THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

Prospective Identification of Significant Prognostic Factors in Patients Treated with Fludarabine, Cyclophosphamide, and Rituximab (FCR) as Initial Therapy for Chronic Lymphocytic Leukemia.

2008-0431

Core Protocol Information

Short Title	Identifying Prognostic Factors in Frontline FCR for Patients with CLL
Study Chair:	William G. Wierda
Additional Contact:	Andrea L. Booker Sheri L. Allred Leukemia Protocol Review Group
Additional Memo Recipients:	**Recipients List** **OPR Recipients** (for OPR use only) None **Study Staff Recipients** Ana B. Ayala Dora E. Villarreal-Reyes Minerva A. Griffin Troy R. Gilchrist
Department:	Leukemia
Phone:	713-745-0428
Unit:	0428
Study Manager:	Ana B. Ayala
Full Title:	Prospective Identification of Significant Prognostic Factors in Patients Treated with Fludarabine, Cyclophosphamide, and Rituximab (FCR) as Initial Therapy for Chronic Lymphocytic Leukemia.
Public Description:	N/A
Protocol Type:	Standard Protocol
Protocol Phase:	Phase II
Version Status:	Activated -- Closed to new patient entry as of 08/05/2019
Version:	16
Document Status:	Saved as "Final"
Submitted by:	Sheri L. Allred--7/17/2019 9:56:07 AM
OPR Action:	Accepted by: Felicia Young -- 7/22/2019 4:13:36 AM

Which Committee will review this protocol?

(●) The Clinical Research Committee - (CRC)

Protocol Body

Frontline FCR Protocol (V2).doc

Prospective Identification of Significant Prognostic Factors in Patients Treated with Fludarabine, Cyclophosphamide, and Rituximab (FCR) as Initial Therapy for Chronic Lymphocytic Leukemia

Version 2

July 9, 2008

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)

Full Title: Prospective Identification of Significant Prognostic Factors in Patients Treated with Fludarabine, Cyclophosphamide, and Rituximab (FCR) as Initial Therapy for Chronic Lymphocytic Leukemia

Short Title: Identifying Prognostic Factors in Frontline FCR for Patients with CLL

1.0 OBJECTIVES

1.1 Primary Objective:

The primary objective of the study is to prospectively evaluate new and traditional prognostic factors and evaluate associations with complete remission (CR) in frontline patients treated with FCR.

1.2 Secondary Objectives:

1. To prospectively evaluate new and traditional prognostic factors and evaluate associations with overall response and time to failure in frontline patients treated with FCR.
2. To prospectively determine the minimal residual disease (MRD)-free rate in bone marrow of patients treated with frontline FCR and to evaluate prognostic factors for MRD.
3. To assess the pharmacokinetics of rituximab in patients treated with frontline FCR.

2.0 BACKGROUND

2.1 Chronic lymphocytic leukemia (CLL) is the most common leukemia in the United States and Western hemisphere. It is a disease of the aging population; the median age at diagnosis is 72 and over two-thirds of patients with CLL are over 60 years of age. Both the incidence and prevalence of this disease increase with age. The natural history for individuals with this disease is diverse. Generally, patients with early Rai stage (stage 0, low-risk) have a median expected survival of more than 10 years. Those with evidence of marrow failure manifested by anemia (stage III) or thrombocytopenia (stage IV) (Rai high-risk) have an estimated median survival of only 2 years. In patients with intermediate-risk disease (Rai stage I and II) the estimated median survival is 7 years.

2.2 There is remarkable clinical diversity in patients with CLL. Following diagnosis, some patients have smoldering, asymptomatic disease that may not progress for many years; others are diagnosed with advanced stage, or early stage disease that rapidly progresses, causing symptoms and/or bone marrow failure and require treatment. Prognostic factors for time to treatment and survival potentially include mutation status of the immunoglobulin heavy chain variable gene (IgV_H); expression of ZAP-70; expression of CD38; plasma level of β2M and soluble CD23; and presence of chromosome abnormalities such as 17p

deletion and 11q deletion by fluorescence *in situ* hybridization (FISH) analysis. Thus far, these factors have been identified in retrospective analyses of highly selected patient populations. There is limited if any prospective validation available for any of the factors. Nevertheless, factors may help delineate patients with smoldering versus progressive disease, thereby providing tools to identify patients for whom early treatment clinical trials may be indicated. They also may prove useful in directing selection of treatment agents; however, prospective clinical trials to evaluate these issues are needed.

