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Protocol Page

A phase I/II clinical trial of fludarabine, bendamustine, and rituximab (FBR) in previously treated patients with chronic lymphocytic leukemia (CLL)
2009-0546

Core Protocol Information

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Study Chair:	William G. Wierda
Additional Contact:	Annette Jalayer Laura K. Fowler Leukemia Protocol Review Group
Department:	Leukemia
Phone:	713-745-0428
Unit:	428
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Protocol Body

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A Phase I/II Clinical Trial of Fludarabine, Bendamustine, and Rituximab (FBR) in Previously Treated Patients with Chronic Lymphocytic Leukemia (CLL)

Version 8

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William G. Wierda, M.D., Ph.D.
Associate Professor of Medicine
Department of Leukemia
Division of Cancer Medicine
UT MD Anderson Cancer Center
1515 Holcombe Blvd., Unit 428
Houston, TX 77030
(713) 745-0428 (ph)
(713) 794-1602 (fax)

Short Title: Phase I/II FBR for Previously Treated Patients with CLL

1.0 OBJECTIVES

Primary objectives:

- Phase I – Evaluate the toxicities and tolerability and identify the maximum tolerated dose of bendamustine combined with fixed-dose fludarabine and rituximab (FBR) in previously treated patients with CLL.

- Phase II – Evaluate efficacy (2008 International Workshop on Chronic Lymphocytic Leukemia [IWCLL] response rate) of the FBR regimen at the tolerated dose of bendamustine in previously treated patients with CLL. This will be compared to the historic data of treatment regimens for previously treated patients with CLL.

Secondary objectives:

- Evaluate safety in the Phase II portion at the tolerated dose of bendamustine in previously treated patients with CLL.

- Evaluate time to treatment failure (TTF) and time-to-progression (TTP) for previously treated patients treated with the FBR combination. These time-to-event endpoints will be compared to historic data of treatment regimens for previously treated patients.

- Correlate pretreatment prognostic factors, including ZAP-70 expression, CD38 expression, β-2 microglobulin, *IGHV* gene mutation status, chromosome abnormalities by FISH, and serum thymidine kinase with response and time to event endpoints.

- Evaluate pharmacodynamic endpoints to determine bendamustine-induced deoxyribonucleic acid (DNA) damage response in quiescent CLL lymphocytes and test the hypothesis that fludarabine triphosphate will enhance this response by inhibiting DNA repair.

2.0 BACKGROUND

2.1 CLL is the most common leukemia in the United States and Western Hemisphere (reviewed in [1]). Nearly two-thirds of patients with CLL are over 65 years of age, and there is a steady increase in the prevalence of this disease in the population over 50 years of age. Interest in newer therapeutic agents was poor until recently, owing to the usual advanced age of patients and often indolent course of disease. However, the natural history of the disease is diverse. Patients with only lymphocytosis have a median survival in excess of 10 years; those with evidence of marrow failure manifested by anemia or thrombocytopenia have a median survival

of only 2 years. Intermediate survival is predicted for patients with lymphadenopathy or organomegaly. The National Cancer Institute Working Group on CLL has described clinical features of "active disease" which are helpful in the decision to treat. These indications for treatment include: 1) unintentional weight loss of more than 10% body weight over the past 6 months; 2) fever or night sweats in the absence of infection; 3) extreme fatigue; 4) worsening anemia or thrombocytopenia; 5) massive (>6 cm below the left costal margin) or progressive splenomegaly; 6) massive (>10 cm in the longest diameter) or progressive lymphadenopathy; 7) progressive lymphocytosis with rapid lymphocyte doubling time; 8) marked hypogammaglobulinemia or paraproteinemia[2-4].

2.2 The mainstay of therapy has been systemic chemotherapy consisting usually of an alkylating agent and a corticosteroid. Chlorambucil plus prednisone has become the standard initial therapy with response rates from 40-77%. Generally, responses have not been complete [5-9]. Therapy in patients who are refractory to alkylating agents is unsatisfactory. The response rates are substantially lower (approximately 30%) with rare complete responses [8-12].

2.3 Fludarabine has shown marked activity in several indolent lymphoproliferative disorders including CLL, low-grade lymphoma, Waldenstroms macroglobulinemia, and prolymphocytic leukemia [13-17].

Treatment of previously treated CLL patients with fludarabine has resulted in a 13% complete response rate and 44% partial response rate [13]. Fludarabine has also been given to patients with previously untreated CLL, resulting in even higher response rates with 33% confirmed complete response, 39% unconfirmed complete response and 6% partial response [16].

Despite attainment of clinical complete response, most patients will experience a recurrence at a median of approximately 2 years [18]. This is likely related to the fact that most patients in complete remission (CR) have residual disease that can be assessed by several parameters. In previously untreated patients, 55% of CR patients have residual nodules on bone marrow biopsy (now referred to as nodular partial remission [nPR]) [16]. At 2 years 87% of CR patients are progression-free, versus 55% of nPR patients[18].

2.4 Fludarabine and prednisone have been combined and used to treat over 200 patients with CLL. Response rates are equivalent to those seen with single agent fludarabine for both previously treated and untreated patients [19]. However, 14 episodes of Listeria sepsis or *Pneumocystis carinii* pneumonia were seen with 4 deaths resulting from these infections.

These infections were not seen in 100 prior patients treated with fludarabine alone.

2.5 Cyclophosphamide has been combined with vincristine (VCR) and prednisone (COP) to treat lymphoproliferative disorders. Liepman and Votaw first reported on this combination to treat CLL in 1978. Thirty-six patients received this regimen, 23 were previously untreated. The response rate was 72%; 18 of 23 (78%) previously untreated patients responded; 8 of 13 (62%) patients previously receiving chlorambucil responded to COP [20]. Oken and Kaplan treated 18 patients with CLL with CVP (cyclophosphamide 800 mg/m^2 I.V. on day 1 or 400 mg/m^2 orally for 5 days, VCR 2 mg I.V. on day 1 and prednisone 60-100 mg/m^2 on days 1-5). All patients were previously treated and 17 of 18 were refractory to chlorambucil. The response rate was 44% [21].

2.6 *In vitro* studies have demonstrated that fludarabine inhibits repair of cyclophosphamide-induced DNA inter-strand cross-links in CLL B cells [22]. This was the basis for a regimen of fludarabine, 25-30 mg/m^2, combined with cyclophosphamide, 250-500 mg/m^2; both drugs were given daily for three days in 4-week courses. Frewin et al [23] first reported their experience giving fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 daily for three days in a limited trial of 7 patients with CLL; two patients achieved a complete remission and the overall response rate was 71%. Toxicities included nausea and vomiting, myelosuppression and infections. O'Brien et al [24] gave this treatment to 94 patients who were previously treated with either an alkylating agent, fludarabine, or both. The complete remission rate was 11% and an overall response rate (ORR) of 69% was reported. Patients that had previously been treated with an alkylating agent and were resistant to fludarabine had a markedly lower, but noteworthy response rate with 3% complete remissions and a 39% overall response rate.

2.7 Rituximab in CLL

The Food and Drug Administration (FDA) approved Rituximab in 1997 for treatment of relapsed or refractory low-grade non-Hodgkin's lymphoma. In the pivotal trial, 33 of 166 patients had small lymphocytic lymphoma (SLL), International Working Formulation (IWF) A (CLL equivalent). Rituximab was given at a dose of 375 mg/m^2 weekly for four weeks, and produced a response rate of 48%. For the patients with IWF A disease the overall response rate was low; 12% compared to 58% for those with IWF B, C, and D (combined) disease [25]. There are several potential explanations for the lower activity of rituximab in patients with CLL/SLL. Leukemia cells from patients with CLL express lower levels of CD20 than that observed on follicular lymphoma cells. In addition, pharmacokinetic analyses conducted during the pivotal trial showed lower plasma levels of rituximab in non-responders and, effectively, in most patients with SLL.

