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Randomized Phase II Trial of Rituximab with Either Pentostatin or Bendamustine for Multiply Relapsed or Refractory Hairy Cell Leukemia

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Investigational Agents: none

Commercial Agents: Pentostatin (Nipent™), Bendamustine (Treanda®) and Rituximab (Rituxan®)

PRÉCIS

Background

- Hairy cell leukemia (HCL) is highly responsive to purine analogs cladribine and pentostatin, without evidence of cure. Neither is standard after 2 courses, due to cumulative marrow and T-cell toxicity and declining remission rates and durations. Once resistant, patients after multiple relapses can die of disease-related cytopenias.
- Rituximab alone in 51 patients from 5 trials who had cytopenias and at least 1 prior purine analog resulted in 10 complete + 10 partial remissions (CR+PR= ORR 39%).
- Rituximab with cladribine gives high CR rates in 1st or 2nd line, but is not standard.
- While cladribine use is more common for 1st and 2nd line, pentostatin is often used for subsequent treatment because of < 100% cross-resistance.
- Retrospective published data for pentostatin plus rituximab in HCL include 7 of 7 responses with 6 (86%) CRs, and there are no prospective data.
- Recombinant immunotoxins targeting CD25 (LMB-2) and CD22 (BL22 and HA22) are highly active in purine analog resistant HCL. Palliative pentostatin-rituximab is often used off-protocol for patients with immunogenicity needing more therapy.
- Bendamustine is approved for early treatment of CLL, and is effective with rituximab for relapsed/refractory CLL. Its use in HCL is unreported.
- CRs with minimal residual disease (MRD) by immunohistochemistry of bone marrow biopsy (BMBx IHC), can relapse early. Tests for HCL MRD in blood or marrow include flow cytometry (FACS) or PCR using consensus primers. The most sensitive MRD test in HCL is real-time quantitative PCR using sequence-specific primers (RQ-PCR).
- Of 5 HCL-specific trials listed on Cancer.gov, 2 are phase II trials of cladribine + rituximab in 1st and 2nd line (1 randomized at NIH, 1 non-randomized at MDA), and 3 NIH phase I-II trials of recombinant immunotoxins BL22, HA22 and LMB-2.

Objective

- Primary: To determine if pentostatin + rituximab and bendamustine + rituximab are each associated with adequate response rates (ORR=PR+CR) in patients with relapsed HCL, and, if so, to select which combination is likely to be superior.

Eligibility

- HCL needing therapy, either $\geq$ 2 prior courses of purine analog, 1 course purine analog plus $\geq$1 course rituximab if < 1 year response to the 1 course purine analog, diagnosis of HCL variant (HCLv), or unmutated IGHV4-34+expressing HCL/HCLv.
- Prior treatment, ineligibility for, or patient refusal of recombinant immunotoxin.

Design

- Rituximab 375 mg/m^2 on day 1, 15 for 6 x 28-day cycles (all 72 patients).
- Initial tolerability study: 12 patients receive rituximab + bendamustine (nonrandom), including 6 at 70 mg/m^2 and 6 at 90 mg/m^2 of bendamustine.
- Randomize: 1) 28 patients to bendamustine 90 mg/m^2/day, days 1 and 2 each cycle
  2) 28 patients to pentostatin 4 mg/m^2 days 1 and 15 of each cycle.

- Non-randomize: up to 4 patients to receive either bendamustine 90 mg/m^2/day, days 1 and 2 each cycle or pentostatin 4 mg/m^2 days 1 and 15 of each cycle.
- Statistics: If > 14/28 respond, can conclude with 90% power that response > 40% in that arm. >80% probability of selecting the better arm if true response probability is approximately 40-50% on the inferior arm and ≥15% higher on the superior arm.
- Stratify to equalize the % of patients/arm refractory to last course of purine analog.
- Accrual Ceiling: 72 evaluable participants

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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

Primary: To determine if pentostatin + rituximab and bendamustine + rituximab are each associated with adequate response rates (ORR=PR+CR) in patients with relapsed HCL, and, if so, to select which combination is likely to be superior.

1.1.2 Secondary Objectives

To compare rituximab plus either pentostatin or bendamustine in terms of MRD-free survival, disease-free survival, overall survival and toxicity, including to CD4+ T-cells.

To compare the 2 regimens in crossover when used after failure of the 1st regimen.

To determine if MRD levels and tumor markers (soluble CD25 and CD22, and RQ-PCR) correlate with response and clinical endpoints, and if bone marrow MRI signal correlates with BMBx results, and whether these tests could in some cases possibly replace BMBx.

To study the mechanism of thrombocytopenia after purine analog plus rituximab.

To study HCL biology by cloning, sequencing and characterizing monoclonal immunoglobulin rearrangements, and other genes.

1.2 BACKGROUND AND RATIONALE

1.2.1 Treatment of HCL with cladribine

The most commonly used purine analog for HCL, particularly for 1st and 2nd line treatment, is cladribine, also called 2-chloro-2'-deoxyadenosine, or CdA. Deoxycytidine kinase phosphorylates cladribine to CdATP, which incorporates into DNA, leading to DNA strand breaks and inhibition of DNA synthesis [1]. CdATP also inhibits ribonucleotide reductase, leading to decreased concentrations of deoxyribonucleotides and further inhibition of DNA synthesis [2]. The resistance of cladribine to adenosine deaminase (ADA) allows low catabolism and increased potency [3]. Cladribine induces complete remission (CR) rates of 75-87%, overall response rates (ORR) of 90-100%, and 4 year disease free survivals (DFS) of 79-84% [4-7]. A full course of cladribine may be administered over 5-7 days either by continuous infusion, daily 2-hr infusions, or by subcutaneous injection [5, 8-12].

1.2.2 HCL Epidemiology and diagnosis

HCL is an indolent B-cell leukemia comprising 2% of all leukemias [13, 14], or approximately 900 of the 44,000 new cases of leukemia/year in the US [15]. Patients present with pancytopenia and splenomegaly, and the malignant cells have cytoplasmic projections resembling hairs [16, 17]. By flow cytometry, B-cell antigens FMC7, CD11c, CD20, CD22 and surface immunoglobulin are strongly positive, and in the typical or classic form, CD103, CD25 and CD123 are also positive [18-20]. The median survival of HCL without effective treatment is about 4 years [21], and early treatments including splenectomy and interferon were of modest benefit [22]. Treatment of HCL was revolutionized with the advent of purine analog therapy in the late 1980s.

1.2.3 Pentostatin treatment of HCL

Pentostatin is less commonly used for early treatment of HCL since it is usually administered every other week, usually for 3-6 months [23-26]. Nevertheless, the CR rates of 72-89% [23, 27-32] were indistinguishable from those achieved by cladribine, and responses appeared as durable. Toxicities from pentostatin and cladribine are similar, including neutropenia and fever usually within the first month [33, 34], depressions of CD4+ T-cells for 40-52 months [35, 36], and neuropathy long-term [5, 7, 37]. HCL patients sometimes receive pentostatin after failure of cladribine, due to anecdotal reports of complete remission to cladribine [31, 38-40] or pentostatin [41] alone after failure of the other purine analog. In summary, while pentostatin is rarely used for 1st and 2nd line treatment of HCL, it is often used for later treatment when purine analog is needed.

1.2.4 Lack of evidence for cure in HCL

With over 20 years elapsing since the introduction of purine analogs for the treatment of HCL, there has been no plateau on the disease-free survival curves [5, 6, 33, 42, 43]. Moreover, while 3rd and 4th CRs are common with repeated

courses of purine analogs, the CR rates decline significantly with each successive course, whether or not the same purine analog is used [5, 43]. Because a single course of cladribine or pentostatin is reported to suppress CD4+ lymphocytes below the lower limit of normal for a median of 40 or 54 months, respectively [35, 36], it may not be safe to use repeated courses of purine analogs alone to maintain HCL patients, particularly at short intervals. Clearly, it would be desirable to lengthen the interval needed between purine analog courses by increasing efficacy, and by using alternative agents which are effective but less toxic, either sequentially or in combination.

1.2.5 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 IgG_1 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) [44]. HCL cells are strongly CD20 positive [18]. Case reports [45-49] documented efficacy of rituximab in HCL. CR rates among the 6 reported rituximab studies in HCL (10-25 patients each, total 97) vary from 10 to 54% [50-55]. However, in the 51 patients from 5 studies who demonstrated a need for treatment based on cytopenias and who had at least 1 prior purine analog, there were 10 (20%) CRs and 10 (20%) PRs [50-54]. In the largest single trial enrolling 24 such patients, there were 3 (13%) CRs and 3 (13%) PRs [52]. Rituximab was usually administered by 4-8 weekly doses of 375 mg/m^2 and some patients required more than 4 doses to respond [53]. Toxicities of rituximab in non-Hodgkin's lymphoma (NHL) were infusion-related (hypotension, bronchospasm, rhinitis, pruritus, rash, urticaria, and tumor-pain) and decreased with repeated dosing [56]. Rituximab appears well tolerated in HCL but the number of patients reported in the above trials are few.

1.2.6 Other treatments for HCL

Other agents with significant systemic efficacy in HCL include the recombinant immunotoxin LMB-2 targeting CD25 [57, 58], and BL22, targeting CD22 [59, 60]. LMB-2 and BL22 have 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. HA22, a high affinity variant of BL22, has completed multicenter phase I clinical testing in HCL, with NIH enrolling 21 of 28 patients. The unconfirmed response rate on this trial includes 12 (43%) CRs and 10 (36%) PRs out of 28 patients. HA22 is being prepared for a pivotal trial in HCL.

1.2.7 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 [5, 7]. 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) [61, 62]. Patients with MRD had shorter relapse free survivals compared to those in CR without MRD after either cladribine or pentostatin [61]. The estimated 4-year relapse-free survival was 55% in patients with MRD compared to 88% in patients without MRD. Tallman et al. reported that 13 and 26% of patients treated with cladribine and pentostatin, respectively, had MRD by this definition. The sensitivity of bone marrow biopsy immunohistochemistry (BMBx IHC) has not been published for HCL, but is considered to be 1% [63], and based on the definition above, would depend on the number of CD3+ normal T-cells [61]. Bastie et al. reported an association between > 5% DBA-44+ cells in the bone marrow 5 months after cladribine and relapse [64]. 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 [65]. Early reports suggested that IHC of BMBx was more sensitive than flow cytometry of marrow aspirate and blood [66], but more recent data [67-69] 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 [69]. While consensus PCR can detect 1 HCL cell in 10^3-10^4 normal cells [70], flow cytometry can detect 1 in 10^4-10^5 [69]. 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 [71], 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.

