Randomized to CdA alone, MRD cleared by 6 months, recurred at 12 months, began rituximab at 12 months, no recurrent MRD.

Blood MRD-free survival: >**24 months**.

↓ (Cladribine)

↓↓↓↓↓↓↓ (Rituximab)

1	2	3			6			9			12			15			18			21			24	
+	+	+			-			-			+	+	+	-			-			-			-	

F. Randomized to CdA alone, MRD cleared by 1 month, recurred at 9 months, began rituximab at 10 months, still MRD+ by 16 months, began 2nd course of delayed rituximab at 17 months, and still MRD- at 24 months.

Blood MRD-free survival: **>24 months**.

↓ (Cladribine)

↓↓↓↓↓↓↓ (Rituximab) ↓↓↓↓↓↓↓ (Rituximab)

1	2	3			6			9			12			15			18			21			24	
-	-	-			-			+		+	+	+			+		+	-			-			-

15.3 APPENDIX C: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

15.4 APPENDIX D: NEW YORK HEART ASSOCIATION CLASSIFICATION

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

15.5 APPENDIX E: GUIDELINES FOR RITUXIMAB PREPARATION AND ADMINISTRATION

15.5.1 How Supplied

Rituximab will be supplied in 50-mL vials containing 500 mg of antibody (50 mL of solution) at a concentration of 10 mg/mL and 10-mL vials containing 100 mg of antibody (10 mL of solution) at a concentration of 10mg/ML. Rituximab vials are sterile, preservative-free, and intended for single use only.

15.5.2 Stability and Storage

Rituximab is biologically and chemically stable at 2°C to 8°C (36°F to 46°F) and has a proposed shelf-life stability of 30 months. Once reconstituted into IV bags, rituximab is chemically stable for up to 24 hours at 2°C to 8°C (36°F to 46°F), followed by up to 24 hours at room temperature (23°C). However, since rituximab solutions do not contain preservative, diluted solutions should be stored refrigerated (2°C to 8°C). No incompatibilities between rituximab and polyvinylchloride or polyethylene bags have been observed. Rituximab vials should be protected from direct sunlight. Rituximab vials are intended for single use only. Do not use beyond the expiration date stamped on the carton.

15.5.3 Dose Calculation

1. Before the first infusion only, calculate the subject's body surface area (BSA). Actual body weight measured within 4 weeks prior to initial treatment with rituximab will be used for calculation of body surface area.

2. Calculate the dose to be administered. The formula for the dose calculation is as follows:

$$\frac{(\text{Subject BSA in } m^2)\ (375 \text{ mg}/ m^2)}{10 \text{ mg/mL}} = \_\_\_ \text{ mL (volume of rituximab for reconstitution)}$$

3. The same volume of rituximab for reconstitution will be used for each subsequent infusion.

15.5.4 Preparation of Rituximab for Intravenous Administration (First Infusion)

1. Using aseptic technique, withdraw the necessary amount of rituximab and dilute to a final concentration of 1 to 4 mg/mL into an infusion bag containing either 0.9% Sodium Chloride, USP, or 5% Dextrose in Water, USP. It is recommended that a dilution of 2 mg/mL be used for ease in calculating dose. Gently invert the bag to mix the solution.

Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

2. **DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.** Do not infuse rituximab concomitantly with another IV solution or other IV medications.

3. The first infusion of rituximab should be administered IV at an initial rate of 50 mg/hr. If hypersensitivity or infusion-related reactions **do not** occur, the infusion rate can be escalated in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If needed the infusion may be slowed to 25 mg/hr or lower for prolonged periods. Such an infusion schedule is listed below for a 1000mg total dose:

Time (Minutes)	**Infusion Rate (mg/h)**	**Dose in 30 minutes (mg)**	**Cumulative Dose (mg)**
0-30	50	25	25
31-60	100	50	75
61-90	150	75	150
91-120	200	100	250
121-150	250	125	375
151-180	300	150	525
181-210	350	175	700
212-240	400	200	900
241-255*	400	200	1000

*Should complete at 255 minutes (4h 15min) to complete a 1000mg total dose.

4. Infusion and hypersensitivity reactions may occur. Premedication consisting of acetaminophen and diphenhydramine should be considered before each infusion of rituximab. Premedication may attenuate infusion reactions. Since transient hypotension may occur during rituximab infusion, consideration should be given to withholding antihypertensive medications 12 hours before rituximab infusion.

5. If a hypersensitivity (non-IgE-mediated) or an infusion reaction develops, the infusion rate should be reduced to half that rate, i.e. from 100 mg/h to 50 mg/h. Subjects who experience a moderate to severe infusion related reaction (fever, chills, or hypotension) should have their infusion interrupted immediately and should receive aggressive symptomatic treatment. The infusion should not be restarted before all the symptoms have disappeared and then the infusion can continue at one-half the previous rate.

6. After the end of infusion, the intravenous line should remain in situ for at least 1 hour in order to be able to administer drugs intravenously if necessary. If there are no adverse events during this period of time, the intravenous line may be removed.

Preparation of Rituximab for Subsequent Intravenous Infusions

1. Using aseptic technique, withdraw the necessary amount of rituximab and dilute to a final concentration of 1 to 4 mg/mL into an infusion bag containing either 0.9% Sodium Chloride, USP, or 5% Dextrose in Water, USP. It is recommended that a dilution of 2 mg/mL be used for ease in calculating dose. Gently invert the bag to mix the solution. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

2. **DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.** Do not infuse rituximab concomitantly with another IV solution or other IV medications. Rituximab infusions should be made through a dedicated line.