Finally, circulating CD20 has been demonstrated in the plasma of patients with CLL [26]. Soluble CD20 may act as a sink for the therapeutic antibody, resulting in more rapid clearance and reducing delivery of the antibody to leukemia cells. Therefore, higher plasma concentrations of rituximab could potentially improve response rates in CLL.

Rituximab has been evaluated as a single agent in the treatment of CLL. The standard dose of 375 mg/m^2 weekly for four weeks was given to 28 patients with CLL who had been previously treated with chemotherapy [27]. There were no complete remissions; 25% of patients had partial responses, 43% had stable disease, and 32% had progressive disease. Smaller studies evaluating rituximab 375 mg/m^2 weekly for four weeks in previously treated patients with CLL report low overall response rates with no complete remissions [27-29].

Two studies of dose-escalated rituximab in CLL yielded encouraging results. O'Brien et al.[30] conducted a study of four weekly doses of rituximab at 500, 650, 825, 1000, 1500, and 2250 mg/m^2. All patients with CLL had been previously treated with chemotherapy. The overall response rate among the 39 evaluable patients was 36% and all remissions were partial. Responses were seen in 5 of 24 (21%) patients receiving 500-825 mg/m^2, 3 of 7 (43%) patients receiving 1000-1500 mg/m^2, and 6 of 8 (75%) patients receiving 2250 mg/m^2 (P=0.03). Byrd et al.[31] conducted a dose-intensified study with 375 mg/m^2 administered thrice weekly for four weeks. Twenty-seven previously treated patients were evaluable for response, 10 (37%) achieved remission. There appears to be increased activity for rituximab in CLL with dose intensified schedules; the optimal dose and schedule for rituximab in CLL is yet to be determined.

2.8 Chemoimmunotherapy in CLL

The increased activity of fludarabine combined with cyclophosphamide (FC) and the potential chemo-sensitization between purine analogue, alkylating agent, and monoclonal antibody was the rationale for combining rituximab with FC. The efficacy, toxicity, and tolerability of chemoimmunotherapy with the combination of fludarabine, cyclophosphamide, and rituximab (FCR) were evaluated in previously treated patients with CLL [32]. The purpose of this study was to improve the CR rate for previously treated patients and evaluate the quality of bone marrow response. One hundred seventy-seven previously treated patients with CLL were evaluated. Treatment consisted of rituximab 375 mg/m^2 day 1 of course 1 and 500 mg/m^2 day 1 of courses 2 to 6; fludarabine 25 mg/m^2/d days 2 to 4 of course 1 and days 1 to 3 of courses 2 to 6; and cyclophosphamide 250 mg/m^2/d days 2 to 4 of course 1 and days 1 to 3 of courses 2 to 6. Courses were repeated every 4 weeks. CR was achieved in 25% of 177 patients, and nPR and partial remission (PR)

were achieved in 16% and 32% of patients, respectively; the overall response rate was 73%. Twelve (32%) of 37 complete responders tested achieved molecular remission in bone marrow. Univariable and multivariable analyses were used to identify pretreatment patient characteristics associated with CR and overall remission, longer time to progression, and overall survival. The FCR regimen was an active and well-tolerated treatment for previously treated patients with CLL. Myelosuppression was the most common toxicity. FCR induced the highest CR rate reported in a clinical trial of previously treated patients with CLL. Furthermore, molecular remissions were achieved in a third of patients achieving CR.

The FCR regimen has been evaluated in two large Phase III randomized trials, one in previously untreated patients (CLL8 trial[33]) conducted by the German CLL Study Group (GCLLSG) and in previously treated patients (REACH trial[34]) conducted as an international trial sponsored by F. Hoffmann La-Roche. Both trials demonstrated superior efficacy for the FCR combination over FC in terms of higher complete and overall response rates and longer progression-free survival. The REACH trial was conducted mainly in Europe and randomized previously treated patients with CLL to either FC or FCR. Patients could have only had one prior treatment that could not have included FC or rituximab. A total of 552 patients were randomized, 276 each received FC or FCR; patients were equally distributed between treatment arms in terms of pre-treatment characteristics. The doses of FC were the same for both treatment arms, fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2, both daily for 3 days of each course. For FCR, rituximab was given at 375 mg/m^2 x1 for course 1 and 500 mg/m^2 x1 for courses 2-6. The CR and OR rates were 24.3 and 69.9% respectively for patients treated with FCR and 13 and 58% respectively for patients treated with FC. The median progression-free survival (PFS) for patients treated with FCR was 30.6 mo, versus 20.6 mo for patients who were treated with FC. Treatment was well tolerated by both arms and there was no significant difference in the rates of grade 3 and 4 neutropenia, anemia, thrombocytopenia, infectious, or other adverse events. These Phase III data clearly demonstrated superiority for the FCR regimen over FC in previously treated patients with CLL.

2.9 Bendamustine in CLL

Bendamustine has recently been approved by the FDA for treatment of patients with CLL[35,36]. Bendamustine has a complex structure with 3 functional groups: an alkylating group, a benzimidazole ring, and a butyric acid side chain[37]. It clearly has alkylating-agent activity, capable of inducing inter-strand and intra-strand DNA cross-links. It may also have anti-metabolite activity conferred by the benzimidazole nucleus. Work by our group[22,38,39] demonstrated that DNA excision repair is inhibited by

fludarabine, giving rationale to combining alkylating agents with fludarabine[24]. Clinical trials have confirmed the superior efficacy with the combination of fludarabine and cyclophosphamide versus single-agent fludarabine[40-42].

The German CLL Study Group conducted a single-arm Phase II clinical trial of bendamustine with rituximab (BR) in previously treated patients (CLL2M) that demonstrated therapeutic activity and acceptable toxicity[43]. In this study, previously treated patients received bendamustine 70 mg/m2 on days 1 and 2 with rituximab 375 mg/m^2 x1 course 1, then 500 mg/m^2 x1 for courses 2-6. The CR and OR response rates for the 81 patients treated on this trial were 14.5 and 77.4%, respectively. The incidence of grade 3 and 4 neutropenia was 12.2%, thrombocytopenia was 9.1%, anemia was 6.1% and infection was 5.2%. The response demonstrated an indication of therapeutic efficacy and the incidence of toxicities were tolerable, considering this was a population of previously treated patients. The GCLLSG is currently conducting a randomized frontline trial of FCR versus BR.

2.10 Rationale for the FBR Combination

This proposal takes advantage of the alkylating-agent activity of bendamustine and inhibition of DNA repair by fludarabine[37] and aims to explore the tolerability and toxicities of this combination with rituximab (FBR regimen) in the Phase I portion and the therapeutic efficacy in the Phase II portion of a clinical trial for previously treated patients with CLL. In Phase I, we will evaluate a fixed dose of fludarabine 20 mg/m^2 daily x 3 and rituximab 375 mg/m^2 x 1 (course 1) and 500 mg/m^2 x 1 (courses 2-6), with bendamustine at increasing doses of 20 mg/m^2 daily x3, 30 mg/m^2 daily x 3, 40 mg/m^2 daily x 3, or 50 mg/m^2 daily x 3. Courses will be every 4 weeks; 6 courses will be given. In Phase I, for the first course, bendamustine will be given alone on Day 1, then on Days 2 and 3 fludarabine will be given before bendamustine; fludarabine will be given alone on Day 4; rituximab will be given on Day 4 (after fludarabine) of this course. For courses 2-6 of Phase I, fludarabine and bendamustine will be given on Days 1-3; rituximab will be given on Day 1. For Phase II, the tolerated dose of bendamustine will be used with fludarabine and rituximab in an expanded population to evaluate clinical efficacy. For Phase II, fludarabine and bendamustine will be given sequentially on the same day.

We have evaluated the fludarabine, cyclophosphamide, rituximab (FCR) combination in over 300 previously untreated patients and nearly 290 previously treated patients. Therefore, we have well-characterized, historic patient populations with which to compare new treatment regimens and assess clinical efficacy of new regimens. These patient

populations will be used as a comparison to evaluate the effectiveness of this regimen.