Studies from the Cancer and Leukemia Group B (CALGB) and the French cooperative group demonstrated that immediate or early treatment did not prolong survival for patients with early-stage disease treated with chlorambucil[1,2]. However, these results were likely influenced by inclusion of patients with smoldering CLL and by inadequate treatment. To readdress this issue, a large US Intergroup trial is will be initiated comparing early versus delayed treatment with fludarabine with rituximab for high-risk patients defined as having an unmutated IgV_H gene (>98% homology to germline). Given available data for prognostic factors and status of therapies for patients with CLL, we continue to follow clinical guidelines for initiating therapy in patients with active and progressive disease. These guidelines were recently updated by the International Workshop on CLL[3] and are consistent with the 1996 National Cancer Institute-Working Group (NCI-WG) guidelines[4].

2.3 Clinical research employs a response-driven approach for clinical trial development. This approach is based on the observation that patients who achieve complete remission (CR) live longer than those who achieve partial remission (PR), or those who fail treatment. In general, clinical trials aim to increase the CR rate and demonstrate prolonged remission duration with the expectation that this will correlate with improved survival.

Response to treatment has been assessed in clinical trials by the NCI-WG criteria published 10 years ago[4]. CR requires no evidence of disease on physical examination or microscopic examination of blood (ALC <4,000/μL) and bone marrow (<30% lymphocytes, no nodules), and recovery of hemoglobin, neutrophil, and platelet counts. Recently, more sensitive tests to evaluate residual disease than microscopic exam have become available, specifically multi-color flow cytometry and allele-specific polymerase chain reaction (PCR) for the immunoglobulin heavy chain variable gene (IgV_H). In some patients achieving CR by NCI-WG criteria, one or both of these methods can demonstrate residual disease, referred to as minimal residual disease (MRD), usually in the bone marrow. Patients free of MRD following treatment have a longer remission duration and longer survival[5]. Therefore, in addition to improving CR rates, investigators are focusing on eliminating MRD. The initial treatment of patients with CLL represents the best opportunity to achieve CR.

2.4 Historically, palliative treatment consisted of an alkylating agent, most commonly chlorambucil, or alkylating agent-based combination. This treatment typically was intermittent and protracted, resulting in PRs. With alkylating agent-based therapy, the CR rate is less than 10%, the overall response (OR) rate is approximately 50-60%, and estimated median survival is 50-70 months.

2.5 Fludarabine is the most extensively studied purine analogue for the treatment of patients with CLL. Randomized, phase III frontline clinical trials have evaluated fludarabine versus single-agent chlorambucil or alkylating agent-based combinations for chemotherapy-naïve patients[6-8]. The US Intergroup trial randomized 509 patients to treatment with fludarabine, chlorambucil, or the combination[8]. The combination arm was closed early due to unacceptable toxicity. The CR rate for patients in the fludarabine arm was 20% versus 4% in the chlorambucil arm ($p<0.001$). In addition, the OR rates were 63% and 37% for the fludarabine and chlorambucil arms, respectively. The median remission duration for patients who received fludarabine was 25 months, nearly double that of patients on the chlorambucil arm. Despite a significantly higher CR rate and longer remission duration with fludarabine, overall survival (OS) was not different.

