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)

John C. Byrd, Bercedis L. Peterson, Vicki A. Morrison, Kathleen Park, Robert Jacobson, Eva Hoke, James W. Vardiman, Kanti Rai, Charles A. Schiffer, and Richard A. Larson

Recent studies have suggested that rituximab has clinical activity and modulates antiapoptotic proteins associated with drug resistance in chronic lymphocytic leukemia (CLL). We performed a randomized phase 2 study to determine the efficacy, safety, and optimal administration schedule of rituximab with fludarabine in previously untreated CLL patients. Patients were randomized to receive either 6 monthly courses of fludarabine concurrently with rituximab followed 2 months later by 4 weekly doses of rituximab for consolidation therapy or sequential fludarabine alone followed 2 months later by rituximab consolidation therapy. A total of 104 patients were randomized to the concurrent (n = 51) and sequential (n = 53) regimens. During the induction portion of treatment, patients receiving the concurrent regimen experienced more grade 3 or 4 neutropenia (74% versus 41%) and grade 3 or 4 infusion-related toxicity (20% versus 0%) as compared with the sequential arm. The consolidation rituximab therapy was tolerated well in both arms. All other toxicities were similar in the 2 arms. The overall response rate with the concurrent regimen was 90% (47% complete response [CR], 43% partial response [PR]; 95% confidence interval [CI], 0.82-0.98) compared with 77% (28% CR, 49% PR; 95% CI, 0.66-0.99) with the sequential regimen. With a median follow-up time of 23 months, the median response duration and survival have not been reached for either regimen. Rituximab administered concurrently with fludarabine in previously untreated CLL patients demonstrates marked clinical efficacy and acceptable toxicity. Phase 3 studies using this combination approach for patients with CLL are warranted. (Blood. 2003;101: 6-14)

Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia occurring in the Western hemisphere. Despite the longer than 10-year life expectancy in early-stage patients, patients who progress or have more advanced stage CLL have a median survival between 18 months and 3 years.[1,2] Therapy with chlorambucil has until recently been the standard treatment for patients with symptomatic CLL. Promising results were observed when fludarabine was used for patients with prior alkylating treatment[3,4] and for those with symptomatic untreated CLL.[5] Three phase 3 studies compared fludarabine with alkylator-based therapies.[6-8] The North American intergroup study examining chlorambucil monotherapy, as compared with fludarabine, demonstrated a significantly higher overall response rate, a higher complete response (CR) rate, and a longer progression free survival (PFS) when fludarabine was used.[7] Toxicities were similar, except for more frequent myelosuppression and infections on the fludarabine arm. Similar findings were noted in 2 European studies,[6,8] demonstrating that fludarabine was superior to alkylator-based therapy (cyclophosphamide, adriamycin, and prednisone) and had an acceptable toxicity profile. The results of these studies provide justification for using fludarabine as first-line therapy in the treatment of CLL.

Despite the observed success with fludarabine, only 20% of previously untreated CLL patients attain a CR, and virtually all of these patients eventually experience a relapse. There is interest in developing new combination therapies for CLL with agents that can be added to fludarabine to increase the CR rate, following strategies that have been successful in such curable malignancies as diffuse large-cell lymphoma, acute leukemia, and testicular cancer.

From the Division of Hematology-Oncology, Department of Medicine, The Ohio State University, Columbus; Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC; Sections of Hematology-Oncology and Infectious Disease, Minneapolis Veterans Affairs Medical Center, MN; Division of Hematology-Oncology, Department of Medicine, Walter Reed Army Medical Center, Washington, DC; Division of Hematology-Oncology, Good Samaritan Hospital, West Palm Beach, FL; Department of Pathology and Department of Medicine, The University of Chicago, IL; Division of Hematology-Oncology, Long Island Jewish Medical Center, New Hyde Park, NY; Division of Hematology-Oncology, Department of Medicine, Wayne State University School of Medicine, Detroit, MI.

Submitted April 26, 2002; accepted June 12, 2002. Prepublished online as *Blood* First Edition Paper, July 5, 2002; DOI 10.1182/blood-2002-04-1258.

Supported by the National Cancer Institute, Kimmel Cancer Research Foundation, Leukemia and Lymphoma Society of America, and D. Warren Brown Foundation. Additional grant support for participating institutions is listed in Appendix 1.

A complete list of the members of the the Cancer and Leukemia Group B appears in Appendix 1.

Reprints: John C. Byrd, Division of Hematology-Oncology, Starling Loving Hall, Rm 302, The Ohio State University, Columbus, OH 43210; e-mail: byrd-3@medctr.osu.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Documented synergy between fludarabine and cyclophosphamide[9,10] has led to a recently initiated phase 3 study of cyclophosphamide and fludarabine compared with fludarabine alone by the Eastern Cooperative Oncology Group. Combinations that include agents with a different toxicity profile from fludarabine would represent a potential advantage over combination strategies with cytotoxic chemotherapy.

Rituximab is a chimeric monoclonal antibody directed against the cell-surface antigen CD20 that has single-agent activity in low-grade and diffuse large-cell non-Hodgkin lymphoma (NHL).[11-15] Since rituximab is selective for B lymphocytes, it does not cause significant cellular immune dysfunction or myelosuppression. In CLL and small lymphocytic lymphoma, the single-agent activity of rituximab with the use of standard NHL doses has been marginal.[16-20] However, recent studies in which the total dose of rituximab was escalated or given thrice weekly have demonstrated significantly more activity with this agent in patients with previously treated CLL.[21,22] On the basis of the safety and efficacy of rituximab used as a single agent and in combination with chemotherapy in NHL, the Cancer and Leukemia Group B (CALGB) conducted a clinical trial to determine the ideal schedule for enhanced safety and efficacy of rituximab when combined with fludarabine for previously untreated patients with CLL.

Patients and methods

Subjects

Patients were enrolled on this multicenter trial after approval by local institutional review boards. All patients gave written informed consent. Patients were required to have histologically and immunophenotypically documented CLL as defined by the modified National Cancer Institute (NCI) 1996 guidelines.[23] Specifically, patients were required to have a lymphocyte count greater than 5×10^9/L, at least 30% bone marrow involvement with CLL, and an immunophenotype with malignant B-cells coexpressing CD5 and CD19 with dim surface immunoglobulin. Patients with bright expression of surface immunoglobulin were excluded. All patients either had Rai stage III/IV disease or required therapy for Rai stage I/II disease as defined by the NCI 1996 guidelines. Eligible patients had received no prior therapy for CLL. Entry requirements included age older than 17 years, a CALGB performance status of 3 or less, no requirement for chronic corticosteroid administration, a negative direct antiglobulin test, and a serum creatinine level of 1.5 times the upper limit of normal or less.