1.2.8 Combination of purine analog with rituximab for HCL

Rituximab combined with either fludarabine [72], or pentostatin [73] has been reported for CLL. Rituximab combined with cladribine has been reported as effective in treating indolent non-Hodgkin's lymphoma (NHL) [74], mantle cell lymphoma [75], and CLL [76]. In a retrospective review, 3 HCL patients received cladribine plus rituximab and 5 received pentostatin plus rituximab after prior therapy with purine analogs [77]. One patient receiving pentostatin plus rituximab achieved PR, and the remaining patients had CR without MRD. In an updated retrospective report, 12 patients received purine analog plus rituximab [43], including 7 with concurrent rituximab plus pentostatin every 2 weeks, 1 with rituximab following pentostatin by 1 month, 2 with cladribine plus rituximab

given weekly, and 1 with rituximab following cladribine by 2 months. Of the 12 patients, 11 (92% CRs) and 1 PR were achieved, and in no cases were the responses less than the previous line of therapy. Of the 12 patients, combination purine analog plus rituximab was administered as 2nd, 3rd, 4th and 6th line therapy in 5, 5, 1 and 1 patients, respectively [43]. This report included neither duration of treatment nor duration of response for patients receiving pentostatin plus rituximab. It also did not state whether the 1 of 12 patients with PR had received pentostatin or cladribine with rituximab, but based on the earlier 2007 report [77], it may be assumed that this patient had pentostatin. Thus, published retrospective data for pentostatin plus rituximab includes 6 CRs and 1 PR out of 7 patients. There are no prospective data of pentostatin plus rituximab for HCL. A phase II trial is currently underway at MD Anderson where patients receive 1st or 2nd-line cladribine by 2 hour i.v. infusion for days 1-5, followed by 8 weekly doses of rituximab beginning on day ~28 [63]. Approximately 28 out of 44 planned patients have been accrued to this trial, and of the first 13 patients, all had CR, including 12 (92%) without MRD [63]. In late March 2009, a prospective randomized trial opened at NCI where patients receive cladribine on day 1 and 8 weekly doses of rituximab beginning either on day 1 or at least 6 months later when MRD is detected in the blood. This trial has quickly accrued 11 patients as of August 2009. These 2 cladribine-rituximab trials are only for 1st and 2nd line treatment and are the only HCL-specific trials listed on Cancer.gov besides trials testing BL22, HA22 and LMB-2. There are no non-immunotoxin trials for multiply relapsed HCL.

1.2.9 Unpublished NCI/LMB data for pentostatin plus rituximab in HCL

In view of the lack of response durability data for patients receiving pentostatin plus rituximab, we decided to collect such data on patients prescreened for or already treated with recombinant immunotoxins. Of 9 such patients who received combination pentostatin-rituximab, response and response duration data were available for 7. The 7 patients previously received 1-6 (median 2) courses of purine analog and were treated with 4-8 (median 6) doses of DCFR. Since bone marrow biopsies were not prospectively obtained on these patients, the 3 endpoints evaluable for these 7 patients included PR, hematologic remission (HR), and MRD-free HR. With respect to normal blood counts, HR required achievement of neutrophils $\geq$ 1500/mm^3, platelets $\geq$ 100/mm^3, and Hgb $\geq$ 11 for at least 4 weeks. PR required HR or at least 50% improvement in neutrophils, platelets and Hgb. MRD-free HR required negative blood flow cytometry in addition to other criteria of HR. Of the 7 evaluable patients, 6 (86%) achieved MRD-free HR and therefore by definition HR and PR as well. Three (42%) of 7 patients remain in MRD-free CR after 6.3-30 (median 6.9) months. The duration of response for the 7 patients is 0-34.5 (median 8.7) months for PR, the same for HR, and 0-30.5 (median 6.0) months for MRD-free HR. Thus, while the number of patients followed was limited, the multiply relapsed HCL patients had high response rates to DCFR, but response durations were usually limited. This

suggests that in the multiply relapsed patient population, it might be feasible to compare response durations produced by DCFR vs. a comparison regimen.

1.2.10 Bendamustine mechanism of action and pharmacology

Bendamustine, synthesized in the 1960s in East Germany, has structural features of both an alkylating drug and a purine analog (Figure 1) [78]. Bendamustine interacts with DNA in a way which is non-cross resistant with other alkylators including cyclophosphamide, carmustine and melphalan [79]. The lack of cross-resistance between bendamustine and other cytotoxic drugs suggested a unique mechanism, or perhaps a combination of mechanisms of action [80]. The specific mechanisms of cytotoxicity identified for bendamustine include 1) activation of DNA-damage stress responses and apoptosis, 2) inhibition of mitotic checkpoints, 3) induction of mitotic catastrophe, 4) activation of a DNA repair pathway involving base excisions, 5) p53-dependent stress pathway initiation leading to apoptosis, and 6) downregulation of genes needed for mitotic checkpoint regulation [80]. Using lymphoma cell lines, bendamustine was synergistic with either cladribine [81] or rituximab [78]. The mean elimination half-life of bendamustine is 49 minutes [82].

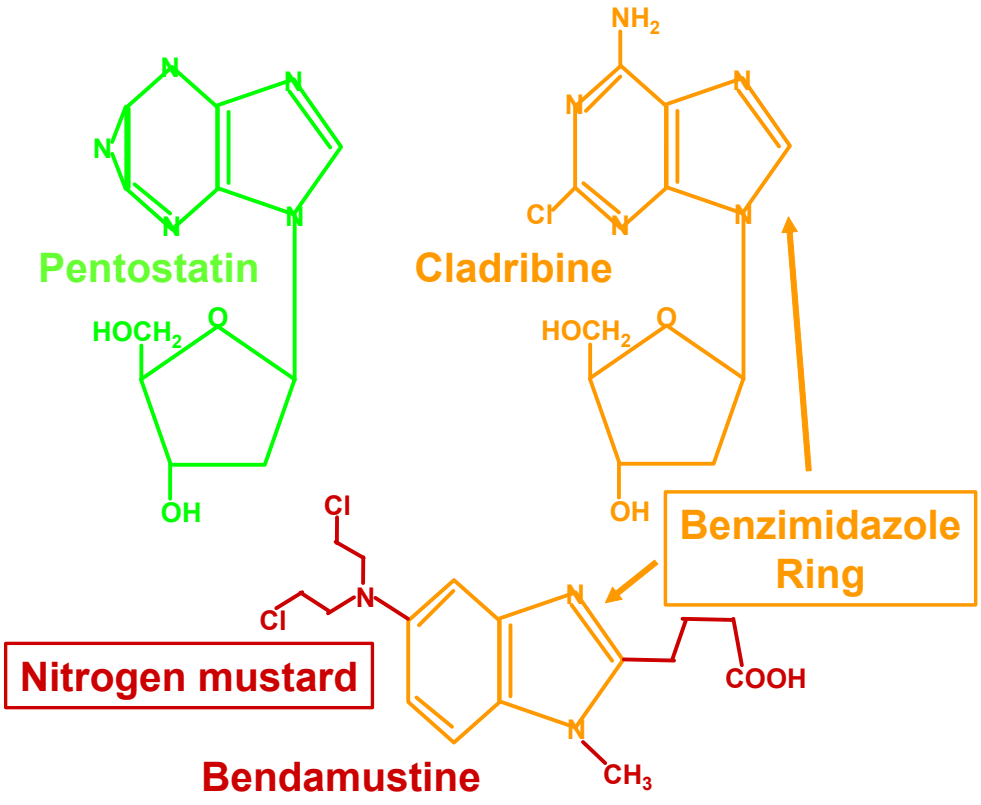


Figure 1: Structure of purine analogs and Bendamustine

1.2.11 Treatment of CLL with Bendamustine with or without rituximab

In a randomized study of 319 previously untreated CLL patients, response rates were 68% for bendamustine vs. 31% for chlorambucil ($p < 0.0001$) [83], leading to approval of bendamustine for newly diagnosed CLL. In this trial, median remission duration was 22 vs. 8 months. The dose of bendamustine used was 100 mg/m^2 days 1-2 every 4 weeks for 6 cycles, and was associated with grade 3-4 toxicity in 40% of patients, including grade 3-4 infections in 8% [83]. A phase II CLL trial of bendamustine 70 mg/m^2 days 1-2 plus rituximab 375 mg/m^2 day 1, with rituximab 500 mg/m^2 for repeat cycles, was associated with an ORR of 77% and CR rate of 15% in the 62 of the 81 patients treated who were evaluable for response [78, 84]. On this trial there were 3 deaths due to infection, including 1 pneumonia, 1 sepsis after diagnosis of Richter's syndrome and 1 urosepsis. An interesting finding from this trial was high response rates in subsets of patients with poor prognostic factors, including unmutated immunoglobulin rearrangements, del(11q22), and del(17p13).

1.2.12 Treatment of NHL with Bendamustine alone

For relapsed/refractory indolent NHL, bendamustine was administered at 120 mg/m2 days 1-2 every 3 weeks. The ORR was 73% with 11% CRs out of 52 patients, median response duration 16 months [85]. Five daily doses of 60 mg/m2 induced a CR rate of 15% with ORR 82% and CR rate 15%. Of 74 evaluable patients with relapsed/refractory indolent and transformed NHL who received bendamustine 120 mg/m2 days 1-2 every 3 weeks x6-12 cycles, the ORR was 77% with 34% CR + CR-unconfirmed (CRu) [86]. Even in the alkylator-refractory patients, the ORR was 61%, indicating non-cross resistance with alkylating agents. Median response duration was 8.3 months for indolent and 4.2 months for transformed NHL. A pivotal trial in 100 patients with rituximab-refractory indolent NHL was conducted with the same dose and schedule for 6-8 cycles [78]. An ORR of 84% with 32% CR+CRu was achieved, and the median response duration was 9.3 months.

1.2.13 Bendamustine + rituximab for indolent NHL

Bendamustine 90 mg/m^2 days 1-2 plus rituximab 375 mg/m^2 every 4 weeks x4 cycles for rituximab-naïve indolent NHL achieved an ORR of 90% with 60% CRs [87]. The median PFS was 24 months, and toxicity included (% of cycles) grade 3-4 leukopenia (16%), thrombocytopenia (3%) and anemia (1%). A follow-up multicenter US trial treated 66 relapsed patients with rituximab 375 mg/m^2 day 1 and bendamustine 90 mg/m^2 days 2-3 every 28 days x4-6 cycles [88]. The ORR was 92% with 41% CRs, 14% CRu, and median duration of response 21 months. A randomized phase III trial compared bendamustine + rituximab with R-CHOP in 315 evaluable patients with untreated indolent and mantle cell NHL [78]. Preliminary analysis of this trial shows similar response rates (ORR 93% vs 93%, CRs 47% vs 42%, respectively. However, toxicity favors bendamustine +

rituximab with lower rates of alopecia (16% vs 94%) and grade 3-4 leukopenia (16% vs 41%).