2.6 Fludarabine inhibits excision repair of DNA inter-strand cross-links induced by cyclophosphamide, thereby enhancing activity and giving a rationale for combining these agents[9]. Results of three large randomized phase III trials comparing efficacy of combined fludarabine and cyclophosphamide (FC) versus single-agent fludarabine or chlorambucil have been reported. In the German CLL Study Group CLL4 trial, previously untreated patients younger than 65 years with indications for treatment were randomized to receive 6 courses of fludarabine (F) at the standard dose versus fludarabine and cyclophosphamide (FC) at 30mg/m^2 and 250mg/m^2, respectively, daily for 3 days of each 4-week course[10]. There were 328 randomized patients evaluable for response; FC produced significantly higher CR (24%) and OR (95%) rates compared to fludarabine (7% CR; 83% OR). The median progression-free survival (PFS) for patients treated with FC was 48 versus 20 months for F ($p=0.001$). The median treatment-free survival for patients treated with FC versus F was 37 versus 25 months ($p=0.001$). The median OS was not reached in both arms. There was an increase in the incidence of myelosuppression with the FC combination; however, there was no increase in the incidence of infection. Results of the Intergroup E2997 and UK Leukemia Research Fund (LRF) CLL4 trials comparing FC to fludarabine have also been reported[11,12]. There were no age restrictions in these randomized, phase III trials; both confirmed superior efficacy for the FC combination over F or chlorambucil in chemotherapy-naïve patients with CLL. Treatment with FC resulted in higher CR and OR rates and longer remission duration. No trial has shown survival advantage for frontline treatment with FC.

2.7 Rituximab, the mAb that targets CD20, is approved by the US FDA for patients with relapsed low-grade non-Hodgkin's lymphoma[13]. Relatively low levels of CD20 are expressed on CLL cells, compared to normal B or neoplastic B cells of

other lymphomas. In addition, soluble CD20 has been demonstrated in plasma of patients with CLL; this may inhibit the capacity of rituximab to bind to CLL cells, thereby resulting in rapid clearance and negatively affecting pharmacokinetics[14]. Standard-dose rituximab (375mg/m^2 weekly for 4 weeks) has very limited activity for patients with CLL giving an overall response rate of 12% in patients with CLL/SLL in the pivotal trial with no compete responders[13,15]. Dose intense[16] and dose-dense[17] single-agent rituximab has been shown to increase efficacy.

2.8 Rituximab enhances the activity of purine analogue-based therapies and has been incorporated into chemoimmunotherapy regimens. The randomized, phase II multi-institutional CALGB 9712 trial evaluated the activity of concurrent versus sequential fludarabine and rituximab as initial treatment of patients with CLL[18]. Induction consisted of either 6 courses of standard-dose fludarabine (sequential group) or standard-dose fludarabine with rituximab (concurrent group). At the end of a 2-month observation period, responders and patients with stable disease in both groups received an additional 4 weekly doses of rituximab. This phase II trial demonstrated a significantly higher CR rate of 47% for the concurrent group versus 28% for the sequential group. The OR rate and PFS were not significantly different between the two groups. All patients in this study received rituximab; the concurrent group received 2.5 times the cumulative dose given to the sequential group. Subsequently, an analysis of all patients treated in the CALGB 9712 trial compared to an historical group of chemotherapy-naive patients treated with single-agent fludarabine in the randomized CALGB 9011 trial (no rituximab) demonstrated statistically significantly higher CR and OR rates, 2-year disease-free survival, and 2-year OS, favoring patients who received frontline fludarabine and rituximab[19].

The combination of fludarabine, cyclophosphamide, and rituximab (FCR) has been evaluated in both chemotherapy-naïve and previously treated patients with CLL[20,21]. In 224 previously untreated patients with CLL, the CR rate with FCR was 70% and the OR rate was 95%, with most patients having no detectable disease by two-color flow cytometry evaluation of the bone marrow at the end of therapy[20]. Over 40% of complete responders were free of disease in the bone marrow by PCR testing for the clonal IgV_H gene. The projected failure-free survival at 4 years was 69%. This was the highest response rate reported for any regimen in previously untreated patients with CLL. The long-term follow-up of this frontline trial was recently updated. The median remission duration for 300 patients treated with FCR was 66 months [22]. An early report of a large, phase III randomized clinical trial of FCR versus FC done in Europe indicates that the response rate, including complete response and progression-free survival are superior for FCR in previously untreated patients. The FCR regimen has become the standard frontline regimen. Clinical trials done at MDACC to improve the activity have included FCR3, consisting of FCR with 3 doses of rituximab with each course of treatment, and FCMR, consisting of FCR with mitoxantrone. Neither of these two trials showed improvement in response rate

or progression-free survival compared to the historic group of 300 patients treated with FCR.