3. If the subject tolerated the first infusion well, subsequent study drug infusions can be administered at an initial rate of 100 mg/hr and increased by 100 mg/hr increments at 30-minute intervals to a maximum of 400 mg/hr as tolerated. Such an infusion schedule is listed below. If the subject did not tolerate the first infusion well, the above guidelines for the first infusion should be followed for subsequent infusions.

Time (Minutes)	**Infusion Rate (mg/h)**	**Dose in 30 minutes (mg)**	**Cumulative Dose (mg)**
0-30	100	50	50
31-60	200	100	150
61-90	300	150	300

91-120	400	200	500
121-150	400	200	700
151-180	400	200	900
181-195*	400	200	1000

Should complete at 195 minutes (3h 15 min) to complete a 1000mg total dose

4. Infusion and hypersensitivity reactions may occur. Premedication consisting of acetaminophen and diphenhydramine should be considered before each infusion of rituximab. Premedication may attenuate infusion reactions. Since transient hypotension may occur during rituximab infusion, consideration should be given to withholding antihypertensive medications 12 hours before rituximab infusion.

5. If a hypersensitivity (non-IgE-mediated) or an infusion reaction develops, the infusion rate should be reduced to half that rate, i.e. from 100 mg/h to 50 mg/h. Subjects who experience a moderate to severe infusion related reaction (fever, chills, or hypotension) should have their infusion interrupted immediately and should receive aggressive symptomatic treatment. The infusion should not be restarted before all the symptoms have disappeared and then the infusion can continue at one-half the previous rate.

6. After the end of infusion, the intravenous line should remain in situ for at least 1 hour in order to be able to administer drugs intravenously if necessary. If there are no adverse events during this period of time, the intravenous line may be removed.

15.6 APPENDIX F: LOCAL PHYSICIAN INFORMATION

***Note**: This list is no longer applicable, but it is being kept for historical purposes.*

Physician Name	Facility Name	Facility Address	Facility Contact Number
ArunV. Sheth, MD	Broome Oncology, LLC	169 Riverside Drive Binghamton, NY 13905	Phone:607-798-5307
Mansoor Javeed, MD (no longer participating)	Folsom-Sierra Hematology & Oncology Medical Center	1580 Creekside Dr., Suite 231 Folsom, CA 95630	Phone: 916-962-1544
Rajeev Kulkarni, MD	Upper Valley Medical Center	3130 North Dixie Hwy., Suite 107 Troy, OH 45373	Phone: 937-440-4210
Ann Von Gehr, MD (no longer participating)	Kaiser-Permanente	270 International Circle San Jose, CA 95119	Phone: 408-972-6560
Shamel Sanani, MD	Notrelli Hematology & Oncology	15031 Rinaldi St., Mission Hills, CA 91345	Phone: 818-365-3099
Jonathan Cohen, MD	University of Miami Hospital	1321 NW 14th St., Suite 207 Miami, FL 33125	Phone: 305-324-7000
Mohammad Pazooki, MD (no longer participating)	Medical Oncology & Blood Disorders LLP	100 Haynes Street, 2nd Floor Manchester, CT 06040	Phone: 860-527-5803

Physician Name	Facility Name	Facility Address	Facility Contact Number
Ehab Atallah, MD (no longer participating)	Cancer Center at Froedtert Hospital	9200 W Wisconsin Ave. Milwaukee, WI 53226	Phone: 414-805-6817
Sandeep Mashru, MD	Kaiser Permanente Northwest	3600 North Interstate Ave., Portland, OR 97227-1191	Phone: 503-331-6500
David C. Benton, MD	Maine Center for Cancer Medicine	81 Medical Center Dr. Brunswick, ME 04011	Phone: 207-729-1148
Charanjeev S. Kapoor, MD	Medical Oncology & Blood Disorders LLP	100 Haynes Street, 2nd Floor Manchester, CT 06040	Phone: 860-527-5803
Isaac Levy, MD (no longer participating)	Memorial Hospital-Pembroke	9937 Pines Blvd, Pembroke Pines, FL 33024	Phone: 954-450-1808
John MacNeill, MD	Lynchburg Hematology Oncology	1701 Thompson Dr. Suite 200 Lynchburg, VA 24501	Phone: 434-200-5925
Amy McMullen, MD	Central Coast Oncology Hematology	1669 Dominican Way Santa Cruz, CA 95065	Phone: 831-475-2220
Robin Locke, MD	Harold Alfond Center for Cancer Care	361 Old Belgrade Rd. Augusta, ME 04330	Phone: 207-621-6100

Physician Name	Facility Name	Facility Address	Facility Contact Number
Benjamin Himpler, MD	Hematology-Oncology Associates for Central New York, P.C.	5008 Brittonfield Parkway East Syracuse, NY 13057	Phone: 315-472-7504
Asim Pati, MD	Spartanburg Regional Health Services District, Inc. (affiliate of Spartanburg Medical Center)	380 Serpentine Drive Spartanburg, SC 29303	Phone: 864- 560-7050
Richard McKittrick, MD	The University of Kansas Cancer Center	1000 E 101st Terrace Kansas City, MO 64131	Phone: 913-588-1227