1.2.14 Secondary malignancies with bendamustine

With toxicity and response showing promising results with and without bendamustine, some investigators have been concerned about the risk of secondary malignancies. This is particularly appropriate because of the combined alkylators and purine analog structural and functional attributes of bendamustine. Two cases each in NHL were reported of myelodysplastic syndrome (MDS) and of myelomonocytic leukemia were reported [78, 86]. It is not clear that these few cases represent an increase risk over and above what was expected from their prior therapy.

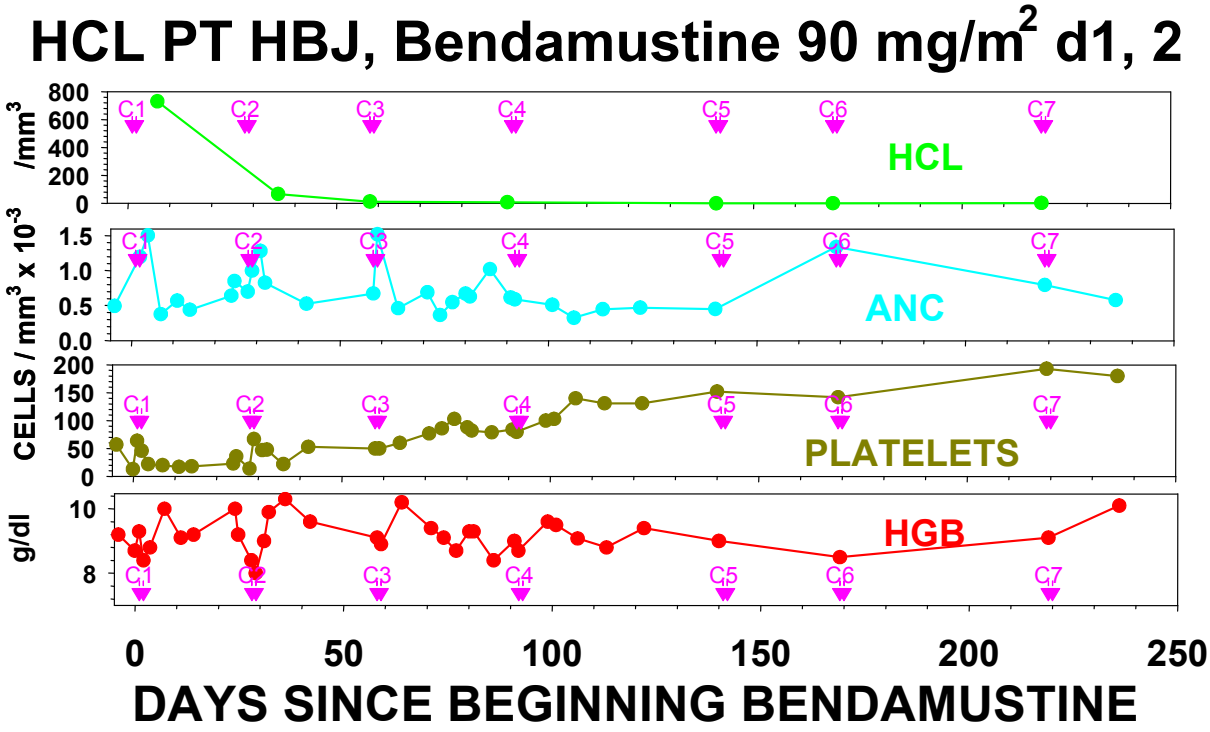


Figure 2: Response of HCL to Bendamustine

1.2.15 Preliminary data on bendamustine in HCL

Although there are no published results of bendamustine in HCL patients at this time, a 57 year old male HCL patient waiting to be enrolled on the HA22 protocol began single-agent bendamustine through his local physician. This patient had previously received treatment with cladribine, splenectomy, rituximab and pentostatin. At the time bendamustine was begun, the patient had a large abdominal mass attributed to HCL, a circulating HCL count of 730 cells/mm^3, and was transfusion dependent for platelets and red cells. As shown Figure 2 above, the transfusion dependence for platelets was resolved after 2 cycles and for red cells after the 4th cycle. The circulating malignant count is 0-1.1 cells/mm^3,

a nearly 99.9% response. Thus, while this patient has not achieved CR, bendamustine was clearly associated with a lifesaving response and was relatively well tolerated. This suggests that bendamustine might have activity in HCL. Moreover, activity might be enhanced when combined with rituximab.

1.2.16 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, some with fatal outcome, 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 disease caused by the reactivation of latent JC virus in the brain. Immunosuppression allows reactivation of the JC virus which causes demyelination and destruction of oligodendrocytes resulting in death or severe disability. Rare cases of PML, some resulting in death, have been reported in patients with hematologic malignancies who have received 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 following the use of rituximab for the treatment of autoimmune diseases. The reported cases had multiple risk factors for PML, including the underlying disease and long-term immunosuppressive therapy or chemotherapy. Most cases of PML were diagnosed 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.

Immunogenicity: Patients may develop a human anti-chimeric antibody (HACA) response with rituximab treatment. The clinical significance of this is unclear.

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 rituximab with either pentostatin or bendamustine

It has now been over 20 years since the advent of purine analogs for HCL [4, 89]. During these last 20 years, purine analogs have kept HCL patients alive without evidence for cure [33]. Therefore, it should not be surprising that multiply relapsed HCL is becoming an increasingly common problem. While many of these patients have been successfully salvaged with immunotoxin therapy [57, 59, 60, 90], other patients have produced neutralizing antibodies or are for other reasons ineligible for this new therapy. The rationale for a randomized trial of pentostatin plus rituximab vs bendamustine plus rituximab is based on the following: 1) Since the Intergroup trial comparing interferon with pentostatin [27], there has not been a randomized trial comparing different agents for HCL, and therefore most treatment recommendations are based on comparisons of small single-arm trials with varying patient populations and inherent bias. 2) The combination of purine analog with rituximab offers very high CR rates and the combination of cladribine plus rituximab is already being tested in the 1st and 2nd line setting [43, 63]. 3) Because pentostatin is < 100% cross-resistant with cladribine [41] and pentostatin use is much less common in the 1st and 2nd line setting, pentostatin may be the more appropriate purine analog to combine with rituximab in the multiply relapsed setting. 4) While published retrospective data on 7 patients suggest that pentostatin plus rituximab is highly active even in patients with multiple relapses [43], there has never been a prospective trial of this combination in HCL, and therefore such a trial needs to be done. 5) While bendamustine with or without rituximab has been reported as effective and well tolerated in a variety of hematologic malignancies [78, 83, 85-88], its use has never been reported in HCL. 6) While examining the activity of pentostatin plus rituximab and bendamustine

plus rituximab in separate cohorts of the same patient population, it would be highly useful and practical to randomize patients to these arms and determine if one regimen is better than the other.

1.3.2 Endpoints by which to evaluate pentostatin + rituximab and bendamustine + rituximab

The initial goal of this trial is simply to determine the response rates in each arm separately, and specifically to determine if response is higher than the 39% expected with rituximab alone. Once each arm meets its accrual goals, the best regimen may be selected using a selection design, described in more detail in the statistical section. Briefly, there is at least 80% probability of selecting the correct arm if the differences in rates of response are at least 15%, i.e. 40% vs. 55%, 45 vs. 60%, and 50% vs. 65%. Using a 2-tailed p-value to compare rates of response, there is at least 80% power to discriminate 36-39% differences in response rates, providing the response rates being compared are between 18 and 82%. While ORR (CR+PR) is normally used to compare arms, more stringent measures of response can be used if necessary, including CR or MRD-free CR rates, and rates of response lasting at least 6 months. Thus, despite the limited patient numbers, this trial is reasonably sized to determine a difference between the 2 regimens. If no difference is observed, the trial will still be very valuable since other factors could guide physicians on which regimen to use for a given patient.

1.3.3 Need to stratify patients with regard to prior purine analog resistance

In randomized trials of this size, it is possible to stratify for one variable so that the data will have bias minimized. It was felt that the most important risk factor would be whether or not patients were refractory to purine analog. This stratification will also include patients with untreated poor-risk disease, namely HCLv and unmutated IGHV4-34+ HCL.

1.3.4 Rationale for feasibility of accrual goals

We have a large and growing multiply relapsed patient population which should be eligible for this trial. A recent survey of 98 patients treated with LMB-2, BL22 or HA22 indicated the following: 1) 44 patients could have received combination purine analog-rituximab after immunotoxin, 2) 18 patients have already received a purine analog after immunotoxin, many of them pentostatin-rituximab, and 3) 34 patients are likely to need pentostatin-rituximab or a similar regimen in the next 1-2 years. Of approximately 100 patients prescreened for immunotoxin protocols, about 10 patients are now in need of a regimen like pentostatin-rituximab and many more are being followed for anticipated need soon. We consider this trial much easier to accrue for compared to the randomized trial of cladribine with or without rituximab for 1st and 2nd line treatment of HCL. This trial has accrued 11 patients in under 5 months, 5 of whom were newly diagnosed and 6 of whom had one prior course of cladribine. It is far easier to find patients with several courses of purine analogs and/or prior rituximab who would be eligible for this trial, since most patient calls we receive are in this category.

1.3.5 Lack of potential conflict with other HCL trials

At this time there are no HCL-specific clinical trials for multiply relapsed HCL except those within LMB, namely LMB-2 (06-c-0150), BL22 (09-c-0076) and HA22 (07-c-0130). The HA22 phase I protocol has completed accrual and we plan to open a phase II HCL trial at NIH while planning proceeds on an international pivotal trial in HCL. The BL22 trial is only for patients who have had prior LMB-2, BL22 or HA22 without immunogenicity who could benefit by more BL22, a small patient population. The LMB-2 trial is mainly for patients with prior HUS with BL22, also a small population. Patients with prior immunotoxin may not enroll on the HA22 trial. Patients with antibodies, either pre-existing or post immunotoxin, are not eligible for our immunotoxin trials. Therefore, this trial fills a large gap in our HCL program where a significant percentage of our patients may continue treatment on a protocol whereas otherwise they could not.

1.3.6 Following tumor markers in HCL patients

We have developed several novel assays quantifying tumor burden in HCL, including soluble IRTA [91, 92] 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 [93, 94], 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. We already have considerable tumor marker data after treatment with rituximab, cladribine, and/or immunotoxins, and believe such data after the agents on this trial will be very useful. We have also determined MRD by PCR using sequence-specific primers (RQ-PCR), and plan to prospectively study protocol patients with this assay. 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.