This is a Phase I/II, open label study to identify the tolerated dose of bendamustine in combination with fixed-dose fludarabine and rituximab in Phase I and in Phase II to evaluate the efficacy of this combination. In the Phase II portion, patients will receive up to 6 courses of treatment at the tolerated dose of bendamustine identified in Phase I and will be evaluated for response.

3.0 BACKGROUND DRUG INFORMATION

The clinical supplies of fludarabine, bendamustine, and rituximab will be commercial drug supply.

3.1 Fludarabine:
The following information applies to fludarabine (Fludara®).

3.1.1 How Supplied:
Sterile, 50 mg prepared as a white lyophilized powder with sodium hydroxide to adjust pH.

3.1.2 Solution Preparation:
Fludarabine for injection should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solidcake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2 – 8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP.

Reconstituted fludarabine for Injection contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution. Care must be taken to assure the sterility of prepared solutions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

3.1.3 Handling and Disposal:
Procedures for proper handling and disposal should be considered. Consideration should be given to handling and disposal according to guidelines issued for cytotoxic drugs. Several guidelines on this subject have been published. There is no general agreement that

all of the procedures recommended in the guidelines are necessary or appropriate.

Caution should be exercised in the handling and preparation of fludarabine for Injection solution. The use of latex gloves and safety glasses is recommended to avoid exposure in case of breakage of the vial or other accidental spillage. If the solution contacts the skin or mucous membrane, wash thoroughly with soap and water; rinse eyes thoroughly with plain water. Avoid exposure by inhalation or by direct contact of the skin or mucous membranes.

3.1.4 Stability:

Fludarabine phosphate is relatively stable in aqueous solution. Over a pH range of approximately 4.5 to 8 in aqueous buffer solutions stored at 65°C, approximately 11% decomposition occurred in one day. From this pH profile, the optimum pH was determined to be approximately 7.7.

At a concentration of 25 mg/ml in distilled water stored at room temperature in normal laboratory light, fludarabine phosphate exhibited less than 20% decomposition in 16 days.

Diluted to concentration of 1 mg/ml in 5% dextrose injection, USP, or in 0.9 sodium chloride injection, USP, less than 3% decomposition occurred in 16 days at room temperature under normal laboratory light.

CAUTION: The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded eight hours after reconstitution.

3.1.5 Route of Administration: Intravenous (IV).

3.1.6 Fludarabine Side Effects:

Common

- Gastrointestinal: loss of appetite (0 to 34%), nausea (1% to 5%), vomiting
- Neurologic: asthenia (9% to 65%), parasthesia (4% to 12%)
- Respiratory: cough (6% to 44%)
- Other: fatigue (10 to 38%), fever (11% to 69%), infectious disease (12% to 44%), pain (5% to 22%), shivering (11% to 19%)

Serious

- Hematologic: decreased hemoglobin (14% to 60%), hemolytic anemia, neutropenia (37% to 59%), pancytopenia, thrombocytopenia (17% to 55%)
- Neurologic: neurotoxicity, progressive multifocal leukoencephalopathy
- Respiratory: pulmonary toxicity
- Other: graft versus host disease, tumor lysis syndrome (.33% to 1%)

3.2 Bendamustine (Treanda):
Bendamustine (TREANDA®) for Injection is indicated for the treatment of patients with CLL. Efficacy relative to first line therapies other than chlorambucil has not been established.

3.2.1 How Supplied:
Bendamustine for Injection single-use vial containing 100 mg of bendamustine hydrochloride (HCl) as white to off-white lyophilized powder.

3.2.2 Solution Preparation:
Aseptically reconstitute each 100 mg bendamustine vial with 20 mL of Sterile Water for Injection, USP. This yields a clear, colorless to a pale yellow solution with a bendamustine HCl concentration of 5 mg/mL. The lyophilized powder should completely dissolve in 5 minutes. If particulate matter is observed, the reconstituted product should not be used.

Aseptically withdraw the volume needed for the required dose (based on 5 mg/mL concentration) and immediately transfer to a 500 mL infusion bag of 0.9% Sodium Chloride Injection, USP (normal saline). The reconstituted solution must be transferred to the infusion bag within 30 minutes of reconstitution. After transferring, thoroughly mix the contents of the infusion bag. The admixture should be a clear and colorless to slightly yellow solution.

Sterile Water for Injection, USP and 0.9% Sodium Chloride Injection, USP must be used as outlined above. Compatibility with other diluents has not been determined. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Any unused solution should be discarded according to institutional procedures for antineoplastic agents.

3.2.3 Handling and Disposal:
Bendamustine is intended for administration as an intravenous infusion over 30 minutes.

As with other potentially toxic anticancer agents, care should be exercised in the handling and preparation of solutions prepared from bendamustine. The use of gloves and safety glasses is recommended to avoid exposure in case of breakage of the vial or other accidental spillage. If a solution of bendamustine contacts the skin, wash the skin immediately and thoroughly with soap and water. If bendamustine contacts the mucous membranes, flush thoroughly with water.

Procedures for the proper handling and disposal of anticancer drugs should be considered. Several guidelines on the subject have been published. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

3.2.4 Stability

Bendamustine contains no antimicrobial preservative. The admixture should be prepared as close as possible to the time of patient administration. Once diluted with 0.9% Sodium Chloride Injection, USP, the final admixture, is stable for 24 hours when stored refrigerated (2-8°C or 36-47°F) or for 3 hours when stored at room temperature (15-30°C or 59-86°F) and room light. Administration of bendamustine must be completed within this period.

3.2.5 Route of Administration

Intravenous

3.2.6 Bendamustine Side Effects

Common

- Dermatologic: rash (all grades, 8% to 16%; grade 3 or 4, less than 1% to 3%)
- Endocrine metabolic: weight loss (all grades, 7% to 18%, grade 3 or 4, 2 %)
- Gastrointestinal: constipation (all grades, 29%), grade 3 or 4 less than 1%), diarrhea (all grades, 9% to 37%; grade 3 or 4, 1% to 3%), loss of appetite (all grades, 23%; grade 3 or 4, 2%), nausea (all grades, 20% to 75%; grade 3 or 4, less than 1% to 4%), stomatitis (all grades, 15%; grade 3 or 4, less than 1%), vomiting (all grades, 16% to 40%; grade 3 or 4, less than 1% to 3%)
- Neurologic: headache (21%)
- Respiratory: cough (all grades, 4% to 22%; grade 3 or 4, less than 1%), dyspnea (all grades,16%; grade 3 or 4, 2%)

- Other: dehydration (all grades, 14%; grade 3 or 4, less than 5%), fatigue (all grades, 9% to 57%; grade 3 or 4, 1% to 11%), fever (all grades, 24% to 34%; grade 3 or 4, 2% to 4%)

Serious

- Cardiovascular: hypertensive crisis
- Dermatologic: dermatologic toxicity, Stevens-Johnson syndrome, toxic epidermal necrolysis
- Endocrine metabolic: hyperuricemia (all grades, 7%; grade 3 or 4, 2%)
- Hematologic: acute myeloid leukemia, anemia (all grades, 88% to 89%; grade 3 or 4, 11% to 13%), febrile neutropenia (6%), leukopenia (all grades, 61% to 94%; grade 3 or 4, 28% to 56%), lymphocytopenia (all grades, 68% to 99%, grade 3 or 4, 47% to 94%), myelodysplastic syndrome, myeloproliferative disorder, myelosuppression, grades 3 and 4 (98%), neutropenia (all grades, 75% to 86%; grade 3 or 4, 43% to 60%), thrombocytopenia (all grades, 77% to 86%; grade 3 or 4, 11 % to 25%)
- Immunologic: anaphylaxis, hypersensitivity reaction (all grades 5%; grade 3 or 4, 1%), infectious disease (all grades 6%; grade 3 or 4, 2%), sepsis, septic shock
- Renal: renal failure
- Respiratory: squamous cell carcinoma of bronchus
- Other: myelodysplastic syndrome, tumor lysis syndrome

3.3 Rituximab:

3.3.1 Investigational Drug Nomenclature

- IDEC Pharmaceuticals code designation Rituxan®
- Generic Name: rituximab
- IND Number: BB-IND 4904

3.3.2 Clinical Formulation:

Clinical supplies for this study will be manufactured by Genentech Incorporated in South San Francisco, CA.