2.9 Bone marrow is the usual site of involvement in patients with residual disease after purine analog-based therapy. Eliminating residual disease may improve remission duration and overall outcome[5,23].

A report of 91 previously treated patients with CLL who received alemtuzumab demonstrated eradication of residual disease in the blood and bone marrow in 20%[5]. Minimal residual disease was evaluated by four-color flow cytometry and those patients who were negative for residual disease in the bone marrow had longer PFS and OS compared with those who had residual disease. The OS for the 18 patients who were free of detectable residual disease was 84% at 60 months. A focus of clinical investigation continues to be the elimination of minimal residual disease with the expectation that this will correlate with improved remission duration and survival.

2.10 Treatment regimen has an impact on the significance of prognostic factors in frontline therapy in predicting response and time-to-event endpoints. Therefore, it is important to evaluate the newer prognostic factors in patients treated uniformly with a specific treatment regimen. This clinical trial will prospectively evaluate the newer prognostic factors, including IgV_H mutation status, ZAP70 expression, CD38 expression, CD49d expression, thymidine kinase, and chromosome abnormalities by FISH analysis along with traditional prognostic factors such as age, beta-2 microglobulin, Rai stage, etc, to identify which are significant, independent predictors of treatment endpoints including response (CR) to treatment and TTF. We will evaluate for MRD and determine the MRD-free rate with the FCR regimen using standardized, validated assays. We will also develop prognostic models and nomograms for these endpoints. Also, we will evaluate pharmacokinetics of rituximab in the FCR regimen in order to more rationally dose rituximab in chemoimmunotherapy regimens. Finally, we will characterize immune reconstitution following frontline FCR treatment.

3.0 BACKGROUND DRUG INFORMATION

3.1 Fludarabine phosphate (Fludara)
Fludarabine phosphate is commercially available as a standard treatment for patients with CLL. Fludarabine is a purine analogue that inhibits DNA synthesis by inhibition of DNA polymerase, ribonucleotide reductase, and DNA primase.

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 2mL of Sterile Water for Injection, USP, the solid cake 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.

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.

3.1.3 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 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.

3.1.4 Route of Administration: Intravenous.

3.2 Cyclophosphamide (Cytoxan)

Cyclophosphamide is commercially available and has activity in treating a variety of malignancies. Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is a cell cycle phase non-specific agent. Cyclophosphamide is a prodrug that must be metabolized to active metabolites in the liver.

Expected toxicities include myelosuppression. Cystitis can be caused by cyclophosphamide and can be dose limiting.

3.2.1 How Supplied: Supplied in quantities of 100 mg, 200 mg, 500 mg, 1 gm, an 2 gm for intravenous use. Maximum concentration is 20 mg/ml.:

3.2.2 Solution Preparation: This is per standard pharmacy practice.

3.2.3 Stability: Room temperature stability is 48 hrs. Refrigeration stability is 28 days.

3.2.4 Route of Administration: Intravenous

3.3 Rituximab (Rituxan®)

Rituximab is commercially available and is an approved treatment for low-grade lymphoma. Rituximab is a monoclonal antibody that binds to CD20.

3.3.1 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.

3.3.2 Storage

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

3.3.3 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.

4.0 PATIENT ELIGIBILITY

4.1 Patients will have a diagnosis of CLL, SLL, or $CD20^+$ low-grade lymphoproliferative disorder.

4.2 All patients with untreated Rai stage III-IV are eligible for this protocol. Prior single-agent rituximab treatment is permitted.

- OR -

Patients with untreated Rai stage 0-II who meet one or more criteria for active disease as defined by the International Working Group for CLL (IWCLL). Prior single-agent rituximab treatment is permitted.

4.3 Patients must have an ECOG performance status of 0-3.

4.4 Patients must have adequate renal and hepatic function (creatinine <2mg%, bilirubin <2mg%). Patients with renal or liver dysfunction due to organ infiltration by lymphocytes may be eligible after discussion with the study chairman.

4.5 Patients may not receive other concurrent chemotherapy, radiotherapy, or immunotherapy. Localized radiotherapy to an area not compromising bone marrow function does not apply.

4.6 Patients must be 16 years of age or older.

4.7 Patients must sign informed consent indicating that they are aware of the investigational nature of this study according to the policies of the UTMDACC IRB.