Pretreatment evaluation

All patients underwent screening that included a history, a physical examination, and laboratory and x-ray studies prior to entry onto the study. These tests included a complete blood count (CBC) with differential; tests for electrolytes, blood urea nitrogen (BUN), creatinine, total protein, albumin, calcium, phosphate, lactate dehydrogenase, uric acid, total bilirubin, and hepatic transaminases alanine aminotransaminase (ALT) and aspartase serine transaminase (AST) immunoglobulins; direct antiglobulin test; chest x-ray; and bone marrow aspirate and biopsy.

Treatment plan

Allopurinol (300 mg orally) was administered to all patients for the first 14 days. Antiemetics were not specified, but could not include corticosteroids. At 30 minutes prior to all rituximab doses, acetominophen (650 mg) and diphenhydramine (50 mg intravenously) were administered. Patients were randomly assigned to 1 of the 2 treatment regimens. All treatment was administered with outpatient intent, although a small proportion of patients received the first treatment with rituximab as an inpatient owing to concerns about prolonged infusion time and toxicity.

Sequential regimen

Patients received fludarabine (25 mg/m^2) intravenously daily over 20 to 30 minutes on days 1 through 5, with the treatment repeated every 28 days for a total of 6 cycles. Patients then underwent clinical restaging (physical examination, CBC with manual differential) followed by 2 months of observation and were again restaged (physical examination, CBC with manual differential, and bone marrow aspirate and biopsy) to determine their induction-therapy response. Patients with stable disease or better, as defined by the NCI criteria,[23] were then treated with 4 weekly doses of rituximab (375 mg/m^2), with the administration procedure identical to that described in the NHL trials.[13-16] Clinical staging was repeated after rituximab therapy; patients were observed for 2 months and then completely restaged (physical examination, CBC with manual differential, and bone marrow aspirate and biopsy) to determine overall response according to the NCI 1996 criteria.[23]

Concurrent regimen

Patients received fludarabine in a dose and schedule similar to those described with the sequential regimen but with the addition of rituximab. Rituximab (375 mg/m^2) was administered on days 1 and 4 of cycle 1 of fludarabine therapy. Owing to the short half-life of rituximab in small lymphocytic lymphoma,[17] 2 doses of rituximab were administered to the first 44 patients with the first cycle to ensure adequate saturation of CD20-binding sites. A single dose of rituximab was then administered on day 1 of cycles 2, 3, 4, 5, and 6. Patients were observed for 2 months after the completion of cycle 6 and then restaged to determine their response to induction therapy. Patients with stable disease or better, as defined by the NCI criteria,[23] were treated with 4 weekly doses of rituximab (375 mg/m^2), with dose escalation identical to that described in the NHL trials. Patients were then observed for 2 months and completely restaged to determine overall response according to the NCI 1996 criteria.[23]

On the basis of the observation that stepped-up dosing improved the tolerability of rituximab,[22] the schedule of administration was modified for the last 7 patients. On day 1 of the first cycle only, the rituximab (50 mg/m^2) was administered intravenously over 4 hours without rate escalation. On day 3 of therapy, rituximab (325 mg/m^2) was administered intravenously at 50 mg/h, and then the infusion rate was escalated in 50-mg/h increments over 30 minutes to a maximum of 400 mg/h as tolerated. On day 5 and during all subsequent cycles of fludarabine, rituximab (375 mg/m^2) was administered at 100 mg/h for the first 15 minutes of the infusion, and then the rate was increased to infuse the entire dose over the next 45 minutes. Rituximab was then administered with this same 1-hour dosing on day 1 of cycles 2 through 6.

Assessment and management of toxicity

Hematologic toxicity was graded according to the modified NCI criteria for CLL,[23] while nonhematologic toxicity was graded according to the NCI Common Toxicity Criteria. Infusion toxicity was assessed according to the criteria shown in Appendix 2. Patients experiencing grade 3 or 4 neutropenia, thrombocytopenia, or anemia were observed without treatment until these hematologic parameters recovered to within 20% of the baseline value. Thereafter, they received either 75% (if they had grade 3 toxicity) or 50% (if they had grade 4 toxicity) of the original fludarabine dose for subsequent cycles. There were no dose reductions for rituximab therapy due to hematologic toxicity.

Acute infusion toxicity following rituximab administration that was reversible did not require subsequent dose reduction of this agent. At the onset of fever, chills, rigors, or other infusional reactions, patients had their infusion discontinued and received an additional dose of diphenhydramine

(50 mg) and acetaminophen (650 mg). For those with rigors, meperidine (12.5 to 25 mg) and promethazine (12.5 to 25 mg) were administered intravenously. After resolution of symptoms, the rituximab infusion was restarted at a rate of 50 mg/h and then escalated as tolerated to 200 mg/h. If significant dyspnea or wheezing (in the absence of true allergic hypersensitivity findings such as urticaria, or tongue or laryngeal edema) occurred, the infusion was discontinued immediately. In this setting, corticosteroids (100 mg hydrocortisone) and histamine-2 (H_2) blockers (cimetidine, ranitidine, or famotidine) were administered. Upon resolution of symptoms, the infusion was restarted at a lower infusion rate (25 mg/h) with close monitoring.

Patients who developed an infection were observed without further CLL treatment until the infection had resolved, but no dose reductions were implemented in the absence of grade 3 or 4 neutropenia. Patients developing autoimmune hemolytic anemia or thrombocytopenia were removed from the study and treated with alternative therapy. Nonhematologic toxicities, including nausea, vomiting, fatigue, diarrhea, and drug-related fever or chills, required no dose reductions. For other reversible nonhematologic toxicities that were grade 2 or greater and that were attributed to fludarabine, the dose was reduced by 50%. For grade 2 irreversible nonhematologic toxicities and other grade 3 or 4 nonhematologic toxicities, each case was evaluated on an individual basis to determine the appropriateness of continuing the fludarabine therapy.

Response evaluation

Patients were assessed with a detailed clinical evaluation (physical examination with lymph node, liver, and spleen measurement; and CBC with differential) at 2 months after completing induction and consolidation therapy (8 and 11 months from starting therapy). For patients attaining a clinical CR, a bone marrow biopsy and aspirate was also performed at these times. Criteria for response used the revised 1996 NCI-sponsored Working Group Guidelines.[23] As specified by these guidelines, a response had to be maintained for 2 months. Progression-free survival was defined from the time of randomization until progression, death, or last follow-up, whichever came first. Survival time was measured from the date of randomization until the time last seen alive (censored) or death (event).

Statistical analysis

Each arm of this randomized phase 2 was initially designed with a target accrual of 35 patients per arm to allow adequate power to detect an improvement in the CR rate from 20% to 40%. The protocol was later amended to allow 15 additional patients per arm so that each arm would have about 35 patients evaluable for response after the consolidation therapy.