Rituximab will be provided to the clinical sites packaged in single use 10 mL (100mg) and 50 mL (500mg) Type I glass vials at a concentration of 10 mg of protein per mL. The product is formulated in 7.35 mg/mL sodium citrate buffer, containing 7 mg/mL polysorbate 80, 9.0 mg/mL sodium chloride and Sterile Water for Injection. The pH is adjusted to 6.5.

Rituximab may be produced by the mammalian (Chinese Hamster Ovary) cell suspension culture in a nutrient medium containing 100 mg/mL of the antibiotic gentamicin. The antibiotic is not detectable in the final product.

3.3.3 Storage

Rituximab for clinical use should be stored in a secure refrigerator at 2-8°C.

3.3.4 Reconstitution and Dilution of Rituximab

Using a sterile syringe and a 21 gauge or larger needle, transfer the necessary amount of rituximab from the vial into a partially filled IV pack containing sterile, pyrogen-free 0.9% Sodium Chloride, USP (saline solution). The final concentration of rituximab should be 1 mg/mL. Mix by inverting the bag gently.

Caution should be taken during the preparation of the drug. Parenteral drug products should be inspected visually for particulate matter prior to administration. Preparations of rituximab containing visible particles should not be used. As with all parenteral drug products, aseptic procedures should be used during the preparation and administration of rituximab.

NOTE: DO NOT USE A VACUUM APPARATUS to transfer rituximab from the syringe to the infusion pack. DO NOT USE evacuated glass containers, which require vented administration sets, because this causes foaming when air bubbles pass through the solution.

3.3.5 Rituximab Side Effects:

Common

- Cardiovascular: hypertension (all grades, 6%; grades 3-4, 1%); hypotension (all grades, 10%; grades 3 and 4, 1%)
- Dermatologic: pruritis
- Gastrointestinal: nausea, vomiting
- Neurologic: asthenia (non-Hodgkin's lymphoma, all grades, 26%; grades 3 and 4, 1%; rheumatoid arthritis, 2%), dizziness (all grades, 10%; grades 3 and 4, 1%), headache (all grades, 19%, grades 3 and 4, 1%), sensory neuropathy (30%)
- Other: fever (all grades, 53%; grades 3 and 4, 1%), shivering (all grades, 33%; grades 3 and 4, 3%)

Serious

- Cardiovascular: cardiac dysrhythmia, cardiogenic shock, heart failure, myocardial infarction, supraventricular arrhythmia, supraventricular tachycardia
- Dermatologic: drug-induced pemphigus, Lichenoid dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis
- Gastrointestinal: bowel obstruction, gastrointestinal perforation
- Hematologic: anemia (all grades, 8%; grades 3 and 4, 3%), aplastic anemia, transient cytopenia, grades 3 and 4 (48%), hemolytic anemia, leukopenia (all grades 14%; grade 3 and 4, 4%, lymphocytopenia (grades 3 and 4, 40%) neutropenia (all grades, 14%; grade 3 and 4, 6%), thrombocytopenia (all grades, 12%; grade 3 and 4, 2%)
- Hepatic: relapsing type B viral hepatitis
- Immunologic: complication of infusion (first infusion, 77%); subsequent infusions, (14% to 30%), immune hypersensitivity reaction
- Neurologic: progressive multifocal leukoencephalopathy (rheumatoid arthritis, rare)
- Renal: nephrotoxicity
- Respiratory: obliterative bronchioilitis, pneumonitis, pulmonary fibrosis
- Other: infectious disease (all grades, 31%, grades 3 and 4, 4%), tumor lysis syndrome

- 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 4 months after the initiation of rituximab and approximately one month after the last dose.

4.0 PATIENT ELIGIBILITY

4.1 Inclusion criteria:

1. Patients must have a diagnosis of CLL/SLL and be previously treated
2. Patients must have an indication for treatment by 2008 IWCLL Criteria
3. Age ≥ 16 years
4. Zubrod performance status ≤ 2
5. Adequate renal and hepatic function as indicated by all the following:
   a. serum creatinine ≤ 2 mg/dL AND;
   b. alanine aminotransferase (ALT) ≤ 2.5 times upper limit of normal AND;

c. total bilirubin ≤ 2.5 times upper limit of normal
6. Patients must give written informed consent
7. Patients of childbearing potential must be willing to practice birth control during the study

4.2 Exclusion Criteria**:**

1. Pregnant or breast-feeding females
2. Significant co-morbidity indicated by major organ system dysfunction
3. Active, uncontrolled infection, including active hepatitis
4. Uncontrolled autoimmune hemolytic anemia (AIHA) or immune thrombocytopenia purpura (ITP)
5. Treatment including chemotherapy, chemoimmunotherapy, monoclonal antibody therapy, radiotherapy, high-dose corticosteroid therapy (prednisone >/= 60 mg daily, or equivalent), or immunotherapy within 21 days prior to enrollment or concurrent with this trial

5.0 TREATMENT PLAN

After patients sign informed consent and screening is complete, all pretreatment evaluations have been done, and eligibility is confirmed, patients may begin treatment. Drug doses for Phase I and Phase II are detailed below. Phase I will consist of fixed doses of fludarabine (20 mg/m^2, days 2-4) and rituximab (375-500 mg/m^2, day 4 or 1) with increasing doses of bendamustine as indicated. Patients will receive 6 courses and will have response assessment prior to course 4 and 3 months after last course of FBR.

Phase I:

<u>Course 1</u>
Fludarabine 20 mg/m^2 IV (fixed), Days 2,3,4
Bendamustine 20, 30, 40, or 50 mg/m^2 IV, Days 1,2,3 (after fludarabine)
Rituximab 375 mg/m^2 IV (fixed), Day 4 (after fludarabine)

<u>Courses 2-6</u>
Fludarabine 20 mg/m^2 IV (fixed), Days 1,2,3
Bendamustine 20, 30, 40, or 50 mg/m^2 IV Days 1,2,3 (after fludarabine)
Rituximab 500 mg/m^2 IV (fixed), Day 1

Courses will be every 4 weeks as permitted by recovery of blood counts, for total of 6 planned courses. The next course of FBR may begin when absolute neutrophil count (ANC) and platelets (PLT) have recovered to within 20% of pretreatment levels or ANC ≥ 1,200/μL and PLT ≥ 75,000/μL. For courses 2-6, bendamustine will be dose reduced to the next lower dose for patients experiencing prolonged myelosuppression defined as Grade 3 or 4 neutropenia or thrombocytopenia lasting longer than Day 42 of the course for

patients who begin treatment with normal neutrophil or PLT counts. For patients who begin treatment with neutropenia (ANC <1,000/μL) or thrombocytopenia (PLT <100,000/μL), dose reduction of bendamustine to the next lower dose will occur if neutrophil or platelet count does not recover to within 20% of the pre-treatment level of the previous course. For courses 2-6, intra-patient dose escalation may occur to the next higher dose level for which there are 3 patients evaluated for toxicity and a decision for dose level expansion or dose escalation has been made. Granulocyte Colony Stimulating Factor (G-CSF growth factor) may be used in courses 2-6.

To prevent Tumor Lysis Syndrome (TLS) in patients with elevated WBC who may develop tumor lysis despite allopurinol therapy, consider the use of rasburicase for C1 if ALC >50K and uric acid >5 and daily labs during chemotherapy to monitor potassium, phosphorus, calcium and uric acid.