5.0 TREATMENT PLAN

5.1 The following are recommended prophylactic medications. For individuals who are allergic, an equivalent replacement may be identified.

Allopurinol 300 mg PO daily for the first 7 days of course 1 is recommended for tumor lysis prophylaxis.

Valacyclovir 500 mg PO daily for all 6 courses and for at least 2 months after completion of treatment is recommended for herpes virus prophylaxis.

PCP prophylaxis may be given at the discretion of the treating physician but is recommended for recent or concurrent corticosteroid use.

Growth factors (neutrophil, erythrocyte, platelet) may be used at the discretion of the treating physician.

5.2 Since transient hypotension has been reported during rituximab infusions, withholding anti-hypertensive medications the day of the rituximab infusion is recommended.

Suggested pre-medication for rituximab: two tablets [375 mg or 500 mg] of acetaminophen orally and 25 to 50 mg diphenhydramine hydrochloride orally or intravenously will be administered prior to starting each infusion of rituximab. Use of corticosteroids will be at the discretion of the treating physician with intent to minimize the infusion-related reactions. Other pre-medications may be appropriate based on the physician or patient experience.

Recommended rituximab administration is according to the standard practice regarding rate of IV infusion, escalation of infusion, and precautions. Suggested administration is as follows: the initial dose rate at the time of the first rituximab infusion should be 50 mg/hr for the first half 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 courses 2-6 will be 100 mg/hour then increased gradually **(100 mg/hour increments at 30-minute intervals)** not to exceed 600 mg/hr.

Drop in Dose Rate	Fever	or	Rigors	or	Mucosal Congestion/ Edema	or	% Systolic BP
Decrease to ½	> 38.5°C		Mild/Moderate		Mild/Moderate		>30 mm Hg

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.

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 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.

First Dose – Week 1

The first dose of rituximab will be 375 mg/m^2. All patients will receive the same first dose.

Subsequent doses will be 500 mg/m^2 for all patients combined with fludarabine and cyclophosphamide.

5.3 Suggested premedication for fludarabine and cyclophosphamide: ondansetron 8 mg IV or 10 mg PO or equivalent.

Course 1 of FC + rituximab-Week 1:

AGENT	DOSE	DAY	ROUTE	TIME
Fludarabine	25 mg/m^2/day	2-4	I.V.	5-30 minutes
Cyclophosphamide	250 mg/m^2/day	2-4	I.V.	5-30 minutes
Rituximab	375 mg/m^2/day	1	I.V.	4-6 hours

Courses 2-6 of FC + rituximab - Weeks 5, 9, 13, 17, 21:

AGENT	DOSE	DAY	ROUTE	TIME
Fludarabine	25 mg/m^2/day	1-3	I.V.	5-30 minutes
Cyclophosphamide	250 mg/m^2/day	1-3	I.V.	5-30 minutes
Rituximab	500 mg/m^2/day	1	I.V.	4-6 hours

Cyclophosphamide will be given after the dose of fludarabine. Rituximab may start two hours before fludarabine. Courses will be repeated every 4 – 6 weeks, depending on neutrophil and platelet recovery.

5.4 Dose modifications:

Dose modifications will be at the discretion of the treating physician. Modification of rituximab dose is not recommended. Dose reduction should be considered for elderly (>69 yr) patients and patients with renal impairment. Recommended dose levels for fludarabine and cyclophosphamide are as follows:

	-2	-1	0
Fludarabine	17.5 x 3	20 x 3	25 x 3
Cyclophosphamide	175 x 3	200 x 3	250 x 3

5.5 Patients will receive fludarabine phosphate 25 mg/m^2 I.V. over 5-30 minutes daily for 3 days. Cyclophosphamide 250 mg/m^2 will be given I.V. over 5-30 minutes daily for 3 days. Rituximab will be given as in section 5.0. Courses will be repeated every 4 – 6 weeks, depending on neutrophil and platelet count recovery. Response evaluation will be conducted after completion of 3 and 6 courses.

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 will come off study treatment.