Under the intention-to-treat principle, this report provides a summary of patient characteristics and outcomes of therapy on all enrolled patients. Response rates and their 95% confidence intervals are reported within each arm separately. Survival probabilities for response duration, PFS, and overall survival were estimated within each arm with the use of the method of Kaplan and Meier. The standard errors for these estimates were obtained by means of the variance estimate. Logistic regression within each arm was used to test the association of toxicity with pretreatment characteristics. Logistic regression was used on the combined data from both arms to test the association of CR rate with pretreatment characteristics. The data used for this analysis were locked on July 30, 2001.

As this was a randomized phase 2 trial, the study was not designed to compare the arms. Thus, it is inappropriate to compare the arms of this trial. A *P* for an arm comparison is presented for the logistic regression model, since a prestudy aim was to perform multivariable modeling of predictors of complete response, and it did not seem reasonable to test Rai stage, age, beta-2 microglobulin (B_2M), or other prognostic factors while leaving out arm of therapy. In accordance with previously published work on appropriate interpretation of randomized phase 2 trials,[24] no other comparisons between the treatment arms of this study were performed.

Results

Patient characteristics

A total of 104 patients were enrolled on this protocol between January 1998 and January 2000; 53 were randomized to the sequential regimen and 51 to the concurrent regimen. The pretreatment features of these patients are summarized in Table 1. The median age was 64 years (range, 36-86 years). All patients met the protocol criteria for having CLL.[23] According to the Rai staging criteria,[1] 61 (59%) of the patients had intermediate-risk (stage I or II) disease, and 43 (41%) of the patients had high-risk (stage III or IV) CLL. In this modified classification, intermediate-risk CLL patients have lymphadenopathy or hepatosplenomegaly without significant cytopenias, while high-risk patients have thrombocytopenia or anemia as previously defined.[1] This staging system is similar to the criteria outlined in the modified response criteria.[23] The median B_2M level was 345 n/M (4.01 mg/L). The pretreatment characteristics for patients randomized to each regimen were similar for all of the features shown in Table 1.

Toxicity

The toxicities observed during induction are summarized in Table 2 for each regimen. All patients were evaluable for toxicity. Overall, treatment was well tolerated in each arm. The 3 most frequent side effects were infusion-related toxicity, myelosuppression, and infections.

Infusion-related side effects were noted in 100% of patients receiving the concurrent regimen during induction. These most commonly consisted of fever, chills/rigors, dyspnea, and hypotension and were generally grade 1 or 2. Only 2 patients (4%) had infusion toxicity with the second administration of rituximab during induction therapy, and none of the patients experienced infusion toxicity during the consolidation therapy with rituximab. Of the first 44 patients enrolled on the concurrent regimen receiving a full dose of rituximab (375 mg/m^2) on day 1, 9 (20%) experienced grade 3 or 4 infusion-related dyspnea, hypoxemia, or

Table 1. Pretreatment characteristics of patients

Clinical feature	Sequential arm (n = 53)	Concurrent arm (n = 51)
Median age, y (range)	63 (36-79)	63 (36-86)
Stage		
No. (%) Rai intermediate risk	31 (58)	31 (61)
No. (%) Rai high risk	22 (42)	20 (39)
Females, %	19	27
CALGB performance status		
0, %	53	61
1, %	42	31
2, %	4	4
3, %	2	0
B_2M, %		
0-3 mg/L	26	37
3.1-5 mg/L	34	24
Greater than 5 mg/L	13	16
Missing	26	24
Median (range) leukocyte count, $\times 10^9$/L	80 (8.8-366)	85 (12.3-436)
Median (range) hemoglobin, g/dL	12.6 (6.3-15.2)	12.9 (6.3-16.1)
Hemoglobin, %		
At least 11 g/dL	82	82
Less than 11 g/dL	18	18
Median (range) platelets, $\times 10^{12}$/L	131 (34-306)	153 (33-316)
Splenomegaly, %	61	60
Hepatomegaly, %	16	15
Adenopathy, %	94	94

Table 2. Maximum toxicity during induction by regimen

Toxicity by treatment type	Toxicity grade, %			
	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia				
Concurrent	2	6	33	43
Sequential	21	21	11	28
Thrombocytopenia				
Concurrent	33	14	14	6
Sequential	36	13	8	2
Anemia				
Concurrent	47	18	4	0
Sequential	45	15	0	0
Infection				
Concurrent	16	27	20	0
Sequential	17	32	21	2
Nausea				
Concurrent	38	10	0	0
Sequential	35	0	2	0
Vomiting				
Concurrent	8	8	0	0
Sequential	6	0	0	0
Dyspnea				
Concurrent	6	6	10	4
Sequential	2	4	6	0
Hypotension				
Concurrent	2	8	6	0
Sequential	2	2	0	0
Fever				
Concurrent	14	18	0	0
Sequential	4	14	0	0
Chills				
Concurrent	28	8	0	0
Sequential	6	2	0	0
Myalgias				
Concurrent	22	6	0	0
Sequential	8	0	0	0
Fatigue/malaise				
Concurrent	48	14	0	0
Sequential	36	22	4	0

hypotension. In contrast, no grade 3 or 4 infusion-related dyspnea, hypoxemia, or hypotension was noted in the 7 patients receiving stepped-up dosing with a lower dose (50 mg/m^2) on day 1 of therapy. None of the pretreatment variables, including age, stage, B_2M level, or leukocyte count, predicted which patients would develop severe infusion toxicity (grade 3 or 4 as defined in Appendix 2). Indeed, the median leukocyte count for patients experiencing grade 3 or 4 infusion toxicity was 70.4×10^9/L versus 90.4×10^9/L for other patients ($P = .32$).

Infusion toxicity during consolidation was noted in 5 patients (9%) in the sequential regimen. Of these, only 1 patient (2%) had grade 3 hypotension after the first dose of rituximab. Two of these patients had elevated lymphocyte counts. None of the patients receiving the concurrent regimen had infusion-related toxicity during their re-exposure to rituximab during consolidation.

Hematologic toxicity, particularly grades 3 and 4 neutropenia, was more commonly noted with the concurrent regimen and occurred throughout the 6-month treatment period. Neutropenia was observed during the induction phase with the use of either regimen (76% when patients received rituximab and fludarabine, and 39% with the use of fludarabine alone), as well as during the consolidation phase (19% versus 8%), where rituximab administration was identical in both arms. Grade 3 or 4 neutropenia in the concurrent treatment during consolidation rituximab was noted only in patients who had had grade 3 or 4 neutropenia during induction. Similarly, 2 of the 3 patients in the sequential arm who developed grade 3 or 4 neutropenia during consolidation rituximab had previously had neutropenia during induction. Grade 3 or 4 thrombocytopenia was noted in 20% and 10% of the patients on the concurrent and sequential arms, respectively, and anemia in 4% and 0% of the patients, respectively.