Phase II:

Course 1
Fludarabine 20 mg/m^2 IV (fixed), Days 2,3,4
Bendamustine 30 mg/m^2 IV (fixed), Days 1,2,3 (after fludarabine)
Rituximab 375 mg/m^2 IV (fixed), Day 4 (after fludarabine)

Courses 2-6
Fludarabine 20 mg/m^2 IV (fixed), Days 1,2,3
Bendamustine 30 mg/m^2 IV (fixed), Days 1,2,3 (after fludarabine)
Rituximab 500 mg/m^2 IV (fixed), Day 1

Courses will be every 4 weeks as permitted by recovery of blood counts, for a total of 6 planned courses. The next course of FBR may begin when ANC and PLT counts have recovered to within 20% of pretreatment level or ANC >/= 1,200/μL and PLT >/= 75,000/μL. Bendamustine will be dose reduced to the next lower dose for patients experiencing prolonged myelosuppression defined as Grade 3 or 4 neutropenia or thrombocytopenia lasting longer than Day 42 of the course for patients who begin treatment with normal neutrophil or PLT counts. For patients who begin treatment with neutropenia (ANC <1,000/μL) or thrombocytopenia (PLT <100,000/μL), dose reduction of bendamustine to the next lower dose will occur if neutrophil or platelet count does not recover to within 20% of the pre-treatment level of the previous course. G-CSF growth factor may be used for courses 1-6 of Phase II.

Dose adjustment for toxicity during Phase II:

Dose Level

-1 Fludarabine 20 mg/m^2 IV (fixed), Days 1,2
Bendamustine 30 mg/m^2, IV, Days 1,2,
Rituximab 500 mg/m^2 IV (fixed), Day 1

-2 Fludarabine 20 mg/m^2 IV (fixed), Days 1,2
Bendamustine 20 mg/m^2 IV (fixed), Days 1,2
Rituximab 500 mg/m^2 IV (fixed), Day 1

The dose of fludarabine may be adjusted in Phase II for patients with renal insufficiency with reduction according to the fludarabine package insert.

5.1 Suggested premedications: Anti-emetic (ondansetron 8 mg IV or equivalent) premedication will be given 30 min prior to chemotherapy (fludarabine/bendamustine). Premedication for rituximab will consist of 325 mg-650 mg acetaminophen orally and 25-50 mg diphenhydramine hydrochloride orally or intravenously. Steroids may also be used at the discretion of the treating physician. Other premedications or modifications of the above may be appropriate based on the physician or patient experience.

5.2 Suggested supportive medications: Allopurinol is recommended for at least the first week of course 1 for tumor lysis prophylaxis. Valacyclovir (or equivalent) is recommended for herpes virus prophylaxis and Bactrim DS (or equivalent) for PCP prophylaxis. For patients who were previously exposed to hepatitis B and are seropositive, consideration should be given for lamivudine prophylaxis.

For courses 2-6, growth factor (Neulasta or Neupogen, Erythropoietin [EPO]) will be allowed and doses of bendamustine may be adjusted as indicated above.

5.3 Administration of fludarabine
Fludarabine will be administered intravenous over 30 minutes after indicated premedication given. On days where fludarabine and bendamustine are to be given, fludarabine will be given prior to bendamustine.

5.4 Administration of bendamustine
Bendamustine will be administered IV over 30 minutes.

5.5 Administration of rituximab:
During the rituximab infusion, the patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored. Available at the

bedside prior to rituximab administration will be appropriate measures for management of anaphylactic reactions.

The recommended first dose administration of rituximab is 50 mg/hr for the first hour. If no toxicity is seen, the dose rate may be escalated gradually **(50 mg/hour increments at 30-minute intervals)** to a maximum of 500 mg/hr. If the first dose of rituximab is well tolerated, the starting flow rate for the administration of doses 2-6 may be 100 mg/hour then increased gradually **(100 mg/hour increments at 30-minute intervals)**.

If the first infusion of rituximab is well-tolerated, then for subsequent infusions (courses 2-6), accelerated administration of rituximab may be given. This consists of 20% of the full dose given over the first 30 min followed by the remaining 80% of the full dose given over the subsequent hour if the first 30 min is tolerated. For accelerated administration place doses less than 1,000 mg in 250 mL of normal saline and run 100mL for the first 30 minutes then the remaining 200 mL over 30 minutes (or until completed). For doses >1000 mg , the infusion should be 500 mL, with 200 mL/hour for the first 30 minutes, then 400 mL/hour for 60 minutes (or until completed).

Patients may experience transient fever and rigors with infusion of chimeric anti-CD20 (rituximab) antibody. When these side effects are noted, the antibody infusion should be slowed or interrupted, the patient should be observed and the severity of the side effects should be evaluated. The patient should be treated according to the best available local practices and procedures. Following observation, when the patient's symptoms improve, the infusion should be continued, initially, at half the previous rate (see Table 1. below). Upon resolution of all side effects and in the judgment of the investigator, the patient's dose may be gradually escalated (50 mg/hr increments at 30 minute intervals) to a maximum rate of 300 mg/hr. Following the antibody infusion, the IV line should be kept open for medications, as needed. If complications occur during the rituximab infusion, the patient should be observed for two hours after the completion of the infusion.

Since transient hypotension has been reported during rituximab infusions, consideration should be given to withholding anti-hypertensive medications the day of the rituximab infusion.

Table 1. Modification of rituximab infusion rate for side effects

Dose Rate	Fever	Rigors	Mucosal Congestion/Edema	% Drop in Systolic Pressure
Decrease	> 38.5C	Mild/		

to ½	Moderate	Mild/Moderate	> 30 mm Hg

5.6 Patients achieving a stable partial response or demonstrating continued response after 3 courses will be given an additional 3 courses. No patient will receive more than 6 courses.

5.7 Patients demonstrating progressive disease or no response after 3 courses of treatment will come off study.

5.8 Dose adjustment to the next lower level will be made if pneumonia, septicemia, or other life-threatening infection occurs with any course. If recovery of the platelet count to the level prior to treatment exceeds 35 days, the dose will be decreased 1 level. If grade 3 or 4 toxicities to other organ systems develop, the dose level will be lowered 1 or 2 levels respectively.

5.9 Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis throughout their study participation.

6.0 PRETREATMENT EVALUATION

Patients will undergo screening to evaluate and confirm eligibility. Upon confirming eligibility, patients will proceed with treatment on this trial. Screening evaluation, including laboratory tests will be done within 2 weeks of starting treatment.

6.1 Screening will consist of medical history and physical examination and pertinent laboratories including SMA12 and CBC with differential, serum β-2 microglobulin, as well as a pregnancy test (serum or urine) for females of childbearing potential. This will confirm eligibility and provide baseline measurements of lymph node, spleen, and liver size and blood counts that will be used for response assessment.

6.2 Patients will be screened for hepatitis exposure and infection by serum HBcAb, HBsAb and HBsAg. Patients will have bone marrow aspirate and biopsy with samples sent for differential and morphology within 2 months of starting treatment. Patients will have their prognostic factors characterized on pretreatment blood or bone marrow including presence of cytogenetic abnormalities (FISH for $13q^-$, +12, $11q^-$, and $17p^-$), expression of ZAP70 and CD38, and serum thymidine kinase. If already known, the following prognostic factors do not need to be reevaluated since they are not expected to change: leukemia cell *IGHV* gene mutational status and ZAP-70 expression.

6.3 Any appropriate radiological and radioisotopic examinations should be performed as clinically indicated.

6.4 Optional blood (20 mL) will be taken to isolate and store pretreatment mononuclear cells, DNA, RNA, and plasma. Optional bone marrow will be taken (5 mL) to isolate and store cells, DNA, ribonucleic acid (RNA) and marrow plasma. Gene methylation analyses will be performed on these pre-treatment samples to correlate with treatment outcome.