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

6.0 PRETREATMENT EVALUATION

6.1 Patients will have a complete history and physical.

6.2 Laboratory studies will include CBC, platelet count, differential count, chemical survey (bilirubin, creatinine, albumin, LDH), β-2 microglobulin, bone marrow aspirate and biopsy with samples sent for morphology.

6.3 Prognostic factors will be characterized including IgV_H gene family and mutation status; leukemia cell expression of ZAP-70, CD38, and CD49d; thymidine kinase; chromosome abnormalities by FISH (13q del, +12, 11q del, and 17p del); soluble CD20, CD52, and CD23; and p53 expression. Because IgV_H and ZAP-70 are thought not to change with time, they do

not need to be repeated if done subsequent to diagnosis, before treatment. Evaluation of all other prognostic factors will be done within 3 months of enrollment on this trial. Blood or bone marrow may be used to evaluate these prognostic factors.

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, RNA and marrow plasma. Gene methylation analyses will be performed on these pre-treatment samples to correlate with treatment outcome.

6.5 Any appropriate radiological and radioisotope examinations should be performed as clinically indicated.

7.0 EVALUATION DURING STUDY

7.1 Patients will be followed with CBC, platelet count and differential every 1 – 2 weeks for the first course and q2 – 4 weeks during therapy thereafter. An SMA (bilirubin, creatinine, albumin, LDH) will be repeated every 1-3 months as clinically indicated. Physical examination every follow-up visit at MDACC.

7.2 Prior to course 4 and 2 months after course 6 (or last course for individuals stopping treatment early) a full evaluation will be performed for response assessment, including CBC, platelet and differential count, SMA (bilirubin, creatinine, albumin, LDH), and bone marrow aspiration and biopsy with samples sent for morphologic and flow cytometry analyses. Blood will be evaluated for soluble CD20 and soluble CD52. CT scans of neck, chest, abdomen, and pelvis are recommended at end of treatment response assessment. In patients achieving CR, nodular PR, or PR, blood or bone marrow will be evaluated for MRD by 4-color flow cytometry or molecular evaluation for the clonal IgV_H gene. Preferentially, this will be done on bone marrow, but may be done on blood if necessary.

7.3 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. 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.

7.3 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.

7.4 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 referring physician in a green-top (heparin) or yellow-top (ACD) tube and mailed to UTMDACC.

7.5 Optional blood (10 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 (heparin) or yellow-top (ACD) tube and mailed to UTMDACC.

7.6 When patients relapse, every attempt will be made to repeat the prognostic factor assessment at relapse: ZAP-70 expression, CD38 expression, CD49d expression, thymidine kinase, beta-2 microglobulin, and FISH.

8.0 RESPONSE CRITERIA

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 (untransfused)	>11,0 g/dl	>11.0 g/dl or >50% improvement from baseline
Lymphocytes	<4,000/μl	>50% decrease
Bone Marrow Aspirate** Biopsy	<30% lymphocytes No lymphocyte infiltrate	N/A for PR < 30% lymphocytes with residual disease on biopsy for nodular PR

* CT scan of neck, chest, abdomen, and pelvis to confirm CR is recommended.

** Evaluation for MRD will be performed either by 4-color flow cytometry or by molecular evaluation.

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 Intolerable treatment-related side effects.

9.3 Patient request.

9.4 Patients will be removed from study if they receive treatment, such as for MRD. In these cases, they will be removed from this study and their time-to-event status will remain censored. They will be followed and evaluated for survival.

10.0 STATISTICAL CONSIDERATIONS

The **primary objective** of the study is to prospectively evaluate new and traditional prognostic factors and validate associations with complete remission (CR) in frontline patients treated with FCR. CR is defined by NCI Working Group / International Working Group for CLL criteria as no evidence of disease on physical examination (no adenopathy or organomegaly) or microscopic examination of blood (ALC <4,000/μL) and bone marrow (<30% lymphocytes, no lymphoid nodules), and recovery of hemoglobin, neutrophil, and platelet counts [3,4]. Response by these criteria will be conducted after completion of 3 and 6 courses. Each planned course is 4 weeks; courses may be delayed for myelosuppression. No patient will receive more than 6 courses. Patients who demonstrate progressive disease or no response after 3 courses will come off treatment and will be counted as treatment failures.