1.3.7 Mechanism of thrombocytopenia after purine analog and rituximab

We have found that patients have an immediate and usually short-lived thrombocytopenia (median ~50,000 cells/mm^3) after receiving purine analog followed by rituximab the same day [95, 96]. We have not seen significant bleeding associated and it is not clear whether platelet margination or consumption is occurring. To investigate the mechanism of this interaction, we

are prospectively drawing coagulation studies as well as plasma which can be used later for cytokine assessment.

1.3.8 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 [57-60]. 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 [71]. The lab is also studying the biology of HCL through patient samples obtained, and has the largest molecular database of HCL patients reported [97-99]. 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 [69, 71]. Finally, this trial can benefit the LMB immunotoxin program by giving patients an option for treatment should immunotoxin not work or be complicated by immunogenicity. We believe these improved options will enhance our accrual for all HCL trials.

1.3.9 Inclusion of patients with HCLv or unmutated IGHV4-34+ HCL

Patients with HCLv or unmutated IGHV4-34+ HCL have been shown to have poor response to cladribine alone for newly diagnosed disease, with ORR <50% and CR <10%, and overall survival from diagnosis is much shorter as well [100]. These patients are not eligible for BRAF/MEK therapy since they are always wild-type for the BRAF V600E mutation [101] We are able to get these patients into complete remission with 1st or 2nd line treatment with cladribine and rituximab. While these remissions can last several years, they often relapse with clinically more aggressive disease. Patients with multiply relapsed HCLv or unmutated IGHV4-34+ HCL have benefitted by this protocol using either Bendamustine-rituximab or Pentostatin-rituximab, but often relapse with more rapidly progressive disease. We believe these patients should be eligible for this protocol regardless of prior therapy. They may be stratified within the existing stratification for resistant HCL/HCLv.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Evidence of HCL by flow cytometry of blood or a solid (lymph node) mass, confirmed by the Laboratory of Pathology, NCI, including positivity for CD19, CD22, CD20, and CD11c. Patients with flow cytometry consistent with HCL

variant (HCLv) are eligible, including those with CD25 and/or CD103 negative disease.

2.1.1.2 BMBx or BMA consistent with HCL, confirmed by NIH Laboratory of Pathology, NCI, or the Department of Laboratory Medicine, Clinical Center, NIH, unless the diagnosis can be confirmed from a solid (lymph node) mass.

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 cladribine 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 cells/µL
- Symptomatic splenomegaly.
- Enlarging lymph nodes > 2cm.
- Repeated infections requiring oral or i.v. antibiotics.
- Increasing lytic bone lesions

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 One of the following:

- At least 2 prior courses of purine analog
- 1 prior course of purine analog plus ≥1 course of rituximab if the response to the course of purine analog lasted < 1 year.
- Diagnosis of HCL variant (HCLv)
- Unmutated (>98% homology to germline) IGHV4-34+expressing HCL/HCLv

2.1.1.5 ECOG performance status [102] of 0-3 (see **Appendix A**).

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

2.1.1.7 Creatinine ≤ 1.5 or creatinine clearance ≥ 60 ml/min.

2.1.1.8 Bilirubin ≤ 2 unless consistent with Gilbert's (total/direct > 5), ALT and AST ≤ 3 x upper limits of normal.

2.1.1.9 No other therapy (i.e. chemotherapy, interferon) for 4 weeks prior to study entry, or cladribine for 6 months prior to study entry, unless progressive disease more than 2 months after cladribine is documented.

2.1.1.10 Age at least 18

2.1.1.11 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.

2.1.1.12 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 B**).

2.1.2.3 Known infection with HIV, hepatitis B or C.

2.1.2.4 Pregnant or lactating women.

2.1.2.5 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.

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

2.1.2.7 Presence of CNS disease

2.1.2.8 Patients with history of non-response to both pentostatin plus rituximab and to bendamustine plus rituximab.

2.1.2.9 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. 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

Recruitment of patients from outside NIH is facilitated by multiple ongoing trials at NIH. Many patients find about NIH trials from NIH websites, such as https://clinicaltrials.gov/ or NIH social media, or from other patients through social media.

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 **9.6.2**.

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.

2.2.3 Tests and procedures for evaluation (needed for eligibility)

2.2.3.1 Within 6 months before starting drug

- BMBx with IHC for CD20 and CD3. BMBx must have been done since last prior therapy and at least 6 months after prior cladribine.
- BMA for FACS and PCR (if evaluable). Blood PCR should be done at the same time as BMA PCR.

2.2.3.2 Within 3 months before starting drug

- Hepatitis B (HBcAB and HBsAg), hepatitis C and HIV test
- 24hour urine for creatinine clearance
- Direct bilirubin, total bilirubin, UPEP and SPEP

2.2.3.3 Within 7 days before starting drug

- Pregnancy test (urine or serum). Women of childbearing potential must have a negative pregnancy test.
- CBC with differential
- Chemistries including acute care panel (sodium, potassium, chloride, CO2, glucose, creatinine, and BUN), mineral panel (calcium, magnesium, phosphorus and albumin), hepatic panel (AST, ALT, bilirubin, alkaline phosphatase), lipid panel (cholesterol, triglycerides, HDL, LDH), direct bilirubin, total protein, creatine kinase (CK), uric acid, LDH, and urinalysis
- History & Physical with documented performance status

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 **Treatment Assignment and Randomization/Stratification Procedures**

Cohorts

Number	Name	Description
1	Cohort 1 (Dose escalation)	Patients with HCL needing therapy, HCLv or unmutated IGHV4-34+ HCL/HCLv enrolled directly to Arm 1 or 2 for initial tolerability study; patients with relapse/no response may crossover to receive rituximab + pentostatin (Arm 4). (Up to 12 evaluable patients) (closed)
2	Cohort 2 (Dose expansion; randomized)	Patients with HCL needing therapy, HCLv or unmutated IGHV4-34+ HCL/HCLv to be randomized, stratified based upon response to prior purine analog, and to be enrolled to Arm 3 or 4; patients with relapse/no response may crossover to the other arm. (Up to evaluable 56 patients; 28 in each arm)
3	Cohort 3 (Dose expansion; non-randomized)	Patients with HCL needing therapy, HCLv or unmutated IGHV4-34+ HCL/HCLv with prior non-response to either pentostatin plus rituximab or to bendamustine plus rituximab not to be randomized and to be enrolled directly to Arm 3 or Arm 4 (Up to 4 evaluable patients)

Arms

Number	Name	Description
1	Arm 1	Rituximab +bendamustine at 70 mg/m^2 for initial tolerability study (closed)
2	Arm 2	Rituximab +bendamustine at 90 mg/m2 for initial tolerability study (closed)
3	Arm 3	Rituximab + bendamustine (at the tolerated dose)
4	Arm 4	Rituximab + pentostatin

Stratifications

Name	Distinct Options	Notes
Response to previous purine analog	Refractory to therapy Non-refractory to therapy	Refractory to therapy also includes untreated poor-risk disease, namely HCLv and unmutated IGHV4-34+ HCL (i.e., unknown)

Randomization and Arm Assignment

At the time of registration for treatment, patients with HCL needing therapy, HCLv or unmutated IGHV4-34+ HCL/HCLv are assigned to:

- Cohort 1: 12 patients in this cohort will not be randomized and will be assigned to Arms 1 or 2 for initial tolerability studies.
- Cohort 2: 56 patients in this cohort are randomized on a 1:1 basis to Arm 3 or 4, to begin either bendamustine plus rituximab or pentostatin plus rituximab, stratifying for prior response to purine analog. The randomization 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. Patients who relapse from or have no response to one arm may cross over to the other arm providing they meet initial eligibility criteria. Patients with progressive disease to one arm may cross over to the other arm at least 2 months after the last dose of purine analog, while those with stable disease after one arm may cross over to the other arm at least 6 months after the last dose of purine analog.
- Cohort 3: Four patients in this cohort, with history of non-response to either pentostatin plus rituximab or to bendamustine plus rituximab will not be randomized and will receive the other regimen on protocol but will not be considered for the primary endpoint of the study.

2.4 BASELINE EVALUATION

2.4.1.1 At the time of enrollment for treatment

- Blood for cloning and sequence immunoglobulin rearrangements.

2.4.1.2 Within 6 months before drug

- MRI Cervical and Thoracic (C- and T-) Spine to correlate the status of the BMBx with the vertebral BMA signal by MRI. May be cancelled at the discretion of the PI, including if patient unable to get MRI at NIH and not covered by insurance outside NIH.

2.4.1.3 Within 3 months before drug

- Thrombin time, Free T4 & T3, D-dimer, beta-2 microglobulin
- 24-hour urine for protein if measurable
- EKG
- Echocardiogram and stress test
- Pulmonary Function Test (PFTs)
- CT neck-pelvis or abdominal MRI.
- Abdominal Ultrasound (U/S) will be done to exclude lymph nodes potentially impacting the biliary tree, and for baseline spleen size which can be subsequently followed by spleen U/S.

2.4.1.4 Within 2 weeks before drug

- Blood FACS, and citrate tube for consensus PCR, IgG, IgA, IgM, haptoglobin, Ferritin, fibrinogen PT, PTT, Lipid panel (HDL, LDL, Cholesterol, Triglyceride), GGT, CRP, TSH, Triiodothyronine, Amylase, Lipase.