Infectious toxicity occurred commonly in both regimens throughout therapy, with similar overall frequencies as shown in Tables 2 and 3. Most of these infections were mucocutaneous infections as previously reported with fludarabine, but opportunistic pathogens were also noted. With the concurrent regimen, 8 opportunistic infections were noted: 2 dermatomal varicella zoster infections, 3 localized herpes simplex infections, and 1 case each of influenza A, Echo virus, and *Pneumocystis carinii* pneumonia. With the sequential regimen, there were 14 opportunistic infections, including 2 dermatomal varicella zoster infections, 7 localized herpes simplex infections, and 1 case each of influenza A, cytomegalovirus pneumonia, and *pneumocystis carinii* pneumonia. While toxicity assessment of treatment was confined to the first 11 months of therapy, no significant opportunistic infections were noted following this period in patients observed in CR or partial response (PR) without further treatment.

Other uncommon toxicities included 3 cases of grade 3 or 4 pulmonary toxicity (isolated interstitial pneumonitis, interstitial pneumonitis with cardiomyopathy, and bronchiolitis obliterans with organizing

Table 3. Maximum toxicity during consolidation treatment by induction regimen

Toxicity by treatment type	Toxicity grade, %			
	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia				
Concurrent	13	18	11	8
Sequential	23	8	0	8
Thrombocytopenia				
Concurrent	24	8	0	0
Sequential	36	5	0	0
Anemia				
Concurrent	14	5	0	3
Sequential	18	3	0	0
Infection				
Concurrent	11	27	3	3
Sequential	5	21	3	0
Nausea				
Concurrent	6	0	0	0
Sequential	10	3	0	0
Vomiting				
Concurrent	0	0	0	0
Sequential	3	3	0	0
Dyspnea				
Concurrent	0	9	6	0
Sequential	3	3	3	3
Hypotension				
Concurrent	6	0	0	0
Sequential	5	0	3	0
Fever				
Concurrent	8	0	0	0
Sequential	5	8	0	0
Chills				
Concurrent	3	0	0	0
Sequential	13	5	0	0
Myalgias				
Concurrent	14	3	0	0
Sequential	16	3	0	0
Fatigue/malaise				
Concurrent	24	6	0	0
Sequential	19	14	0	0

pneumonia) on the concurrent arm after 2, 3, and 5 cycles of therapy, respectively. This was successfully treated in all patients by stopping fludarabine therapy and administering a short course of corticosteroid treatment. One of these patients went on to receive consolidation rituximab without any subsequent toxicity. Two cases of autoimmune hemolytic anemia were noted in the sequential regimen during fludarabine therapy, and one case each of idiopathic thrombocytopenic purpura and pure red cell aplasia were observed with concurrent fludarabine and rituximab. Three cases of neurotoxicity were noted that required cessation of fludarabine therapy (1 patient on the concurrent regimen and 2 on the sequential regimen). These consisted of transient confusion (n = 1), isolated headache (n = 1), and headache and confusion (n = 1). These toxicities were reversible with cessation of fludarabine therapy.

Response to treatment and treatment outcome

All patients enrolled on this trial were evaluable for response. Response evaluation occurred 2 months following completion of induction (fludarabine or fludarabine plus rituximab) therapy (induction response) and then again 2 months after completion of all therapy (comprehensive response). The treatment responses for each regimen at these 2 different time points are shown in Table 4. With the use of an intent-to-treat analysis for the 51 patients enrolled on the concurrent regimen, the induction CR rate was 33% (95% CI, 0.20-0.46), and the overall response rate (CR plus PR) was 90%. Of the 21 patients with a PR due to disease (8 nodular PR, 13 PR) after induction, the responses in 2 (10%) were converted to a CR after the rituximab consolidation therapy. These conversions occurred only in patients with a nodular PR. Four additional patients on the concurrent regimen had no evidence of CLL after induction therapy (including morphologically normal bone marrow biopsies), but had persistent cytopenias that prevented a CR classification following induction but that resolved during consolidation. The comprehensive response rate for the patients in the concurrent regimen included a 47% (95% CI, 0.33-0.61) CR rate and an overall response rate of 90% (95% CI, 0.82-0.98). No patients in the concurrent arm had a further reduction in their measurable lymph node disease beyond the final posttreatment response evaluation that might have resulted in a conversion to a PR or CR. One patient with minimal residual nodules in the bone marrow at 2 months after treatment had no evidence of nodular disease at 1 year after completion of therapy.

The response rate for the 53 patients enrolled on the sequential regimen included an induction CR rate of 15% (95% CI, 0.05-0.25) and an overall response rate of 77%. Of the 32 patients with a PR due to disease (12 nodular PRs, 20 PRs) after induction who received consolidation rituximab, 7 (22%) had a conversion to a CR following consolidation therapy. Six of these conversions occurred in patients with nodular PR. The comprehensive response rate for these patients included a 28% (95% CI, 0.16-0.40) CR rate and an overall response rate of 77% (95% CI, 0.66-0.89). No patients with stable disease following fludarabine therapy had a conversion to a PR or CR following receipt of rituximab therapy during the consolidation therapy. No patients had further reduction in their measurable lymph node disease beyond the 2-month posttreatment response evaluation that might have resulted in conversion to a PR or CR.

Table 4. Treatment outcome

Response evaluation	Concurrent, % (95% CI)	Sequential, % (95% CI)
Induction CR	33 (20-46)	15 (5-25)
Induction CR + PR	90 (82-98)	77 (66-89)
Consolidation PR to CR*	10 (2-18)	22 (13-36)
Overall CR	47 (33-61)	28 (16-40)
Overall PR + CR	90 (82-98)	77 (66-89)

CR indicates complete response; and PR, partial response.

*Includes patients with PR after induction except for those with residual cytopenias, who are excluded.

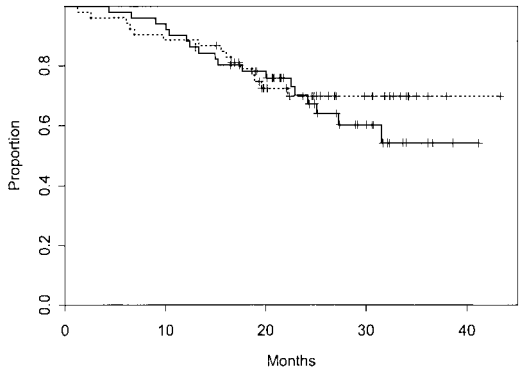

Figure 1. Progression-free survival for CLL patients receiving concurrent or sequential treatment. Progression-free survival for 51 patients with CLL treated with a concurrent fludarabine and rituximab regimen (unbroken line) and 53 similar patients treated with a sequential fludarabine and rituximab regimen (dashed line) on CALGB 9712. Eighteen patients on the concurrent arm have experienced a relapse, and 15 patients on the sequential arm have experienced a relapse.