The **secondary objectives** for the study are:

1. To prospectively evaluate new and traditional prognostic factors and validate associations with overall response and time to failure in frontline patients treated with FCR.
2. To prospectively determine the minimal residual disease (MRD)-free rate in bone marrow of patients treated with frontline FCR and to evaluate prognostic factors for MRD.
3. To assess the pharmacokinetics of rituximab in patients treated with frontline FCR.

FCR regimen is the standard frontline regimen. Historical data for 300 previously untreated patients with CLL indicate that the CR rate after 6 courses of FCR was 72%, overall response rate was 95%. Prognostic factors for CR include traditional ones such as age, beta-2 microglobulin, Rai stage, etc and the newer ones such as IgV_H mutation status, ZAP70 expression, CD38 expression, CD49d expression, thymidine kinase, and chromosome abnormalities by FISH analysis. Prognostic factors will be characterized including IgV_H gene family and mutation status; leukemia cell expression of ZAP-70, CD38, and CD49d; thymidine kinase; chromosome abnormalities by FISH (13q del, +12, 11q del, and 17p del); soluble CD20, CD52, and CD23; and p53 expression. In the current study, we will prospectively evaluate these newer prognostic factors, along with the traditional ones, in 300 patients with CLL treated frontline with FCR.

Sample size

The sample size calculation was based on fitting a multiple logistic regression model for the binary endpoint of CR with various prognostic factors as covariates. For each new prognostic factor of interest, let π be the percent prevalence in CLL patients, and P_1 and P_0 be the probability of CR in patients with and without the expression (or mutation) of the factor. We then computed the power of detecting this difference between P_0 and P_1 with a sample size of 300 patients, after adjusting for the association between this factor and other prognostic factors in the same model. The prevalence level for the factor of interest,π, was also taken into account in the computation. Table 1 below shows the power calculation results for each of the newer prognostic factors of interest, assuming a sample size of 300 and an R-Squared of 0.40 obtained from the multiple regression of the factor of interest on other prognostic factors. For example, given the these assumptions and assuming a 48% prevalence level of ZAP-70 in CLL patients, we could achieve 79% power at a two-sided 0.05 significance level to detect a change in CR rate from 0.80 for patients without ZAP-70 expression to 0.57 for patients with ZAP-70 expression. PASS 2005 was used for all sample size calculations.

Table 1. Power calculations assuming a sample size of 300 and an R-squared of 0.40 from the multiple regression of the factor of interest on other prognostic factors using PASS 2005 software.

Prognostic Factors		% in CLL patients (π)	CR within each group (%)	Power
ZAP-70 expression				0.79
	No	52	80	
	Yes	48	57	
CD38 expression				0.77
	No	80	81	
	Yes	20	59	
IgV_H				0.77
	Unmutated	66	84	
	Mutated	34	66	
17p deletion				0.89
	No	91	70	
	Yes	9	31	

No interim analysis for futility is planned since all drugs are FDA approved and the FCR combination is a standard treatment regimen for frontline patients.

Analysis Plan

Descriptive statistics, such as frequency (percentage) or median (range), will used to summarize the prognostics factors. Intent-to-treat analysis will be conducted for the primary endpoint of CR. The CR rates, along with 95% confidence intervals, will be estimated in all patients and in subgroups categorized by prognostic factors. A multiple logistic regression model will be fit to assess the predictive effect of newer (eg, ZAP-70, IgV_H, CD38 expression and 17p deletion, etc.) and traditional prognostic factors (eg, age, beta-2 microglobulin, Rai stage, etc.) on CR. Shrinkage estimates will be used in the final fitted model. Based on this final fitted model, a nomogram will be developed as a tool to predict the probability of CR for each individual patient. The nomogram provides a graphical presentation of the effect of each covariate in the fitted model and it can be used to calculate the probability of CR for an individual patient by adding the points associated with each prognostic factor. For model validation, we will assess both discrimination and calibration capabilities. Discrimination refers to the ability of the model to rank patients by their risk, such that patients with higher probability of CR should be more likely to experience CR. The discrimination will be quantified using the area under the ROC curve. We will measure the calibration of the model through visual examination of plots of predicted vs. actual probabilities. Bootstrapping will be used to generate the calibration plot and obtain more generalizable estimates of expected future performance.