2.4.1.5 Within 1 week before drug

- TBNK (B-cell, CD4_CD8+ and NK cells) Pre Tx draw will suffice for cycle 1. May skip pre-cycle blood FACS if neg x1. TBNK needed only on patients at NIH.
- EKG

2.4.1.6 Within 0-1 days before drug

- CBC, diff
- Acute care panel, mineral panel, hepatic panel, LDH, direct bilirubin, CK, uric acid, and total protein.
- Urinalysis
- TBNK
- Any of these tests performed for screening purposes (Section **2.2.3.3**) within 0-1 days before drug may also count for baseline purposes.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Overview

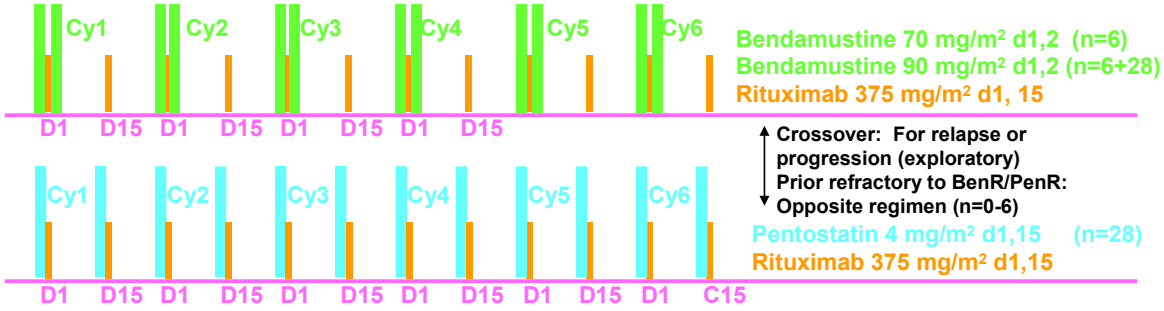
This is a randomized study of pentostatin plus rituximab versus bendamustine plus rituximab for multiply relapsed HCL. The primary objective of the study is to determine if response in each arm is higher than the 39% expected from rituximab alone, and to determine which regimen might be better. To determine whether the proposed dose of bendamustine plus rituximab is well tolerated in HCL, 6 non-randomized patients will initially receive bendamustine 70 mg/m^2 days 1-2 plus rituximab 375 mg/m^2 days 1 and 15 for six 4-week cycles. An additional 6 patients will receive the same regimen with 90 rather than 70 mg/m^2 of bendamustine. Randomization will begin using the higher of the 2 dose levels of bendamustine providing 5-6 of the 6 patients can tolerate the 6 cycles without delays > 4 weeks. If neither dose level is tolerated to this extent, the trial may need to be amended prior to randomization. If tolerance of the 90 mg/m^2 dose level of bendamustine is acceptable, subsequent patients will be randomized to receive either this dose of bendamustine + rituximab (n=28), or pentostatin 4 mg/m^2 plus rituximab 375 mg/m^2 days 1, and 15 for six 4-week cycles (n=28). Once randomization begins, patients will be stratified to equalize the percent of patients in each arm who were refractory to their last course of purine analog, meaning lack of response lasting at least 1 year. This stratification will also include patients with untreated poor-risk disease, namely HCLv and unmutated IGHV4-34+ HCL.

3.1.1.1 Crossover

Patients who relapse from or have no response to one arm may cross over to the other arm providing they meet initial eligibility criteria. Patients with progressive disease to 1 arm may cross over to the other arm at least 2 months after the last dose of purine analog,

while those with stable disease after 1 arm may cross over to the other arm at least 6 months after the last dose of purine analog. Up to four patients with a history of non-response to 1 arm before enrollment may be treated in the other arm without being randomized, and will not be able to cross over. These non-randomized patients will not be considered for the primary endpoint of the study.

3.1.2 Schema



3.1.3 Definition of MRD positivity

To determine MRD rates for the secondary endpoints, the following 3 CLIA-certified MRD tests will be used:

- Blood FACS and consensus PCR
- Bone marrow aspirate (BMA) FACS and consensus PCR
- Bone marrow biopsy immunohistochemistry (BMBx IHC)
- Patients will be considered blood MRD-free providing the blood FACS is negative and the blood counts are consistent with CR (ANC $\geq$ 1500/mm^3, Plt $\geq$ 100,000/mm^3, and Hgb $\geq$ 11 g/dL). 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. Flow cytometry studies which are not positive (i.e. suspicious for MRD) will be considered negative for protocol purposes. 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

- 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

Participants will receive treatments at NIH.

3.2.1 Bendamustine by 30-60 minute infusion days 1-2 at 70 mg/m^2/dose for 1st 6 patients, and 90 mg/m^2/dose for additional patients.

3.2.2 Pentostatin 4 mg/m^2 by rapid (<45 min) infusion days 1 and 15.

3.2.3 Rituximab 375 mg/m^2 i.v. infusion days 1 and 15. When rituximab is given on the same day as bendamustine or pentostatin, it will be given after the other drug. Actual body weight measured within 4 weeks prior to initial treatment will be used for calculations of body surface area. See **Appendix C: Guidelines for Rituximab Preparation and Administration** for infusion times.

3.2.4 Cycles will repeat every 28 days (+/- 2 days).

3.2.5 The day 15 dose of Pentostatin and/or Rituximab may be given on day 13 or 14 if needed for logistical reasons.

3.3 DOSE MODIFICATIONS

3.3.1 Dosing may be delayed up to 4 weeks for toxicity or logistical reasons, including inability of the patient to get to clinic.

3.3.2 Patients with excessive delay (delay > 4 weeks) or dose-limiting toxicity (DLT) are off-treatment and may be followed but not retreated with the current regimen. They will receive the cross-over regimen if and when eligible.

3.3.3 Treatment with rituximab or bendamustine 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 anaphylaxis or hypersensitivity reactions to rituximab may receive desensitization using standard methods [103], but otherwise should be removed from treatment.

3.3.4 If creatinine resolves to grade >1, the following pentostatin doses should be used:

If eGFR is:
>40 and ≤60: treat with 3 mg/m^2 of pentostatin
>20 and <40: treat with 2 mg/m^2 of pentostatin

≤20: treat with 1 mg/m^2 of pentostatin

eGFR may be obtained from CRIS or it may be calculated using the following formula:

$$\frac{(140\text{-age})(\text{weight in Kg})(0.85 \text{ if female})}{(72)(\text{Serum creatinine in mg/dL})}$$

3.3.5 DLT criteria: Grade III-IV bendamustine, pentostatin or rituximab related toxicity except:

- Grade 3-4 hematologic toxicity resolving in 8 weeks.
- Grade 3-4 lymphopenia, leukopenia, or CD4 reduction.
- Hematologic toxicity managed with platelet transfusion and/or other support.
- Grade 3 toxicity resolving within the 4-week allowable treatment delay.
- Grade 4 AST, ALT and GGT for < 5 days
- Allergic reactions prevented by desensitization.
- Grade 3-4 tumor lysis syndrome with significant decrease in tumor burden. Patients may continue without dose reduction providing laboratory abnormalities resolve to grade 0-1 or baseline within the 4-week allowable delay.

3.3.6 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.

3.4 STUDY CALENDAR

3.4.1 Randomization and Initial Crossover Treatment Calendar

	Randomization and Initial Crossover Treatment												
	Screening/Baseline (time interval before drug)						**Treatment**						
							Cycles 1-6					**Pre Cycle 4 (at NIH)**	**End of Tx[12]**
Time point	**6 months**	**3 months**	**2 weeks**	**1 week**	**0-1 days**	**Anytime**	**D1**	**D2**	**D3**	**D5**	**D15**		
Treatment													
Bendamustine							X	X					
Pentostatin							X				X		
Rituximab							X				X		
Procedures													
NIH Advance Directives Form						X[11]							
Bone Marrow Biopsy/aspiration[1], Blood PCR[2]	X											X	X
Echocardiogram, stress test, PFTs		X											
HCL Imaging[4]		X										X[5]	X
EKG		X		X[7]								X	X
History & Physical				X[7]								X	X
MRI Cervical and Thoracic Spine[3]	X											X	X
Laboratory Evaluations													
HBsAg, HBcAB, & HCV, HIV		X											
24hr urine protein, creatinine clearance and protein		X											

	Randomization and Initial Crossover Treatment												
	Screening/Baseline (time interval before drug)						**Treatment**						
							Cycles 1-6					**Pre Cycle 4 (at NIH)**	**End of Tx[12]**
Time point	**6 months**	**3 months**	**2 weeks**	**1 week**	**0-1 days**	**Anytime**	**D1**	**D2**	**D3**	**D5**	**D15**		
Direct bilirubin, UPEP and SPEP		X											
Thrombin time, Free T4 & T3, D-dimer, Beta-2 microglobulin		X											
Pregnancy test (if relevant)				X									
IgG, IgA, IgM, IFE[13], haptoglobin, ferritin, fibrinogen, PT, PTT, Lipid panel, GGT, CRP, TSH, Triiodothyronine, Amylase, Lipase			X									X	X
TBNK				X	X		X[7]				X[7, 15]	X[7]	X[7]
CBC, diff				X	X		X	X[7]	X[7]	X[7]	X[15]	X	X
Chemistries[8]				X	X		X[7]				X[7,14]	X	X
Urinalysis				X	X		X[7]						
Research Evaluations[15]													
Blood for cloning and sequencing immunoglobulin rearrangements[15]						X							
Blood FACS			X[7]				X[6]					X	X
Coagulation labs[9] and Cytokine labs[10]							X[7,15]	X[7,15]					

[1] At NIH, aspirate for FACS, PCR-molecular diagnostics, and RQ-PCR.
[2] Blood PCR should be done at the same time as BMA PCR.

[3] MRI of C- and T-Spine (only at NIH) Spine to correlate marrow uptake in vertebra with response status, may be cancelled at discretion of PI.
[4] CT neck-pelvis or abdominal MRI for Baseline Evaluation. If no nodes, subsequent imaging may be done with spleen or abdominal U/S, abdominal MRI, or CT. If nodes >2cm in short axis are present, subsequent imaging should include CT or MRI of that area.
[5] Pre-cycle 2 and 3 on patients at NIH
[6] Pre-cycle FACS, may be done 0-4 days before drug, and FACS may be skipped if negative x1.
[7] When patients are at NIH
[8] Chemistries=Acute Care Panel, Mineral Panel, Hepatic Panel, lipid panel, total protein, direct bilirubin, CK, Uric Acid. Precycle day 1 chemistries may be done 0-2 days before drug.
[9] Cycle 1: Coagulation labs: Thrombomodulin, P-selectin, plasminogen activator inhibitor type 1, vWF activity, vWF antigen, d-dimer, fibrin degradation products (FDP), fibrinogen, retic, LDH, and haptoglobin, to be drawn day1 and 2 (1st 5 on ice)
[10] Cycle 1: Sodium Heparin Green Top Tubes for cytokines are drawn Pre-Bendamustine or Pre-Pentostatin, Pre-Rituximab, and 2, 4 and 8 (each +/- 1) hours from the start of the Rituximab Infusion, then next AM, each to be placed in Dr. Kreitman's bin in the refrigerator until picked up by Research Nurse or other appropriate staff. Draw 1 extra if rigors or temp >38.3.
[11] As indicated in Section **9.3**, all subjects ≥ age 18 will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended but is not required.
[12] End of treatment visit will occur approximately 30 days after the last dose of study drug. If the patient cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs from a local physician or laboratory. If this is not possible, patients may be assessed by telephone for symptoms.
[13] IFE, Serum immunofixation electrophoresis and free light chains when drawn at NIH.
[14] Acute Care Panel only for patients receiving Pentostatin; it may be done 0-2 days before receiving Pentostatin. Patients receiving Bendamustine do not need chemistries on day 15.
[15] 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.