7.0 EVALUATION DURING STUDY

Table 2. Schedule of Events – Phase I / II

Test and Evaluations	Screening Visit Day ≤ -14	C1 D1	C1 D2	C1 D3	C1 D4	C1 D8	C1 D15	C1 D22	C2-6 D1	C2-6 D2	C2-6 D3	C2-6 D15	Prior to C4	End of Tx
Informed consent	X													
Medical history	X													
Interval history		X							X**					
PE including VS	X	X							X**					
Pregnancy test	X													
HBcAb, HBsAb, HBsAg	X													
Bendamustine (Ph I/II doses)		X	X	X					X	X	X			
Fludarabine (20mg/m^2)			X	X	X				X	X	X			
Rituximab (mg/m^2)					375				500					
Adverse event screening		X				X	X	X	X			X		
CBC with diff, PLT	X	X				X	X	X	X**			X		
SMA 12	X	X				X*	X*	X*	X**					
PK & PD, optional samples****	X	X	X	X	X				X		X			
Response assessment (2008 IWCLL criteria, staging CT scan, and BM for MRD)***													X	After C6, then 6, 12, 24 mo

BM=bone marrow evaluation; C=course; D=day; PE=physical examination; VS=vital signs; CBC=complete blood count; PK=pharmacokinetic; PD=pharmacodynamic; IWCLL=International Working Group for CLL; MRD=minimal residual disease; All courses are 4 weeks, depending on recovery of ANC and PLT; Tx=treatment; X*=for Phase I only; **indicates ± 3 days; ***before C4 and after 6 courses or the last course if fewer than 6 given; ****not all samples will be collected in all patients at all time-points

7.1 Patients will be followed with CBC, platelet count and differential weekly (±3 days) for the first course and q2 – 4 weeks (±3 days) during therapy

thereafter for both Phase I and II. An SMA 12 (bilirubin, creatinine, albumin, LDH) will be done weekly (±3 days) for the first course for Phase I. For Phase I, courses 2-6 and for patients on Phase II, SMA 12 will be done before each course and as clinically indicated. Physical examination every follow-up visit at MD Anderson Cancer Center (MDACC).

7.2 Before course 4 and after 6 courses of treatment or the last course if fewer than 6 given, a full evaluation for response assessment will be performed including CBC, platelet and differential count, SMA (bilirubin, creatinine, albumin, LDH), and bone marrow aspiration and biopsy with samples sent for differential, flow cytometry, and morphologic analysis. Bone marrow will be evaluated by flow cytometry for minimal residual disease (4-color flow will be done). A confirmatory CT of neck, chest, abdomen, and pelvis will be done for patients in clinical complete remission.

7.3 Myelosuppression and associated complications are expected events during leukemia therapy and are part of the treatment success (marrow emptying of leukemia cells). Therefore, myelosuppression and associated complications such as fever, infections, bleeding, and related hospitalizations, will not be reported as individual ADRs, but will be summarized in the updated and final reports. Only prolonged Grade 3-4 myelosuppression, as defined by the 2008 IWCLL criteria specific for leukemia, i.e., marrow cellularity <5% on day 42 or later (6 weeks) from start of therapy without evidence of leukemia, will be reported as ADR and considered in defining the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of particular agents or regimens.

7.4 Repeat response assessments by physical examination, blood counts, and bone marrow evaluation, including for MRD will be done 6, 12, and 24 months after last treatment course until relapse. Evaluation for MRD may be done on blood. Blood or marrow for MRD evaluation may be taken by referring physician and mailed to UTMDACC. Follow-up will be annually thereafter and bone marrow examinations will be done at the discretion of the treating physician for those visits.

7.5 Optional blood (10 ml) may be taken to determine rituximab levels prior to rituximab dose with each course and on day 4 (±2 days) of course 1 and day 3 (±1 day) of courses 2-6.

Optional blood may be taken by the referring physician in a green-top (heparin) tube and shipped overnight to UTMDACC. For shipping, each tube will be labeled with the patients' name, MDACC MRN, date and time of drawing. Samples will be shipped same day they are drawn. Tubes will be covered with absorbent wrap and placed in a plastic bag then into a shipping kit provided. Pre-printed shipping labels will be provided for shipping.

Samples will be shipped to:

Attention of Ruth LaPushin
MD Anderson Cancer Center
1515 Holcombe Blvd
T6.3849
Houston, Texas 77030
Phone: 713-792-3690

7.6 Optional blood (20 mL) may be taken at response assessment (end of treatment) and follow-up visits after completion of treatment to monitor for immune reconstitution. Immune reconstitution samples will be evaluated for T cell and normal B cell populations, T cell receptor repertoire, T cell functional subsets defined by multi-color flow cytometry. Optional blood may be taken by referring physician in a green-top tube (heparin) and mailed to UTMDACC.

8.0 TOXICITY EVALUATION AND RESPONSE CRITERIA

Non-hematologic toxicity will be described and graded by the Common Terminology Criteria for Adverse Events (CTCAE) Version 3. Hematologic toxicity will be graded according to the 2008 IWCLL criteria for grading (Table 3)[4].

Table 3. – Grading of Myelosuppression

Grade	Decrease in PLT* or HGB** (nadir) from pretreatment value, %	Absolute neutrophil count (ANC)/μl*** (nadir)
0	< 10%	≥ 2000
1	11 – 24%	≥ 1500 – < 2000
2	25 – 49%	≥ 1000 – < 1500
3	50 – 74%	≥ 500 – < 1000
4	≥ 75%	< 500

Death occurring as a result of toxicity at any level of decrease from pretreatment will be recorded as grade 5.

* PLT counts must be below normal levels for grades 1-4. If, at any level of decrease, the PLT count is < 20K/μl, this will be considered grade 4 toxicity, unless there was severe or life-threatening low initial PLT count (< 20K/μl) pretreatment, in which case the patient is not evaluable for toxicity referable to PLT count.

** HGB levels must be below normal levels for grades 1-4. Baseline and subsequent HGB determinations must be performed before any given transfusions.

*** If the ANC reaches <1000/μl, it should be judged to be grade 3 toxicity. If the ANC was <1000/μl before therapy, the patient is not evaluable for toxicity referable to the ANC.

Responses will be evaluated by the updated 2008 IWCLL Response Criteria (Table 4), including staging with CT scan and bone marrow evaluation for minimal residual disease for patients in complete remission[4]. Bone marrow will be evaluated for minimal residual disease by 4-color flow cytometry for patients in clinical complete remission. For patients in clinical complete remission, a confirmatory CT scan of neck, chest, abdomen, and pelvis will be done. Lymph nodes 1.5 cm in diameter or smaller will be considered normal and consistent with complete remission.

Table 4. 2008 IWCLL Response Criteria Summary

SITE	CR	PR
Nodes	None	≥ 50% decrease
Liver/Spleen	Not palpable	≥ 50% decrease
Symptoms	None	N/A
PMN	>1,500/μl	> 1,500/μl or >50% improvement from baseline
Platelets	>100,000/μl	>100,000/μl or 50% improvement from baseline
Hemoglobin (non-transfused)	>11.0 gm/dl	>11.0 g/dl or >50% improvement from baseline
Lymphocytes	<4,000/μl	>50% decrease
Bone Marrow aspirate	<30% lymphocytes	N/A for PR
Bone Marrow biopsy	No lymphocyte infiltrate	< 30% lymphocytes with residual disease on biopsy for nodular PR
Bone Marrow aspirate flow	Research	N/A
CT scan of chest, abdomen, pelvis	Lymph nodes <1.5 cm	Lymph nodes >/= 50% reduced (sum product of lymph nodes

9.0 REMOVAL FROM STUDY

9.1 Progressive or Relapsed Disease

Progressive disease (PD) will be characterized by at least one of the following:

a. ≥ 50% increase in the sum of the products of at least two nodes on two consecutive examinations two weeks apart (at least one node must be ≥ 2 cm). Appearance of new palpable lymph nodes.

b. ≥ 50% increase in the size of liver and/or spleen as determined by measurement below the respective costal margin; appearance of palpable hepatomegaly or splenomegaly, which was not previously present.

c. ≥ 50% increase in absolute number of circulating lymphocytes and at least 10,000/μl.