Outcome data relative to PFS and overall survival are shown in Figures 1 and 2. After a median of 23 months of follow-up, 18 patients (35%) have experienced a relapse on the concurrent regimen and 15 (28%) on the sequential regimen. The estimated 2-year progression-free survival is 70% for each regimen. No deaths unrelated to CLL were documented during this period. Among the 104 patients enrolled on this trial, only 8 (6 on the concurrent regimen and 2 on the sequential regimen) have died. Of the 6 deaths on the concurrent regimen, 2 patients died in CR (1 of a wasting syndrome not attributed to therapy or CLL, and 1 of a pulmonary embolism). Two other patients received only 1 day of protocol therapy owing to toxicity (1 with neurotoxicity, and 1 with infusion-related toxicity) and ultimately died of progressive CLL. The final 2 patients died of progressive CLL or Richter transformation following receipt of the prescribed protocol therapy. The 2 deaths in the sequential regimen were attributed to progressive CLL.

Clinical features predicting response and toxicity to treatment

We examined whether regimen, age, sex, Rai stage (intermediate versus high), lactate dehydrogenase (LDH) level, or B_2M level

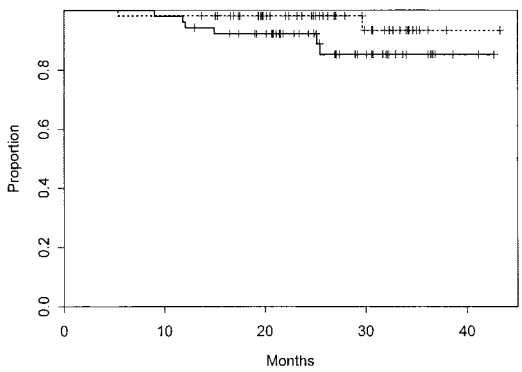

Figure 2. Overall survival for CLL patients receiving concurrent or sequential treatment. Overall survival for 51 patients with CLL treated with a concurrent fludarabine and rituximab regimen (unbroken line) and 53 similar patients treated with a sequential fludarabine and rituximab regimen (dashed line) on CALGB 9712. Six patients on the concurrent arm have died, and 2 patients on the sequential arm have died.

predicted CR during induction and/or consolidation therapy. The concurrent regimen had a significantly higher CR rate when compared with the sequential regimen ($P = .048$). A higher CR rate was not correlated with intermediate versus high Rai stages (43% versus 29%; $P = .15$) or with sex, age, LDH level, or B_2M level ($P > .40$ for all of these).

For each arm, we examined factors that predict for both hematologic and infectious toxicity by examining the variables of age, Rai stage, B_2M level, performance status, leukocyte count, and spleen involvement. With the concurrent regimen, only poor performance status (2 or 3) ($P = .02$) predicted for hematologic toxicity, and no variable predicted for infectious toxicity. With the sequential regimen, both high-risk Rai stage disease ($P = .0006$) and increased B_2M level ($P = .0003$) predicted for hematologic toxicity, and no variable predicted infectious toxicity.

Discussion

In this randomized phase 2 trial, we have demonstrated that the anti-CD20 monoclonal antibody rituximab can be safely administered concurrently with fludarabine in previously untreated CLL patients. This concurrent regimen yields an overall response rate of 90% and a CR rate of 47%. The CR rate with the concurrent regimen was higher than the 28% CR rate noted when rituximab was administered sequentially following 6 cycles of fludarabine alone. In the latter arm, we demonstrated that weekly rituximab did not covert the response of any patients with stable disease after receiving 6 cycles of fludarabine to a partial response or better. To our knowledge, the present study represents the first direct randomized comparison of concurrent versus sequential rituximab in combination with chemotherapy. Despite the increased efficacy with the concurrent regimen, only granulocytopenia was more common. Indeed, neither infections, including opportunistic pathogens, nor other toxicities, such as thrombocytopenia and anemia that are generally associated with chemotherapy-induced myelosuppression, were more common with the concurrent regimen. For CLL, this trial establishes that the concurrent administration of rituximab and fludarabine is quite effective at inducing a high incidence of CR not previously attainable and appears to be superior to both fludarabine alone and sequential fludarabine followed by rituximab. The long-term benefit of this therapy as related to PFS and overall survival is not yet known. To answer this question, CALGB is planning to test this regimen against monotherapy with fludarabine in a phase 3 clinical trial. On the basis of the previous CALGB intergroup trial[7] and 2 European trials,[6,8] fludarabine is the appropriate standard therapy to compare against new regimens, such as the concurrent fludarabine and rituximab regimen described here.

Rituximab causes profound and prolonged depletion of B lymphocytes. Similarly, fludarabine treatment in CLL causes profound and prolonged depletion of T-cells. Several prior studies have identified[25-27] the increased risk of opportunistic infections observed with fludarabine-based therapies. Combining fludarabine and rituximab as administered in either the concurrent or sequential regimen of this study was therefore performed with great attention to infectious morbidity. The frequency of infections was similar in the 2 treatments, with 8 opportunistic infection noted in the concurrent regimen and 14 in the sequential treatment. The majority of these opportunistic infections were viral in origin and often localized. *Pneumocystis carinii* pneumonia, another opportunistic pathogen associated with purine nucleoside analog combination therapies, was noted in only 2 patients. On the basis of the infectious data derived from this trial, preventive strategies (prophylaxis) against herpes virus infections would appear warranted. In contrast, the low frequency of pneumocystis carinii pneumonia in this patient population does not justify empiric antimicrobial therapy targeting this organism.

One concern about the use of rituximab in CLL is infusion-related toxicity. This concern arises from several studies demonstrating that rituximab can cause severe infusion-related toxicity in a minority of patients and that a high number of circulating tumor cells might predispose patients to this.[18,22,28-32] Subsequent studies demonstrated that rituximab-infusion toxicity does not correlate with blood tumor cell count but is directly mediated by cytokines such as tumor necrosis factor–alpha (TNF-α), interferon-γ (IFN-γ), interleukin 6 (IL-6), and IL-8.[18,22] As these cytokines are likely to be more elevated in patients who have not received prior chemotherapy, we required close observation during the first rituximab treatment. Following the first and second treatments for each patient with the concurrent regimen, information was obtained about any reactions with the treatment. Infusion toxicity was assessed by means of the criteria outlined in Appendix 2, to provide useful information to practicing oncologists about toxicities that would be likely to extend office observation time or lead to hospitalization. Infusion toxicity was manageable in almost all cases, with or without stepped-up dosing. However, stepped-up dosing may have diminished early-infusion toxicity of rituximab-based therapy in CLL, as previously reported,[22] although larger studies will be required to confirm these findings. Furthermore, prior treatment with fludarabine greatly diminished the infusion toxicity observed with subsequent rituximab treatment. Only 9% of patients on the sequential regimen had any toxicity, and only 2% of patients experienced grade 3 or 4 toxicity.