3.4.2 Follow-up Calendar (for patients waiting to cross over or patients who have not developed progressive disease).

	Follow-up								
Time point	**9mo**	**1yr[1]**	**15mo[1]**	**1.5yrs[1]**	**21mo[1]**	**2yr[1]**	**27mo[1]**	**2.5yrs[1]**	**>2.5yrs[1]**
Procedures									
Bone Marrow Biopsy/aspiration, Blood PCR[2]				X				X	X[4,5]
MRI Cervical and Thoracic Spine[3]				X				X	X
HCL Imaging[6]				X				X	X[4]
EKG				X				X	X[4]
Laboratory Evaluations									
GGT, PT, PTT, fibrinogen, IFE[11], quantitative immunoglobulins, lipid panel, CRP, ferritin, TSH, Free T3, Free T4, amylase, lipase, haptoglobin				X				X	X[4]
TBNK[10]		X		X		X		X	X[8]
CBC, diff	X	X	X	X	X	X	X	X	X[8]
Urinalysis, chemistries[7]				X				X	X[4]
Research Evaluations[12]									
Blood FACS		X		X		X		X	X[9]

[1]From the beginning of initial treatment, acceptable time windows for follow up procedures may be +/- 1 month up to 1.5 y, then +/- 2 mo. Time points will remain from initial treatment even if end of treatment is before the 6-month time point.
[2]At NIH, aspirate for FACS, PCR-molecular diagnostics, and RQ-PCR. Blood PCR should be done at the same time as BMA PCR.

[3]MRI of C- and T-Spine (only at NIH) Spine to correlate marrow uptake in vertebra with response status, may be cancelled at discretion of PI.
[4]Continue every 2 years
[5]May delete bone marrow if not in CR.
[6]CT neck-pelvis or abdominal MRI needed only for baseline. If no nodes, subsequent imaging may be done with spleen or abdominal U/S, abdominal MRI, or CT. If nodes >2cm in short axis are present, subsequent imaging should include CT or MRI of that area and imaging is optional if no spleen at baseline.
[7]Chemistries=Acute Care Panel, Mineral Panel, Hepatic Panel, LDH, total protein, CK, Uric Acid
[8]Continue every 6 months for those in CR, otherwise every 3 months
[9]Continue every year
[10]When patients are at NIH
[11]IFE, Serum immunofixation electrophoresis and free light chains when drawn at NIH
[12] 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.

3.5 PROTOCOL EVALUATION

3.5.1 Evaluation during study

- Day 1 and 2 of 1st cycle (if at NIH): Coagulation labs (Thrombomodulin, P-selectin, plasminogen activator inhibitor type 1, vWF activity, vWF antigen, d-dimer, fibrin degradation products {FDP}, fibrinogen, retic, LDH, and haptoglobin, 1st 5 on ice) are drawn days 1 and 2.
- Sodium Heparin Green Top Tubes for cytokines are drawn Pre-Bendamustine or Pre-Pentostatin, Pre-Rituximab, and 2, 4 and 8 (each +/- 1) hours from the start of the Rituximab Infusion, then next AM. Draw 1 extra cytokine sample if rigors or temp >38.3.

  Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

  For sample pickup, page 102-11964.

  For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

  For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

  The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator. See **5.4.3** for additional details.

- Days 0-1 and 14-15 (i.e., the day of or the day before the day 15 dose) of each cycle and every week for the first cycle: CBC, diff
- 0-2 days before each cycle: Acute care panel, mineral panel, hepatic panel, total protein, CK, uric acid, LDH, direct bilirubin, urinalysis
- 0-4 days before each cycle: blood FACS (until negative)
- 0-2 days before Day 15 of each cycle: CBC with diff and acute care panel
- Prior to Cycle 4, then 4-8 weeks after last dose of arm (end of treatment restaging): Blood citrate tube for consensus PCR, BMBx with IHC for CD20 and CD3, and bone marrow aspirate (BMA, if obtainable) for FACS, PCR, and RQ-PCR.
- Prior to Cycle 4 and at end of treatment restaging: CT or other imaging study of spleen and any other site of known disease, acute care panel, mineral panel, hepatic panel, total protein, uric acid, Lipid panel, GGT, PT, PTT, fibrinogen, quantitative immunoglobulins, creatinine kinase (CK), CRP, ferritin, TSH, Free T3, Free T4, amylase, lipase, haptoglobin, and urinalysis. Acceptable time windows for all post 6-mo time points (even CBCs) should be +/- 1 month up to 1.5 y, then +/- 2 months, unless individual change needed for logistical reasons.
- At end of treatment restaging: CBC with diff.
- At end of treatment restaging: blood FACS, and if patient is at NIH, TBNK (B-cells, CD4+, CD8+, and NK cells).
- Cervical and Thoracic (C- and T-) Spine MRI with each restaging that includes BMBx, so that the status of the BMBx can be correlated with the vertebral BMA signal by MRI. May be cancelled at the discretion of the PI

- EKG- Pre cycle 4 and at 4-8 weeks after last dose (end of treatment)
- History and Physical- Pre-Cycle 4 and 4-8 weeks after last dose of drug (end of treatment).

3.5.2 Evaluations during the follow up period

All end of treatment time points (even CBCs) should occur +/- 1 month up to 1.5 years after initial treatment, then +/- 2 months.

3.5.2.1 Yearly for 2 years (i.e. 1.5 and 2.5 years after initial treatment), then every 2 years

- Blood citrate (4-6 ml blue top) tube for consensus PCR
- BMBx with IHC for CD20 and CD3, and bone marrow aspirate (BMA, if obtainable) for FACS, PCR, and RQ-PCR. (while in CR)
- Cervical and Thoracic (C- and T-) Spine MRI with each restaging that includes BMBx, so that the status of the BMBx can be correlated with the vertebral BMA signal by MRI. May be cancelled at the discretion of the PI
- CT or other imaging study of spleen and any other site of known disease,
- acute care panel, mineral panel, hepatic panel, total protein, uric acid, Lipid panel, GGT, PT, PTT, fibrinogen, quantitative immunoglobulins, creatinine kinase (CK), CRP, ferritin, TSH, Free T3, Free T4, amylase, lipase, haptoglobin, and urinalysis.
- EKG

3.5.2.2 9 months after initial treatment, then every 3 months. If in CR, every 6 months after 2.5 years after treatment

- CBC with diff.

3.5.2.3 1, 1.5, 2, and 2.5 years after initial treatment, then yearly (also 6 months after initial treatment if end of treatment evaluation occurred prior to 5 months after initial treatment)

- Blood FACS
- TBNK (B-cells, CD4+, CD8+, and NK cells (if patient is at NIH).

If patient progresses during follow up period and has already undergone or will not undergo crossover treatment, or otherwise begins other treatment, or voluntarily withdraws from follow-up testing, an annual phone call (or other contact) will be made to patient or their treating physician to assess survival and obtain data about other treatments (dates and names of treatments) and response.

3.6 CONCURRENT THERAPIES

3.6.1 Patients may not receive other treatments for HCL while on treatment or in the active follow-up portion of the study. If splenectomy is done after initial randomization regimen, then progressive disease will need to be documented using a post-splenectomy baseline, prior to cross-over.

3.6.2 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.

3.7 RADIATION THERAPY GUIDELINES

Radiation therapy for HCL is not permitted while on protocol unless follow-up portion of the study, i.e., while patients are off of active follow-up testing and being followed for survival.

3.8 COST AND COMPENSATION

3.8.1 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.

3.8.2 Compensation

Participants will not be compensated on this study.

3.8.3 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.

3.9 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.

3.9.1 Criteria for removal from protocol therapy (off treatment), for either arm

- Subject has completed cross-over treatment
- Progressive disease or DLT and ineligible for future cross-over treatment
- Intercurrent illness or medical circumstances
- Radiation therapy or other treatment for HCL other than splenectomy
- Voluntary withdrawal from treatment regimen
- PI determines further treatment on this study is not in patient's best interest
- Patients may still be eligible for crossover after going off-treatment from their randomized regimen.
- Patients who are off-treatment but still on-study will be followed as per the schedule in Sections **3.4** and **3.5**, until they begin alternative therapy or voluntarily withdrawal from follow-up testing. Thereafter, they may be followed on-study for survival and other secondary endpoints.
- Positive pregnancy test

3.9.2 Off Study Criteria

- Voluntary patient withdrawal from study
- Death

- Non-compliance with study treatment and/or testing, or lost to follow-up, at discretion of PI
- Screen failure
- 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 maintained to minimize this occurrence.

4 SUPPORTIVE CARE

4.1 INFECTIONS/FEVER AND NEUTROPENIA

Febrile Neutropenia is a common side-effect of pentostatin or bendamustine 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 **13.3.2**.

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 chemotherapy 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 pentostatin or rituximab. Tumor lysis syndrome associated with bendamustine treatment has been reported in patients in clinical trials and in spontaneous reports. The onset tends to be within the first treatment cycle of bendamustine and, without intervention, may lead to acute renal failure and death. Preventive measures include maintaining adequate volume status, and close monitoring of serum chemistry, particularly potassium and uric acid levels. Allopurinol has also been used prior to or at the beginning of bendamustine therapy. However, there may be an increased risk of severe skin toxicity when bendamustine and allopurinol are administered concomitantly. 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.

4.6 SKIN REACTIONS RELATED TO BENDAMUSTINE

Skin reactions have been reported in clinical trials and post-marketing spontaneous reports. These events have included rash, toxic skin reactions, and bullous exanthema. Some events occurred when bendamustine was given in combination with other anticancer agents, so the precise relationship of the skin reactions to bendamustine treatment is uncertain.
In a study of bendamustine (90 mg/m2) in combination with rituximab (study SDX-105-02), 1 case of TEN occurred. TEN has been associated with treatment with rituximab. Spontaneous reports of SJS and TEN, some fatal, have been reported when bendamustine was administered concomitantly with allopurinol and other medications known to cause these syndromes. The relationship to bendamustine cannot be determined.
When skin reactions occur, they may be progressive and increase in severity with further treatment. Therefore, patients with skin reactions should be monitored closely. If skin reactions are severe or progressive, bendamustine treatment should be withheld or discontinued.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

5.1.1 Documentation of dosage and timing of drug administration

5.1.2 Outside laboratory, radiologic, and pathology results will be faxed to the PI and entered into the database at NIH

5.1.3 The original signed consent goes to Medical Records; copy placed in research record

5.1.4 NIH labs and tests will be downloaded into the database

5.1.5 A pre-existing (baseline) laboratory abnormality will be considered the last one obtained prior to the first dose of drug, unless the PI considers an abnormality of higher grade occurring within 100 days prior to the first dose to be a truer baseline.