9.2 Patient request.

9.3 Active HBV infection or hepatitis.

10.0 STATISTICAL CONSIDERATIONS

10.1 Phase I

A standard 3+3 design will be used to evaluate for tolerability and toxicities of combined bendamustine (increasing doses), fludarabine, and rituximab in previously treated patients with CLL. The DLT and MTD will be evaluated based on the information from the first course (4 weeks) of the combination therapy.

DLT will be defined as treatment-related, Grade ≥ 3 non-hematologic toxicity. Hematologic toxicity grade ≥ 3 that lasts longer than 42 days will be considered a DLT (see Section 8). Since TLS is associated with therapeutic activity of this regimen and since it is medically manageable, TLS will not be considered a DLT (see Section 5). Fixed doses of fludarabine and rituximab will be given. There will be up to 4 dose levels for bendamustine including: 20, 30, 40 and 50 mg/m^2 daily x3.

Applying the "3+3" design, the first cohort of 3 patients will be treated at the first dose level and evaluated for toxicity. The algorithm is as follows: (1) If 0 out of 3 patients experiences DLT, the next cohort of 3 patients will be treated at the next higher dose level. (2) If 1 out of 3 patients develop DLT, an additional 3 patients will be treated at the same dose level. At this level, if 1 out of 6 patients had DLT, the dose escalation continues. If 2 out of 6 patients experience DLT, the dose level exceeds the MTD and 3 more patients will be treated at the next lower dose if there are less than 6 patients already treated at that dose. (3) If greater than 1 out of 3 patients experiences DLT, the dose level exceeds the MTD and 3 more patients will be treated at the next lower dose if there are less than 6 patients already treated at that dose. In summary, MTD is defined as the highest dose level in which 6 patients have been treated with less than or equal to 1 patient experiencing DLT. Toxicity evaluation will be completed within 1 course of therapy for all 3 patients. No patient will be enrolled in the next dose level until the toxicity is fully assessed in all 3 patients enrolled at the previous dose level. For courses 2-6, intra-patient dose escalation may occur to the next higher level for which there are 3 patients evaluated for toxicity and a decision for dose level expansion or dose escalation has been made. Given these 4 dose levels, the maximum number of patients required is 24 for this Phase I trial. The 6 patients treated at the tolerated dose will be included in the Phase II analysis.

10.2 Phase II

The efficacy will be assessed in the Phase II part, using the Simon's two-stage MinMax design. Overall response will be assessed 2 months after the 6th or last course if patients are not able to receive all 6 intended courses of treatment. The treatment will be considered promising if the complete remission rate is 40% or higher, and will be considered unworthy of further investigation if the complete remission rate is 25% or lower. The

sample size of 64 is chosen to differentiate between the complete remission rate of 40% and 25% with 90% power at a significance level of 0.1. In particular, 39 patients will be enrolled at the first stage. If 9 or fewer of the first 39 patients respond, the trial will be terminated; otherwise another 25 patients will be treated for a total of 64 patients. If 20 or fewer patients respond among 64 patients, the treatment will be concluded ineffective. The probability of early termination due to futility is 0.48.

In addition, the probability of toxicity will be monitored based on the Bayesian model (beta-binomial), assuming a priori that p = Prob(toxicity) ~ beta(1,1). The trial will be terminated if Prob(p > .30 | data) > .80. This rule will be applied continuously starting from the 6th evaluable patient and will stop the trial if [number of toxicities]/[number of patients evaluated] is greater than or equal to: 3/6, 4/8, 5/10, 6/13, 7/16, 8/19, 9/22, 10/25, 11/28, 12/31, 13/34, 14/37, 15/40, 16/43, 17/46, 18/49, 19/52, 20/55, 21/58, 22/61.

The expected rate of accrual onto the trial is 0.2 patients per month. The maximum sample size in Phase I is 24. The patients in Phase I at a dose level of MTD will be used for the Phase II part since this dosage is used in the Phase II. The maximum number of patients expected to enter in to this Phase I/II is 82 (= 24+ 64 -6).

Phase II will include 64 patients to evaluate the efficacy and tolerability of the FBR combination in previously treated patients with CLL. We will estimate the complete, partial, and overall response rate with 95% confidence interval. In addition, we will determine the median PFS and TTF and 95% confidence interval. We will also evaluate associations between the prognostic factors and response and time-to-event endpoints. Efficacy endpoints for Phase I and II will be evaluated by the 2008 IWCLL criteria. We will evaluate the complete, nodular partial and partial response rates. We aim to improve the complete remission rate from the expected 25% for the FCR regimen to 40% as the primary objective. Safety end-points will be tolerability and toxicity of therapy. The tolerated dose of bendamustine will be the highest dose at which fewer than 2 of 6 patients experience grade 3, non-hematologic, non-infusion related toxicity involving a major organ system. The maximum tolerated dose will have been exceeded if 2 or more of 6 patients experience grade 3 or higher, non-hematologic, non-infusion related toxicity a major organ system.

11.0 PHARMACODYNAMIC AND PHARMACOKINETIC ENDPOINTS

These investigations will be performed in Drs. Gandhi and Plunkett's laboratory. Patients with more than 5,000 WBC/µl of peripheral blood who agree to participate in pharmacokinetic and pharmacodynamic

investigations will be evaluated. Not all samples will be collected in all patients at all time-points.

Please page Yuling Chen (713-404-2550), Min Fu (713-606-2212), or call 713-792-3336 to inform of registration date and to arrange for blood sampling.

Blood samples (10 ml) will be collected at the following times:

Day 1 Bendamustine alone:

- Pre treatment (0 h), 2 h, 4 h, and 6 h, after the start of the first bendamustine infusion.

Day 2 Fludarabine followed by bendamustine:

- 24 h (prior to fludarabine), 26.5 h, 28.5 h, and 30.5 h (2 h, 4 h, and 6 h, after start of bendamustine infusion).

Day 3 Fludarabine followed by bendamustine:

- 48 h (prior to fludarabine)

These samples will be processed to collect plasma and cells. Plasma will be stored for future pharmacokinetic analyses, if needed, by Cephalon. Cells will be processed for the following endpoints.

1. DNA damage response using H2AX phosphorylation as a biomarker
2. Cell death measurements by Annexin V assay
3. Quantitation of intracellular level of fludarabine triphosphate by HPLC (only in 28.5 hr sample)
4. Cell pellet for immunoblot assays for DNA damage response, DNA repair, and recovery of DNA damage.

These assays will primarily measure bendamustine induced DNA damage response and recovery of the DNA repair. In addition, we will determine if circulating leukemia cells undergo apoptosis. These studies will also compare if there is an increase in DNA damage response and/or cell death when bendamustine is infused alone (day 1) versus when bendamustine is administered with fludarabine (day 2). For these comparative analyses, each patient serves as his or her own control. Augmentation of DNA damage response will be compared with intracellular fludarabine triphosphate peak (peak occurs 4 h after fludarabine infusion[44]. Correlations will be sought between these laboratory endpoints and cytoreduction and/or clinical response to therapy.