Previous phase 2 single-agent studies and combination-treatment studies of rituximab with alkylator-based chemotherapy in lymphoma have not demonstrated myelosuppression as a defined toxicity of rituximab.[11-21,33-35] It is notable that in this study 74% of the patients receiving the concurrent combination of rituximab and fludarabine had grade 3 or 4 neutropenia. Possible explanations for this include the compromised marrow reserve present in most CLL patients when therapy is required or the excess plasma TNF-α levels noted in this disease,[36,37] both factors known to predispose to chemotherapy-induced cytopenias.[38] However, a preliminary report on low-grade NHL noted similar enhanced myelosuppression when these 2 agents were combined, suggesting a more complicated mechanism may exist that is currently not well understood.[39] It is notable, nonetheless, that this excess neutropenia during induction with the combination of rituximab and fludarabine did not predispose to an excess number of neutropenic fever episodes or life-threatening infections when compared either with the sequential arm in this trial or with the previous intergroup trial, which administered fludarabine alone.

The low CR rate achieved with previous therapies used for CLL probably relates to several different mechanisms of disrupted apoptosis.[40,41] Overexpression of several antiapoptotic proteins (mcl-1, bag-1) and the presence of specific genetic aberrations [p53 mutations, ataxia telangiectasia gene mutations, and interphase cytogenetic abnormalities including del(17p13)] have been associated with poor response to fludarabine-based therapy.[42-50] One of the attractive features of immunotherapy with monoclonal antibodies, such as rituximab, is that the proposed mechanism of cell clearance is different from that of cytotoxic chemotherapy, involving both complement-mediated cell lysis and antibody-dependent cellular cytotoxicity.[51-53] However, recent evidence shows that a portion of CLL patients receiving rituximab treatment have

in vivo activation of caspase-9, caspase-3, and poly-adenosine-5′-diphosphate-ribose polymerase (PARP) cleavage in blood leukemia cells immediately following treatment.[54] Activation of caspase-9 and caspase-3 occurs with a variety of chemotherapy agents in CLL, including fludarabine, so enhanced response would not necessarily be expected. However, it was noted that significant down-modulation of the antiapoptotic proteins XIAP and Mcl-1 occurred in the majority of patients receiving rituximab irrespective of response.[54] Favorable modulation of these antiapoptotic proteins by rituximab may explain the enhanced response observed with the concurrent regimen, while less benefit was observed with the sequential regimen.

Risk stratification of treatment based upon pretreatment biologic factors has become standard for patients with acute myeloid leukemia[55] and acute lymphoblastic leukemia.[56] Various clinical features, including age, Rai stage (which includes anemia and thrombocytopenia), and serum B_2M levels, have been associated with inferior response and poor long-term treatment outcome after alkylator- and purine analog–based therapy for CLL.[57,58] Recent studies examining molecular aberrations, including p53 mutations, unfavorable cytogenetics, CD38 expression, and somatic variable-heavy (VH) gene mutational status, have demonstrated that these are also important determinants for treatment outcome in CLL.[42-50,59-66] It is of interest that several standard prognostic factors, including age, Rai stage, and B_2M level, were not shown to be important in predicting treatment outcome for the group of patients treated in this trial. Similar results with respect to the lack of importance of age to treatment outcome have been observed in the 2 trials examining altered dosing of rituximab therapy in previously treated patients with CLL.[22,22] From clinical features alone, we cannot prospectively identify subsets of patients who have a low likelihood of responding to combination therapy with fludarabine and rituximab. Examination of additional prognostic factors is warranted. It is hoped that the results of these studies will profile those patients who have the greatest chance of gaining benefit from fludarabine and rituximab–based combination therapy.

The addition of rituximab to fludarabine in this trial builds upon the single-agent results of the intergroup CALGB trial that were recently reported.[7] Several pilot studies have demonstrated that the addition of cyclophosphamide to fludarabine enhances the CR rate or the rate of negative results by flow cytometry in previously untreated CLL.[9,10] A phase 3 intergroup study is now ongoing to determine if fludarabine and cyclophosphamide administered together are more efficacious than fludarabine monotherapy. Investigators at the MD Anderson Cancer Center (Houston, TX) have combined rituximab with fludarabine and cyclophosphamide and have reported preliminary results showing a CR rate of 66% in previously untreated CLL.[67] While the CR rate in this single-institution trial is higher than we observed with fludarabine and rituximab, the 95% confidence intervals around these values overlap. Because both the concurrent rituximab regimen of the CALGB study described here and studies previously reported by others using fludarabine and cyclophosphamide have shown more granulocytopenia than with fludarabine alone, it will be important to carefully define the importance of cyclophosphamide to improving overall response and remission duration prior to proceeding with randomized studies of all 3 agents. The ultimate goal in treating CLL should be the achievement of a high CR rate that translates into prolonged remissions and possibly cure. Addition of less effective components to up-front treatment regimens not only has the potential to increase toxicity, but may also diminish the ability to add other active agents to the regimen.

In conclusion, we have demonstrated that fludarabine and rituximab administered concurrently are active in CLL as measured by CR rate. Treatment with this regimen was associated with a higher incidence of initial infusion reactions and granulocytopenia than fludarabine alone, but there was no increase in infections. The long-term benefit of this therapy will require continued follow-up examining both PFS and overall survival. However, these data clearly support future phase 3 studies of rituximab combined with fludarabine compared with other fludarabine-based combinations in CLL.

Appendix 1

Grant support, participating institutions, principal investigators, and views expressed in the study.