5.1.6 Patients beginning crossover treatment will be assessed for new baseline signs and symptoms.

5.1.7 All adverse events (AEs) will be collected except grade 1 events which are unrelated or unlikely related to the research.

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.

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 dose of any study drug through 30 days after the last dose of any study drug. Beyond 30 days after the last dose of study drug, only adverse events which are serious and related to the study drug need to be recorded.
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. Data from NIH will be located in the CRIS. Data managers can enter the data from both sources electronically into the database.

An abnormal laboratory value will be considered 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, i.e., at least possibly related to drug.

AEs need not be collected retroactively after the 30 day time point after the last dose, even if the patient subsequently receives crossover treatment.

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 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 or lack of response unless they are consistently low, i.e. at least 2 low counts > 1 week apart.
- For determining response durations, if a given response (i.e. PR, CR or MRD-negative CR) appears to end but then later resumes in the absence of intervening therapy, it will be considered to have continued.

5.2.2 Complete remission (CR): All of the following for at least 4 weeks

- Absolute neutrophil count (ANC) ≥ 1500/mm^3
- Platelets ≥ 100,000/mm^3
- Hgb ≥ 11 g/dL
- Spleen non-palpable below the costal margin, or not below costal margin on CT.
- Circulating HCL cells either non-visible on Wright stain or < 1% by flow cytometry
- Lymph nodes ≤ 2 cm by short axis, unless larger lymph nodes not due to HCL
- Absence of hairy cells on BM aspirate smears and H/E stain of the bone marrow biopsy negative for HCL The date CR begins will be either the date of the BMBx or the date at which normal blood counts remain adequate for at least 4 weeks off growth factors or transfusions without 2 unacceptable CBCs in a row, whichever comes last.
- CR with MRD in the BMBx by IHC is defined as CR and a ratio of CD20+ cells to T cells of at least 1, and most of the CD20+ cells having morphology consistent with HCL.
- CR with MRD in the peripheral blood is defined as CR and a positive peripheral blood FACS or PCR. Suspicious flow is considered negative per protocol.
- 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.

5.2.3 Partial Response (PR): All of the following for at least 4 weeks

- 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 in evaluable (> 2cm) lymphadenopathy.
- ≥ 50% reduction in extent of spleen below costal margin by CT or physical exam, if abnormal at baseline.

5.2.4 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.
- The baseline for determination of PD for the purpose of best response to each arm of the protocol will be the patient's baseline prior to beginning that arm of the protocol. However, the baseline for determination of PD for the purpose of crossing over will be the lowest (nadir) tumor burden achieved. That baseline, if after enrollment, should not be assessed less than 27 days after beginning the last cycle.

5.2.5 Stable disease (SD): None of the above

5.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through 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.

5.4.1 Description of data/specimens

Blood and bone marrow samples

5.4.2 Timeframe and location of storage

- Samples will be stored and cataloged longer than a year, in alarmed freezers at a number of locations, including Frederick, MD, in NCI contracted space and Building 37.
- Confidentiality: Patient names or identifiers will not be used in publications resulting from testing of patient samples. Other than described above, no germline testing will be done which may impact disease risk in the patient's relatives. Studies will only be done if the subject provides consent for future use and the studies to be done have been approved by the IRB.
- Samples at Leidos Biomedical Research, Inc. laboratory in Frederick will be tracked in a secure electronic database.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of Section **10.2.1.**

5.4.3 Sample Handling, Data Collection and Sample Disposition for the Blood Processing Core (BPC)

5.4.3.1 Sample Data Collection

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 U may be stored, tracked and disposed of as specified in investigator's companion protocol, 10-C-0066.

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.4.3.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used

for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested).

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.4.4 Procedures for storage and disposition of serum specimens at the Clinical Support Laboratory, Leidos Biomedical Research, Inc.in Frederick, MD

- The Clinical Support Laboratory, Leidos Biomedical Research, Inc.-Frederick, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. The laboratory is CLIA certified for anti-IL15 and certain cytokine measurements and all laboratory areas operate under a Quality Assurance Plan with documented Standard Operating Procedures that are reviewed annually. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:
- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
- The database resides on a dedicated program server that is kept in a central, locked computer facility.
- The facility is supported by two IT specialists who maintain up to date security features including virus and firewall protection.
- Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
- The database sample entry program itself is accessed through a password protected entry screen.

- The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID.
- Inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.
- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long term storage.
- Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, the protocol Principal Investigator, specifies who has access to the collection. Specific permissions will be required to view, input or withdraw samples from a collection.
- Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. The repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.
- The Clinical Support Laboratory does perform testing services that may be requested by clinical investigators including, but not limited to, immunophenotyping by flow cytometry and cytokine testing using ELISA or multiplex platforms.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that appropriate approvals and/or agreements are in place to cover the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

6 CORRELATIVE STUDIES FOR RESEARCH

6.1 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.

Tissue Sample Type	Test	Lab	Volume	Tube	Storage	Timing
Blood	Blood FACS	Flow Lab B1B58	18cc	3- Na Heparin	Room Temperature	Pre-treatment D1 of each cycle x 6 Pre-cycle 4 Restaging, end of -treatment restaging **Post-treatment;** with every bone marrow, every 6 months x 2 years then yearly
Blood	LDH	Main Lab: Chemistry	6cc	1- Lithium Heparin	Room Temperature	Pre-treatment Cycle 1- D1-D2, (D3 and D5 if at NIH) D1 of each cycle x 6 Pre-cycle 4 Restaging 4-8 week post-treatment restaging, **Post-treatment;** Every year x 2 years, then every 2 years if in CR otherwise continue yearly.
Blood	P-selectin, plasminogen activator inhibitor type 1, vWF activity, vWF antigen, thrombomodulin, d-Dimer, FDP, fibrinogen,	Main Lab Coagulation	13.5cc	3- Na-Citrate	On Ice	Cycle 1- D1-D2 (D3 and D5 if at NIH)

Tissue Sample Type	Test	Lab	Volume	Tube	Storage	Timing
Blood	Haptoglobin	Main Lab: Chemistry	6cc	1-Lithium Heparin	Room Temperature	Pre-treatment Cycle 1- D1-D2 (D3 and D5 if at NIH) Pre-cycle 4 Restaging 4-8 week post-treatment restaging **Post-treatment**; Every year x 2 years, then every 2 years if in CR otherwise continue yearly.
Blood	Reticulocytes	Main Lab: Hematology	4.5cc	1-EDTA	Room Temperature	Cycle 1- D1-D2 (D3 and D5 if at NIH)
Blood	Cloning and sequence immunoglobulin rearrangements	Kreitman or Figg Lab, please page designated person in orders to pick up.	5 green top tubes x 3 sets	Na-Heparin (total of 15 tubes)	Room Temperature	3 sets drawn on different days prior to starting treatment if at NIH
Blood	Cytokines	Kreitman or Figg Lab, please page designated person in orders to pick up.	2cc	6ml Na-Heparin tube	Room Temperature	Pre-Bendamustine or Pre-Pentostatin, Pre-Rituximab, and 2, 4 and 8 (each +/- 1) hours from the start of the Rituximab Infusion, then next AM. Draw 1 extra cytokine sample if rigors or temp >38.3.

Tissue Sample Type	Test	Lab	Volume	Tube	Storage	Timing
Note: Samples collected during the course of this study will be stored, tracked, and disposed of as specified in investigator's companion protocol, 10C0066, on which all subjects will be co-enrolled.						

7 STATISTICAL CONSIDERATIONS

- The primary objective of this randomized phase II trial is to determine if the combination of pentostatin + rituximab and the combination of bendamustine + rituximab is associated with an adequate clinical response rate (PR+CR) in patients with relapsed hairy cell leukemia (HCL) and, if so, to select for further investigation, the combination which is likely to be superior.
- Data from 51 patients who were similar to the patients to be enrolled on this study have exhibited a 39% response rate (20/51 responses) to rituximab alone. As such, since each arm would be testing a combination of agents which would include rituximab, it would be desirable to rule out a response rate which is somewhat greater than 39%.
- Prior to randomization, 12 patients will be treated with bendamustine + rituximab at 2 dose levels and evaluated for toxicity in order to determine if each combination is safe for use in subsequent patients. Randomization will begin using the higher of the 2 dose levels of bendamustine providing 5-6 of the 6 patients can tolerate the 6 cycles without delays > 4 weeks. If neither dose level is tolerated to this extent, the trial may need to be amended prior to randomization, or accrual will be discontinued.
- Patients will be stratified according to whether they are purine analog refractory vs. sensitive vs. unknown. Within the refractory category will be patients with untreated poor-risk disease, namely HCLv and unmutated IGHV4-34+ HCL.
- Patients will be randomized between bendamustine + rituximab or pentostatin + rituximab. For each arm of the trial, the study will be conducted using an optimal two-stage phase II design (Simon R, Controlled Clinical Trials 10:1-10, 1989), in order to rule out an unacceptably low 40% clinical response rate (PR+CR; p0=0.40) in favor of a higher response rate of 65% (p1=0.65). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), the study will initially enroll 13 evaluable (randomized) patients onto each arm, and if 0-5 of the 13 on a given arm have a clinical response (CR+PR), then no further patients will be accrued to that arm. If 6 or more the first 13 have a response, then accrual would continue until a total of 28 evaluable patients have enrolled on that arm. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 6-14 clinical responses

in 28 patients on an arm, this would be an uninterestingly low response rate, while if there were 15 or more responses in 28 patients on that arm, then this would be considered a sufficiently interesting response rate. Under the null hypothesis (40% response rate), the probability of early termination for each arm is 57%.

- If accrual stops early to one arm because of insufficient activity, patients will continue to be accrued in a non-randomized fashion to the other arm. If both arms end accrual early, neither combination would be pursued further. If 28 patients are treated on each arm, and if one arm has at least 15 responses and the other does not, then only the arm with 15 or more responses would be worthy of further study. If both arms have at least 15 responses, then a selection design approach will be used to determine which of the two combinations is worthy of further investigation. Using this approach, the arm with the greater total number of responses (CR+PR) will be selected for further study. If there is a tie in the number of responses, then secondary criteria, such as the number of CRs, the duration of response, or the amount of toxicity noted will be used to select the superior arm. Taking into consideration the two-stage nature of the design, the following table provides the probability that the truly superior arm will be selected following the rule stated above:

True response probability in Inferior arm	Superior arm	Probability of correctly selecting the superior arm
0.40	0.55	87.0%
0.40	0.60	93.3%
0.40	0.65	97.1%
0.45	0.55	77.2%
0.45	0.60	87.0%
0.45	0.65	93.5%
0.50	0.60	77.4%
0.50	0.65	87.2%

- Thus, with 28 patients on each arm, the inferior arm having a true 40-50% response rate, and the superior arm having a true response rate of 55-65%, there is at least a 77% probability of correctly selecting the superior arm.
- To select the best arm, ORR (PR+CR) would normally be used, but more stringent measures of response could be used to discriminate the 2 arms, including CR rate, MRD-free CR rate, and rates of patients who have a 6 month duration of response.

