Consolidation and Maintenance Immunotherapy With Rituximab Improve Clinical Outcome in Patients With B-cell Chronic Lymphocytic Leukemia

Giovanni Del Poeta, MD[1]
Maria Ilaria Del Principe, MD[1]
Francesco Buccisano, MD[1]
Luca Maurillo, MD[1]
Giovanni Capelli, MD[2]
Fabrizio Luciano, PhD[1]
Alessio Pio Perrotti, MD[1]
Massimo Degan, PhD[3]
Adriano Venditti, MD[1]
Paolo de Fabritiis, MD[1]
Valter Gattei, MD[3]
Sergio Amadori, MD[1]

[1] Department of Hematology, University "Tor Vergata," S. Eugenio Hospital, Rome, Italy.

[2] Department of Motor Science and Health, University of Cassino, Cassino, Italy.

[3] Clinical and Experimental Hematology Research Unit, Oncology Referral Center, Aviano, Italy.

Supported in part by the Italian Ministry of University, Research, Science, and Technology Program, 2005.

Presented in part at the 48th Annual Meeting of the American Society of Hematology, Orlando, Florida, December 9–12, 2006.

Address for reprints: Giovanni Del Poeta, MD, Cattedra di Ematologia, Università "Tor Vergata," Ospedale S. Eugenio, Via Fiume Giallo, 430 MA, 00144 Rome, Italy; Fax: (011) 39 06 5914745; E-mail: g.delpoeta@tin.it

Received May 16, 2007; revision received August 2, 2007; accepted August 9, 2007.

BACKGROUND. Rituximab in sequential combination with fludarabine (Flu) allowed patients with B-cell chronic lymphocytic leukemia (B-CLL) to achieve higher remission rates and longer response duration. Based on their recent experience in indolent non-Hodgkin lymphomas, in this study, the authors attempted to demonstrate whether consolidation/maintenance therapy with rituximab could prolong the response duration in this patient population.

METHODS. This Phase II study was based on a consolidation/maintenance therapy with rituximab for patients in complete remission (CR) or partial remission (PR) who were positive for minimal residual disease (MRD), as determined by flow cytometry. Seventy-five symptomatic, untreated patients with B-CLL received 6 monthly cycles of Flu (25 mg/m^2 for 5 days) followed by 4 weekly doses of rituximab (375 mg/m^2). Then, 28 patients who were positive for MRD were consolidated with 4 monthly cycles of rituximab (375 mg/m^2) followed by 12 monthly low doses of rituximab (150 mg/m^2).

RESULTS. Based on National Cancer Institute criteria, 61 of 75 patients (81%) achieved a CR, 10 of 75 patients (13%) had a PR, and 4 of 75 patients (5%) had either no response or disease progression. MRD-positive patients in CR or PR who received consolidation therapy (n = 28 patients) had a significantly longer response duration (87% vs 32% at 5 years; $P = .001$) compared with a subset of patients who did not receive consolidation therapy (n = 18 patients). All patients experienced a long progression-free survival from the end of induction treatment (73% at 5 years). It was noteworthy that, within the subset of ZAP-70-positive patients, MRD-positive, consolidated patients (n = 12 patients) had a significantly longer response duration (69% vs 0% at 2.6 years; $P = .007$) compared with MRD-positive, unconsolidated patients (n = 11 patients).

CONCLUSIONS. The addition of a consolidation and maintenance therapy with rituximab prolonged response duration significantly in patients with MRD-positive B-CLL. ***Cancer* 2008;112:119–28.** © *2007 American Cancer Society.*

KEYWORDS: B-cell chronic lymphocytic leukemia, minimal residual disease, consolidation and maintenance therapy with rituximab, ZAP-70, CD38, immunoglobulin heavy-chain variable-region status, cytogenetics, response duration.

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the Western world, and it affects mainly elderly individuals. Patients with CLL have variable outcomes, and survival ranges from months to decades.[1,2]

Promising results were observed when fludarabine was introduced for patients who were treated with alkylator agents and for those with symptomatic, untreated CLL.[3,4] However, despite response rates as high as 70%, complete remissions (CR) were

DOI 10.1002/cncr.23144
Published online 12 November 2007 in Wiley InterScience (www.interscience.wiley.com).

infrequent, and the longer progression-free survival (PFS) in the fludarabine arm from 3 randomized studies[5–7] did not produce significantly improved overall survival (OS).