Time to failure (TTF) is defined as the time from start of treatment to disease progression or death or lost of follow-up, which ever occurs first. Kaplan-Meier

plots will be used to assess the probability of failure-free survival and log-rank tests will be performed to assess the difference in TTF between subgroups of patients. A multiple Cox proportional hazards model will be fit for TTF, with newer and traditional prognostic factors as potential covariates. Shrinkage estimates will be used in the final fitted model. Similarly as in the case for CR, a nomogram for the TTF will be developed and calibrated[24]. Calculating the concordance index for censored data will assess the discrimination ability of the model. Again, in order to adjust for the bias associated with evaluating the performance of a model on the same group of patients used to build the nomogram, the assessment of calibration and discrimination was repeated for 500 bootstrapped samples.

Similar methods described above for analyzing CR will be used to assess overall response (OR) or minimal residual disease (MRD)-free rate in bone marrow. Descriptive statistics will be used to summarize pharmacokinetics of rituximab.

Missing data

Firstly, we will analyze complete data only, ie, patient with any missing covariate will not be included in the model fitting. Secondly, we will perform multiple imputations for missing data, assuming missing completely at random (MCAR). We will then assess the results from both methods and determine whether the result is severely biased when ignoring missing data.

11.0 REFERENCES

1. Shustik C, Mick R, Silver R, Sawitsky A, Rai K, Shapiro L. Treatment of early chronic lymphocytic leukemia: intermittent chlorambucil versus observation. Hematol Oncol. 1988;6:7-12.
2. Dighiero G, Maloum K, Desablens B, et al. Chlorambucil in indolent chronic lymphocytic leukemia. French Cooperative Group on Chronic Lymphocytic Leukemia. N Engl J Med. 1998;338:1506-1514.
3. 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 (IWCLL) updating the National Cancer Institute-Working Group (NCI-WG) 1996 guidelines. Blood. 2008;Epub ahead of print.
4. 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. 1996;87:4990-4997.
5. Moreton P, Kennedy B, Lucas G, et al. Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. J Clin Oncol. 2005;23:2971-2979.
6. Johnson S, Smith AG, Loffler H, et al. Multicentre prospective randomised trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for

treatment of advanced-stage chronic lymphocytic leukaemia. The French Cooperative Group on CLL. Lancet. 1996;347:1432-1438.
7. Leporrier M, Chevret S, Cazin B, et al. Randomized comparison of fludarabine, CAP, and ChOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. Blood. 2001;98:2319-2325.
8. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N Engl J Med. 2000;343:1750-1757.
9. Yamauchi T, Nowak BJ, Keating MJ, Plunkett W. DNA repair initiated in chronic lymphocytic leukemia lymphocytes by 4-hydroperoxycyclophosphamide is inhibited by fludarabine and clofarabine. Clin Cancer Res. 2001;7:3580-3589.
10. 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. 2006;107:885-891.
11. 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. 2007;25:793-798.
12. 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. 2007;370:230-239.
13. 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. 1998;16:2825-2833.
14. 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. 2003;101:2507-2513.
15. Huhn D, von Schilling C, Wilhelm M, et al. Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. Blood. 2001;98:1326-1331.
16. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol. 2001;19:2165-2170.
17. 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. 2001;19:2153-2164.
18. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). Blood. 2003;101:6-14.
19. Byrd JC, Rai K, Peterson BL, et al. Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. Blood. 2005;105:49-53.
20. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol. 2005;23:4079-4088.

21. 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. 2005;23:4070-4078.
22. Tam CS, O'Brien S, Wierda W, et al. Long term results of the fludarabine, cyclophosphamide & rituximab regimen as initial therapy of chronic lymphocytic leukemia. Blood. 2008; E-pub ahead of print.
23. Bosch F, Ferrer A, Lopez-Guillermo A, et al. Fludarabine, cyclophosphamide and mitoxantrone in the treatment of resistant or relapsed chronic lymphocytic leukaemia. Br J Haematol. 2002;119:976-984.
24. Harrell FE Jr: Regression Modeling Strategies With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York, NY, Springer Verlag, 2001