- The study is being designed as a selection trial. Only large differences, on the order of 35% to 40% or more, can be detected between the arms with 80% power (2-way p=0.05).
- Accrual was expected to be 16-24 patients per year; however, that has not held true. Instead, the accrual has been about 6 per year and is likely to continue at that rate. There are 3 groups of patients in the study:

    1. Twelve evaluable non-randomized patients in the Dose Escalation group
    2. Fifty-six evaluable randomized patients in the Dose Expansion group
    3. Four evaluable patients who were previously refractory to therapy or untreated with poor-risk disease in the Dose expansion, non-randomized group.

    At this rate, 72 evaluable patients will be enrolled in approximately 12 years. The accrual ceiling is set at 200, which includes patients consented for screening and enrolled.

- The trial would be a single-institution trial at NIH.
- The most important secondary endpoint of the study is to compare rituximab plus either pentostatin or bendamustine in terms of MRD-free survival and disease-free survival, and toxicity, including to CD4+ T-cells. Patients will also be followed for overall survival.
- Another secondary endpoint is to compare the 2 regimens in crossover when used after failure of the 1st regimen. It may be determined whether the 1st regimen in each patient was more effective than the 2nd regimen, or vice versa, and it may also be determined whether one regimen is better than the other in crossover, considering all patients crossed over. However, it is recognized that this endpoint is only exploratory since crossover is not universal and not at a fixed time.
- Other secondary endpoints on this study include: 1) To determine if MRD levels and tumor markers (soluble CD25 and CD22, and RQ-PCR) correlate with response and clinical endpoints, and could possibly replace BMBx, 2) 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, 3) To enhance the study of HCL biology by cloning, sequencing and characterizing monoclonal immunoglobulin rearrangements, and other genes, and 4) To study the mechanism of thrombocytopenia after purine analog plus rituximab. These endpoints would be considered exploratory.

8 COLLABORATIVE AGREEMENTS

8.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

CRADA #02753 between Genentech and NCI was executed 11/8/2012 and amended 11/3/2017. CTA #00819 between NCI and Cephalon was executed on 07/20/2017.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Both men and women and members of all races and ethnic groups are eligible for this trial. Pregnant women are not allowed to enroll because both bendamustine and pentostatin may pose significant risks to the fetus.

9.2 PARTICIPATION OF CHILDREN

Children will not be eligible for this study. HCL is a disease of adults, not children, and it is unlikely that any children will be diagnosed.

9.3 PARTICIPATION OF SUBJECTS UNABLE TO 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 **9.5**), all subjects ≥ age 18 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 **9.6.1** for consent procedure.

9.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

9.4.1 Potential benefits

Purine analog in combination with rituximab may lead to durable responses including CR in patients.

9.4.2 Risks/discomforts

Purine analogs have many toxicities, some of which can be long term. Rituximab has mainly short-term toxicities and 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 purine analog.

9.5 RISK/BENEFIT ANALYSIS

Patients are multiply relapsed from purine analog and have either received or are ineligible for immunotoxin treatment. They will have limited options and may obtain better response to purine analog plus rituximab as compared with purine analog or rituximab alone.

9.5.1 Risks from Study Procedures

9.5.1.1 Blood draws

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

9.5.1.2 Urine collection

There is no physical risk involved with urine collection.

9.5.1.3 Bone marrow aspirate and/or 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.

9.5.1.4 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.

9.5.1.5 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.

9.5.1.6 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.

9.5.1.7 Stress test

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

9.5.1.8 Pulmonary function tests (PFTs)

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

9.5.1.9 Ultrasound

No physical risks are associated with ultrasound procedures.

9.5.1.10 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.

9.5.1.11 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.

9.5.2 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%.

9.6 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.

<u>Manual (non-electronic) signature on electronic document</u>:

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

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

For participants addressed in Section **9.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **9.6**.

9.6.2 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 waiver 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.

10 NIH REPORTING 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 regular basis (weekly) when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator and members of the research team. 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 DEFINITIONS

11.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

11.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see Section **11.1.3**)
  - Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32).

11.2 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
  Forward all SAEs also to the IRB and PI as directed in Section **10.2.**

11.3 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.

11.4 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
- **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 identifiers (i.e. D.O.B. 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 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.
- **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.

11.5 PRODUCT COMPLAINT REPORTING

11.5.1 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.

11.5.2 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.clinops.sae@tevapharm.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.).

11.6 SAFETY REPORTING REQUIREMENTS FOR IND EXEMPT STUDIES (GENENTECH INFORMATION)

- For Investigator Sponsored IND Exempt Studies, there are some reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 314.80.
- Postmarketing 15-Day "Alert Report": The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of Rituximab. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be submitted to the FDA (2 copies) at the following address: Central Document Room, 12229 Wilkins Avenue, Rockville, MD 20852.
- All Postmarketing 15-Day "Alert Reports" submitted to the FDA by the Sponsor-Investigator must also be faxed to: Genentech Drug Safety, Fax:(650) 225-4682 or (650) 225-4683, (Please use the safety reporting fax cover sheet attached to this document for your fax transmission. Also provided on Rituxan CD-ROM).
- For questions related to safety reporting, contact: Genentech Drug Safety, Tel:1-888-835-2555 or Fax: (650) 225-4682 or (650) 225-4683 (Please use the safety reporting fax cover sheet attached to this document for your fax transmission).
- Principal investigator contact information and fax #: Robert J. Kreitman, M.D., Chief, Clinical Immunotherapy Section, Laboratory of Molecular Biology

(LMB), Centers for Cancer Research (CCR), National Cancer Institute (NCI), NIH (9000 Rockville Pike, Bethesda, MD 20892), building 37/5124b, Phone 301-480-6187, email kreitmar@mail.nih.gov

11.7 REQUIREMENTS FOR REPORTING TO TEVA FOR PATIENTS GETTING BENDAMUSTINE

- "Serious Adverse Event or Adverse Drug Reaction (AE/ADR)" means any AE/ADR occurring at any dose that results in any of the following outcomes:
  (a) Death;
  (b) A life-threatening AE/ADR (i.e., the patient/subject was, in the view of the initial reporter/investigator, at immediate risk of death from the AE/ADR as it occurred. It does not refer to an AE/ADR that hypothetically might have caused death if more severe);
  (c) Inpatient hospitalization or prolongation of existing hospitalization (i.e., hospitalization was required to treat or diagnose the AE/ADR; excludes hospitalization for unrelated reasons);
  (d) A persistent or significant disability or incapacity (*disability* here means that there is a substantial disruption of a person's ability to conduct normal life functions;
  (e) A congenital anomaly/birth defect.
  (f) An important medical event (i.e., AEs/ADRs that might not be immediately life-threatening, or result in death or hospitalization might be considered serious when, based upon appropriate medical and scientific judgment, they might jeopardize the patient/subject or might require medical or surgical intervention to prevent one of the other serious outcomes listed above);
  (g) Any suspected transmission via a medicinal product of an infectious agent;
- Sponsor-Investigator shall use his/her judgment to determine the relationship between the Serious Adverse Drug Experience and the Study Drug.
- Institution shall notify the IRB and Teva within 15 business days, by facsimile at 215-619-3825 or Email to us.clinops.sae.tevapharm.com, upon learning of the occurrence during the Study of:
  (a) All Serious AE/ADRs, regardless of causality;
  (b) Any exposure of a pregnant Study participant to the Study Drug within thirty (30) days of exposure;
  (c) A female partner of a male Study participant becoming pregnant within thirty (30) days of exposure;
  (d) Any medical event which may reasonably be believed to impair the integrity, validity or ongoing viability of the Study.
- All such occurrences listed in this section shall be reported to Teva using the MedWatch 3500A Form
- In the event the IRB requests additional safety information from Sponsor-Investigator, Sponsor-Investigator shall notify Teva of such request within 15 business days.

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 and the Institutional Review Board (IRB) 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 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, 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.

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

The study monitor, 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 or Institutional policies.

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 BENDAMUSTINE

Please, refer to package insert.

13.1.1 Source

Bendamustine is commercially available under the name of Treanda and obtained from Teva Pharmaceutical Industries Ltd.

13.1.2 Administration Procedures

Bendamustine is administered as a 30-60-minute intravenous (IV) infusion. Precautions should be taken to avoid extravasation, including monitoring of the intravenous infusion site for redness, swelling, pain, infection and necrosis during and after the administration.

13.2 PENTOSTATIN (NIPENT)

Please, refer to package insert.

13.2.1 Source

Pentostatin is commercially available under the trade name Nipent, and will be obtained from commercial sources from the Clinical Center Pharmacy.

13.2.2 Administration Procedures

Pentostatin may be given intravenously by bolus injection or diluted in a larger volume (25 to 50 mL) with 5% dextrose injection or 0.9% sodium chloride injection. Dilution of the entire contents of a reconstituted vial with 25 mL or 50 mL provides a pentostatin concentration of 0.33 mg/mL or 0.18 mg/mL, respectively, for the diluted solutions. Although the dose level of pentostatin is 4 mg/m^2, patients who are at least 2.5 m^2 will only receive a maximum of 10 mg total dose.

13.3 RITUXIMAB (RITUXAN) FROM GENENTECH

Please, refer to package insert.

13.3.1 Source

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.

13.3.2 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 C in Section **15.3** 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.
- 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.

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15 APPENDICES

15.1 APPENDIX A: 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.

15.2 APPENDIX B: 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.

15.3 APPENDIX C: GUIDELINES FOR RITUXIMAB PREPARATION AND ADMINISTRATION

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.

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.

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.

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. 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.

15.4 APPENDIX D: 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**
Elizabeth Bengtson, MD	Dartmouth College	1 Medical Center Dr. Lebanon, NH 03756	603-650-5529
Pamela Oster, MD	Fremont-Rideout Cancer Center	618 5th Street Marysville, CA 95901	530-749-4400
Michael Crump, MD	Princess Margaret Cancer Centre	610 University Ave, Toronto, ON Canada M5G 2M9	416-946-4501
Philip Kuriakose, MD	Henry Ford West Bloomfield Hospital	6777 West Maple Rd. West Bloomfield Township, MI 48322	800-436-7936
Paul Hendrie, MD	University of Washington/ Seattle Cancer Care Alliance*	825 Eastlake Ave. E PO Box 19023 Seattle, WA 98109	206-541-6426