Institution	Location	Principal investigator	Institutional NCI grant support
CALGB statistical office	Durham, NC	Stephen George, PhD	CA33601
Dana Farber Cancer Institute	Boston, MA	George P. Canellos, MD	CA32291
Duke University Medical Center	Durham, NC	Jeffrey Crawford, MD	CA47577
Georgetown University Medical Center	Washington, DC	Edward Gelmann, MD	CA77597
Illinois Oncology Research Association	Peoria, IL	John W. Kugler, MD	CA35113
Medical University of South Carolina	Charleston, SC	Mark Green, MD	CA03927
Memorial Sloan-Kettering Cancer Center	New York, NY	George Bosl, MD	CA77651
Mount Sinai School of Medicine	New York, NY	Lewis Silverman, MD	CA04457
North Shore–Long Island Jewish Medical Center	Manhasset, NY	Daniel R. Budman, MD	CA35279
Roswell Park Cancer Institute	Buffalo, NY	Ellis Levine, MD	CA02599
State University of New York Upstate Medical University	Syracuse, NY	Stephen L. Graziano, MD	CA21060
The Ohio State University Medical Center	Columbus, OH	Clara D. Bloomfield, MD	CA77658
University of Chicago Medical Center	Chicago, IL	Gini Fleming, MD	CA41287
University of Illinois at Chicago	Chicago, IL	David Gustin, MD	CA74811
University of Iowa	Iowa City, IA	Gerald Clamon, MD	CA47642
University of Maryland Cancer Center	Baltimore, MD	David Van Echo, MD	CA31983
University of Minnesota	Minneapolis, MN	Bruce A. Peterson, MD	CA16450
University of Missouri/Ellis Fischel Cancer Ctr	Columbia, MO	Michael C. Perry, MD	CA12046
University of Nebraska Medical Center	Omaha, NE	Anne Kessinger, MD	CA77298
University of North Carolina at Chapel Hill	Chapel Hill, NC	Thomas C. Shea, MD	CA47559
University of Tennessee Memphis	Memphis, TN	Harvey B. Niell, MD	CA47555

Appendix 1 (continued)

Institution	Location	Principal investigator	Institutional NCI grant support
Vermont Cancer Center	Burlington, VT	Hyman B. Muss, M.D	CA77406
Wake Forest University School of Medicine	Winston-Salem, NC	David D. Hurd, MD	CA03927
Walter Reed Army Medical Center	Washington, DC	Joseph J. Drabeck, MD	CA26806
Washington University	St Louis, MO	Nancy Bartlett, MD	CA77440
Weil Medical College of Cornell University	New York, NY	Michael Schuster, MD	CA07968

The research for CALGB 9712 was supported, in part, by grants from the National Cancer Institute (CA31946) to the Cancer and Leukemia Group B (Richard L. Schilsky, MD, chairman), listed in tabular form above. The contents of this study are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

Appendix 2

Infusion-toxicity grading system

	Toxicity grade				
Infusion-related events	0	1	2	3	4
Fever	None	T < 38°C, 100.4°F	T ≥ 38°C, 100.4°F	NA	NA
Chills	None	Mild, requires symptomatic treatment (eg, blanket) or nonnarcotics	Prolonged or pronounced, requires interruption of infusion and narcotics	Prolonged or pronounced, not responsive to narcotics	NA
Hypotension/hypertension	None or no change	Asymptomatic, transient, requires no intervention	Requires cessation/interruption of infusion	Requires therapy (ie, fluid resuscitation, medication)	Requires step-up in care (ie, hospitalization or transfer to ICU)
Dyspnea, drop in pO_2 wheezing, bronchospasm	None	Asymptomatic, transient drop in pO_2, requires no intervention	Requires cessation/interruption of infusion and O_2 therapy	Requires nebulizer, parenteral medications, or corticosteroids	Requires step-up in care (ie, hospitalization or transfer to ICU)
Rash, pruritus, hives, angioedema	None	Transient, requires no therapy	Requires cessation/ interruption of infusion and medication	Persists beyond 24 hours despite therapy	NA
Vomiting	None	1 episode/24 h over pre-tx	2–5 episodes/24 h over pre-tx	≥ 6 episodes/24 h over pre-tx; or needs intravenous fluids	Requiring TPN or step-up in care
Nausea	None	Present, but no impairment in appetite	Decreased appetite	Requires intravenous supplementation	NA

pO_2 indicates O_2 pressure; T, temperature; NA, not applicable; ICU, intensive care unit; tx, treatment; and TPN, total parenteral nutrition.

References

1. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood. 1975;46: 219-234.
2. Binet JL, Auguier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981;48:198-216.
3. Grever MR, Kopecky KJ, Coltman CA, et al. Fludarabine monophosphate: a potentially useful agent in chronic lymphocytic leukemia. Nouv Rev Fr Hematol. 1988;30:457-459.
4. Keating MJ, Kantarjian H, Talpaz M, et al. Fludarabine: a new agent with major activity against chronic lymphocytic leukemia. Blood. 1989;74: 19-25.
5. 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. 1991;9:44-49.
6. Johnson S, Smith AG, Loffler H, et al. Multicentre prospective randomized trial of fludarabine versus cyclophosphamide, doxorubicin, and prednisone (CAP) for treatment of advanced-stage chronic lymphocytic leukemia. Lancet. 1996;347: 1432-1438.
7. 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.
8. 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.
9. O'Brien SM, Kantarjian HM, Cortes J, et al. Results of the fludarabine and cyclophosphamide combination regimen in chronic lymphocytic leukemia. J Clin Oncol. 2001;19:1414-1420.
10. Flinn IW, Byrd JC, Morrison C, et al. Fludarabine and cyclophosphamide with filgrastim support in patients with previously untreated indolent lymphoid malignancies. Blood. 2000;96:71-75.
11. Maloney DG, Liles TM, Czerwinski DK, et al. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood. 1994;84:2457-2466.
12. Maloney DG, Grillo-Lopez AJ, Bodkin DJ, et al. Results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J Clin Oncol. 1997;15:3266-3274.
13. Maloney DG, Grillo-Lopez AJ, White CA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood. 1997;90: 2188-2195.
14. Coiffier B, Haioun C, Ketterer N, et al. Rituximab for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood. 1998;92:1927-1932.
15. Davis TA, White CA, Grillo-Lopez AJ, et al. Single agent monoclonal antibody efficacy in bulky non-Hodgkin's lymphoma: results of a phase II trial of rituximab. J Clin Oncol. 1999;17:1851-1857.
16. 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.
17. Berinstein NL, Grillo-Lopez AJ, White CA, et al. Association of serum rituximab concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol. 1998;9:1-7.
18. Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A. Cytokine release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with the anti-CD20 monoclonal antibody. Blood. 1999; 94:2217-2224.
19. Nguyen DT, Amess JA, Doughty H, Hendry L, Diamond LW. IDEC-C2B8 anti-CD20 immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoproliferative disorders: evaluation of response in 48 patients. Eur J Haematol. 1999;62:76-82.
20. Foran JM, Rohatiner AZ, Cunningham D, et al. European phase II study of rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly diagnosed mantle-cell lymphoma and previously treated mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma. J Clin Oncol. 2000;18:317-324.

21. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol. 2001;19:2165-2170.
22. 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.
23. 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.
24. Liu PY, LeBlanc M, Desai M. False positive rates of randomized phase II designs. Controlled Clin Trials. 1999;20:343-352.
25. Byrd JC, Hargis JB, Kester KE, Hospenthal DR, Knutson SW, Diehl LF. Opportunistic pulmonary infections with fludarabine in previously treated patients with low-grade lymphoid malignancies: a role for Pneumocystis carinii pneumonia prophylaxis. Am J Hematol. 1995;49:135-142.
26. Morrison VA, Rai KR, Peterson BL, et al. Impact of therapy with chlorambucil, fludarabine, or fludarabine plus chlorambucil on infections in patients with chronic lymphocytic leukemia: Intergroup Study Cancer and Leukemia Group B 9011. J Clin Oncol. 2001;19:3611-3621.
27. Byrd JC, Houde-McGrail LL, Hospenthal DR, Howard RS, Dow N, Diehl LF. Herpes virus infections occur frequently following treatment with fludarabine: results of a prospective natural history study. Br J Haematol. 1999;105:445-447.
28. Jensen M, Winkler U, Manzke O, Diehl V, Engert A. Rapid tumor lysis in a patient with B-cell chronic lymphocytic leukemia and lymphocytosis treated with an anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab). Ann Hematol. 1998;77: 89-91.
29. Yang H, Rosove MH, Figlin RA. Tumor lysis syndrome occurring after the administration of rituximab in lymphoproliferative disorders: high-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Am J Hematol. 1999;62:247-250.
30. Dilman RO. Infusion reactions associated with the therapeutic use of monoclonal antibodies in the treatment of malignancy. Cancer Metastasis Rev. 1999;18:465-471.
31. Kunzmann V, Ruediger T, Hallek M, Mueller-Hermelink HK, Wilhelm M. Tumor cell agglutination and not solely cytokine release as mechanism of adverse reactions during anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) treatment. Blood. 2001;98:1991-1992.
32. Byrd JC, Waselenko JK, Maneatis TJ, et al. Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. J Clin Oncol. 1999;27: 791-795.
33. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346:235-242.
34. Czuczman MS, Grillo-Lopez AJ, White CA, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol. 1999;17:268-276.
35. Vose JM, Link BK, Grossbard ML, et al. Phase II study of rituximab in combination with chop chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2001;19:389-397.
36. Adami F, Guarini A, Pini M, et al. Serum levels of tumour necrosis factor-alpha in patients with B-cell chronic lymphocytic leukaemia. Eur J Cancer. 1994;30:1259-1263.
37. Waage A, Liabakk N, Lien E, Lamvik J, Espevik T. p55 and p75 tumor necrosis factor receptors in patients with chronic lymphocytic leukemia. Blood. 1992;80:2577-2583.
38. Voog E, Bienvenu J, Warzocha K, et al. Factors that predict chemotherapy-induced myelosuppression in lymphoma patients: role of the tumor necrosis factor ligand-receptor system. J Clin Oncol. 2000;18:325-331.
39. Czuczman MS, Fallon A, Mohr A, et al. Rituximab in combination with CHOP or fludarabine in low-grade lymphoma. Semin Oncol. 2002;29:36-40.
40. Reed JC. Molecular biology of chronic lymphocytic leukemia: implications for therapy. Semin Hematol. 1998;35:3-13.
41. Bannerji R, Byrd JC. Update on the biology of chronic lymphocytic leukemia. Curr Opin Oncol. 2000;12:22-29.
42. Kitada S, Andersen J, Akar S, et al. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with in vitro and in vivo chemoresponses. Blood. 1998;91:3379-3389.
43. Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. N Engl J Med. 1990;323:720-724.
44. Callet-Bauchu E, Salles G, Gazzo S, et al. Translocations involving the short arm of chromosome 17 in chronic B-lymphoid disorders: frequent occurrence of dicentric rearrangements and possible association with adverse outcome. Leukemia. 1999;13:460-468.
45. Matutes E, Oscier D, Garcia-Marco J, et al. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical, and laboratory features in 544 patients. Br J Haematol. 1996;92:382-388.
46. Bigoni R, Cunio A, Roberti MG, et al. Chromosome aberrations in atypical chronic lymphocytic leukemia: a cytogenetics and interphase cytogenetic study. Leukemia. 1997;11:1933-1940.
47. Dohner H, Stilgenbauer S, James MR, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood. 1997;89:2516-2522.
48. Dohner H, Stilgenbauer S, Dohner K, Bentz M, Lichter P. Chromosomal aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogenetic analysis. J Mol Med. 1999;77:266-281.
49. Dohner H, Fischer K, Bentz M, et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. Blood. 1995;85:1580-1589.
50. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343: 1910-1916.
51. Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. 1994;83: 435-445.
52. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med. 2000;6:443-448.
53. Bellosillo B, Villamor N, Lopez-Guillermo A, et al. Complement-mediated cell death induced by rituximab in B-cell lymphoproliferative disorders is mediated in vitro by a caspase-independent mechanism involving the generation of reactive oxygen species. Blood. 2001;98:2771-2777.
54. Byrd JC, Kitada S, Flinn IW, Pearson M, Shinn C, Reed J. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood. 2002;99: 1038-1043.
55. Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 1998;58:4173-4179.
56. Ma SK, Wan TS, Chan LC. Cytogenetics and molecular genetics of childhood leukemia. Hematol Oncol. 1999;17:91-105.
57. Cheson BD, Zwiebel J. Chronic lymphocytic leukemia: staging and prognostic factors. Semin Oncol. 1998;25:42-59.
58. Molica S, Levato D, Cascavilla N, Levato L, Musto P. Clinico-prognostic implications of simultaneous increased serum levels of soluble CD23 and beta2-microglobulin in B-cell chronic lymphocytic leukemia. Eur J Haematol. 1999, 62:117-122.
59. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig VH genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood. 1999;94:1848-1854.
60. Damie RN, Wassil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood. 1999;94:1840-1847.
61. Maloum K, Davi F, Merle-Beral H, et al. Expression of unmutated VH genes is a detrimental prognostic factor in chronic lymphocytic leukemia. 2000;96:377-380.
62. Thunberg U, Johnson A, Roos G, et al. CD38 expression is a poor predictor for VH gene mutational status and prognosis in chronic lymphocytic leukemia. Blood. 2001;97:1892-1894.
63. Ibrahim S, Keating M, Do KA, et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. Blood. 2001;98: 181-186.
64. Wattel E, Preudhomme C, Hecquet B, et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood. 1994;84:3148-3157.
65. Fenaux P, Preudhomme C, Lai JL, et al. Mutations of the p53 gene in B-cell chronic lymphocytic leukemia: a report on 39 cases with cytogenetic analysis. Leukemia. 1992;6:246-250.
66. Cordone I, Masi S, Mauro FR, et al. p53 expression in B-cell chronic lymphocytic leukemia: a marker of disease progression and poor prognosis. Blood. 1998;91:4342-4349.
67. Wierda W, O'Brien S, Albitar M, et al. Combined fludarabine, cyclophosphamide, and rituximab achieves a high complete remission rate as initial treatment for chronic lymphocytic leukemia [abstract]. Blood. 2001;98:771a.