12.0 REFERENCES

1. Wierda WG, Keating MJ, S.M. OB: Chronic lymphocytic leukemias, in DeVita VT, Lawrence TS, Rosenberg SA (eds): DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology. Chronic Leukemias (ed 8th Edition). Philadelphia, PA, Lippincott Williams & Wilkins, 2008, pp 2278-2292
2. Cheson BD, Bennett JM, Rai KR, et al: Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendations of the National Cancer Institute-sponsored working group. Am J Hematol 29:152-63, 1988
3. Cheson BD, Bennett JM, Grever M, et al: National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 87:4990-7, 1996
4. Hallek M, Cheson BD, Catovsky D, et al: Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 111:5446-56, 2008
5. Han T, Ezdinli EZ, Shimaoka K, et al: Chlorambucil vs. combined chlorambucil-corticosteroid therapy in chronic lymphocytic leukemia. Cancer 31:502-8, 1973
6. Sawitsky A, Rai KR, Glidewell O, et al: Comparison of daily versus intermittent chlorambucil and prednisone therapy in the treatment of patients with chronic lymphocytic leukemia. Blood 50:1049-59, 1977
7. Idestrom K, Kimby E, Bjorkholm M, et al: Treatment of chronic lymphocytic leukaemia and well-differentiated lymphocytic lymphoma with continuous low- or intermittent high-dose prednimustine versus chlorambucil/prednisolone. Eur J Cancer Clin Oncol 18:1117-23, 1982
8. Montserrat E, Alcala A, Parody R, et al: Treatment of chronic lymphocytic leukemia in advanced stages. A randomized trial comparing chlorambucil plus prednisone versus cyclophosphamide, vincristine, and prednisone. Cancer 56:2369-75, 1985
9. Keller JW, Knospe WH, Raney M, et al: Treatment of chronic lymphocytic leukemia using chlorambucil and prednisone with or without cycle-active consolidation chemotherapy. A Southeastern Cancer Study Group Trial. Cancer 58:1185-92, 1986
10. Binet JL, Lepoprier M, Dighiero G, et al: A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 40:855-64, 1977
11. Kempin S, Lee BJ, 3rd, Thaler HT, et al: Combination chemotherapy of advanced chronic lymphocytic leukemia: the M-2 protocol (vincristine, BCNU, cyclophosphamide, melphalan, and prednisone). Blood 60:1110-21, 1982
12. Keating MJ, Kantarjian H, Talpaz M, et al: Fludarabine therapy in chronic lymphocytic leukemia (CLL). Nouv Rev Fr Hematol 30:461-6, 1988

13. Keating MJ, Kantarjian H, Talpaz M, et al: Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood 74:19-25, 1989
14. Kantarjian HM, Alexanian R, Koller CA, et al: Fludarabine therapy in macroglobulinemic lymphoma. Blood 75:1928-31, 1990
15. Kantarjian HM, Childs C, O'Brien S, et al: Efficacy of fludarabine, a new adenine nucleoside analogue, in patients with prolymphocytic leukemia and the prolymphocytoid variant of chronic lymphocytic leukemia. Am J Med 90:223-8, 1991
16. Keating MJ, Kantarjian H, O'Brien S, et al: Fludarabine: a new agent with marked cytoreductive activity in untreated chronic lymphocytic leukemia. J Clin Oncol 9:44-9, 1991
17. Redman JR, Cabanillas F, Velasquez WS, et al: Phase II trial of fludarabine phosphate in lymphoma: an effective new agent in low-grade lymphoma. J Clin Oncol 10:790-4, 1992
18. Robertson LE, Huh YO, Butler JJ, et al: Response assessment in chronic lymphocytic leukemia after fludarabine plus prednisone: clinical, pathologic, immunophenotypic, and molecular analysis. Blood 80:29-36, 1992
19. O'Brien S, Kantarjian H, Beran M, et al: Results of fludarabine and prednisone therapy in 264 patients with chronic lymphocytic leukemia with multivariate analysis-derived prognostic model for response to treatment. Blood 82:1695-700, 1993
20. Liepman M, Votaw ML: The treatment of chronic lymphocytic leukemia with COP chemotherapy. Cancer 41:1664-9, 1978
21. Oken MM, Kaplan ME: Combination chemotherapy with cyclophosphamide, vincristine, and prednisone in the treatment of refractory chronic lymphocytic leukemia. Cancer Treat Rep 63:441-7, 1979
22. Yamauchi T, Nowak BJ, Keating MJ, et al: DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. Clin Cancer Res 7:3580-9, 2001
23. Frewin R, Turner D, Tighe M, et al: Combination therapy with fludarabine and cyclophosphamide as salvage treatment in lymphoproliferative disorders. Br J Haematol 104:612-3, 1999
24. O'Brien SM, Kantarjian HM, Cortes J, et al: Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol 19:1414-20, 2001
25. McLaughlin P, Grillo-Lopez AJ, Link BK, et al: Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 16:2825-33, 1998
26. Manshouri T, Do KA, Wang X, et al: Circulating CD20 is detectable in the plasma of patients with chronic lymphocytic leukemia and is of prognostic significance. Blood 101:2507-13, 2003
27. Huhn D, von Schilling C, Wilhelm M, et al: Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. Blood 98:1326-31, 2001

28. Winkler U, Jensen M, Manzke O, et al: Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). Blood 94:2217-24, 1999
29. Nguyen DT, Amess JA, Doughty H, et al: IDEC-C2B8 anti-CD20 (rituximab) immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoproliferative disorders: evaluation of response on 48 patients. Eur J Haematol 62:76-82, 1999
30. O'Brien SM, Kantarjian H, Thomas DA, et al: Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 19:2165-70, 2001
31. Byrd JC, Murphy T, Howard RS, et al: Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 19:2153-64, 2001
32. Wierda W, O'Brien S, Wen S, et al: Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol 23:4070-8, 2005
33. Hallek M, Fingerle-Rowson G, Fink A-M, et al: Immunochemotherapy with Fludarabine (F), Cyclophosphamide (C), and Rituximab (R) (FCR) Versus Fludarabine and Cyclophosphamide (FC) Improves Response Rates and Progression-Free Survival (PFS) of Previously Untreated Patients (pts) with Advanced Chronic Lymphocytic Leukemia (CLL). Blood 112:325-, 2008
34. Robak T, Moiseev SI, Dmoszynska A, et al: Rituximab, Fludarabine, and Cyclophosphamide (R-FC) Prolongs Progression Free Survival in Relapsed or Refractory Chronic Lymphocytic Leukemia (CLL) Compared with FC Alone: Final Results from the International Randomized Phase III REACH Trial. Blood 112:lba-1-, 2008
35. Knauf WU, Lissichkov T, Aldaoud A, et al: Bendamustine Versus Chlorambucil in Treatment-Naive Patients with B-Cell Chronic Lymphocytic Leukemia (B-CLL): Results of an International Phase III Study, 2007, pp 2043-
36. Knauf WU, Lissitchkov T, Aldaoud A, et al: Bendamustine Versus Chlorambucil as First-Line Treatment in B Cell Chronic Lymphocytic Leukemia: An Updated Analysis from An International Phase III Study, 2008, pp 2091-
37. Gandhi V: Metabolism and mechanisms of action of bendamustine: rationales for combination therapies. Semin Oncol 29:4-11, 2002
38. Rao VA, Plunkett W: Activation of a p53-mediated apoptotic pathway in quiescent lymphocytes after the inhibition of DNA repair by fludarabine. Clin Cancer Res 9:3204-12, 2003
39. Sandoval A, Consoli U, Plunkett W: Fludarabine-mediated inhibition of nucleotide excision repair induces apoptosis in quiescent human lymphocytes. Clin Cancer Res 2:1731-41, 1996

40. Eichhorst BF, Busch R, Hopfinger G, et al: Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood 107:885-91, 2006
41. Catovsky D, Richards S, Matutes E, et al: Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet 370:230-9, 2007
42. Flinn IW, Neuberg DS, Grever MR, et al: Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol 25:793-8, 2007
43. Fischer K, Stilgenbauer S, Schweighofer CD, et al: Bendamustine in Combination with Rituximab (BR) for Patients with Relapsed Chronic Lymphocytic Leukemia (CLL): A Multicentre Phase II Trial of the German CLL Study Group (GCLLSG). Blood 112:330-, 2008
44. Gandhi V, Kemena A, Keating MJ, et al: Cellular pharmacology of fludarabine triphosphate in chronic lymphocytic leukemia cells during fludarabine therapy. Leuk Lymphoma 10:49-56, 1993