Rituximab is a chimeric monoclonal antibody directed against the cell-surface antigen CD20, which is active in low-grade and diffuse large-cell non-Hodgkin lymphoma (NHL).[8,9] Moreover, experimental data indicate a potential additive effect of combining rituximab with fludarabine because of enhanced sensitivity of tumor cells to chemotherapy-induced apoptosis.[10] Three Phase II studies combining rituximab with fludarabine have achieved a much higher CR rate than the rate reported previously with any other therapeutic approach in CLL.[11–13] Although to our knowledge there is no proof to date of an improvement in OS, disease-free progression has been prolonged. Significantly, not only has the CR rate increased dramatically, but it also has proved possible to eradicate minimal residual disease (MRD) in an increasing proportion of patients. In 224 patients with untreated CLL who received combined fludarabine, cyclophosphamide, and rituximab, Keating et al.[14] demonstrated that flow cytometry-negative or minimally positive bone marrow (BM) at the completion of therapy was correlated with improved OS. Therefore, studies using MRD assays by 4-color flow cytometry or molecular methods may be better for confirming a true CR, which should be the major therapeutic objective to improve PFS and OS in patients with CLL. Recently, the eradication of detectable MRD by 4-color flow cytometry with the combination of alemtuzumab and fludarabine was associated with improved OS and treatment-free survival.[15]

In addition, it has been demonstrated that rituximab maintenance therapy provides a significant PFS benefit in patients with indolent B-cell NHL.[16,17] Therefore, the drug potentially may be useful as maintenance therapy in patients with indolent NHL or CLL.[18–21]

Recent literature data indicate that unmutated V_H genes, CD38 or ZAP-70 protein tyrosine kinase overexpression, and positive MRD status may predict either a lower response, a shorter PFS, or a shorter OS.[22–27] In the current study, our objective was to demonstrate that a consolidation and maintenance therapy with rituximab administered to symptomatic, untreated patients in clinical CR or partial remission (PR) but with detectable MRD, as determined by flow cytometry, may prolong PFS. Finally, we evaluated the different clinical impact of relevant biologic features, such as ZAP-70, CD38, and immunoglobulin heavy-chain variable-region (*IgVH*) status and cytogenetics, on response duration.

MATERIALS AND METHODS

From March 2000 to February 2005, 46 patients were registered in this prospective Phase II study on consolidation/maintenance with rituximab. The study was conducted in accordance to the Declaration of Helsinki. The current analysis was performed in July 2006. All eligible patients provided written informed consent and had received prior induction therapy for CLL based on sequential combination of fludarabine with rituximab, as described previously.[13] Patients were required to have histologically and immunophenotypically documented CLL, as defined by the modified National Cancer Institute (NCI) 1996 guidelines.[28] An Eastern Cooperative Oncology Group (ECOG) performance status from 0 to 2 was required. Patients were excluded if they had active opportunistic infections, any other severe infection that was not controlled by medical therapy, or major organ dysfunction.

Cellular Immunophenotypic Analysis

Peripheral blood mononuclear cells were analyzed for surface expression of CD19/CD5/CD38 and CD19/CD5/CD23 by triple-color immunofluorescence, as described previously.[24] ZAP-70 protein determination was performed by flow cytometry, as described elsewhere.[24,25] Flow cytometric analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif), and CellQuest software was used to acquire and analyze data. The threshold of positivity both for CD38 and for ZAP-70 was set at >20% CD19-positive (CD19+)/CD5+ B cells.

MRD Flow Cytometric Study

Between 50,000 and 300,000 total cells were analyzed in each test performed in BM approximately 1 to 2 months after the completion of induction therapy. The antibodies used were anti-CD5-allophycocyanin, anti-CD20-phycoerythrin (PE), anti-CD19-peridin-chlorophyll protein, anti-CD38-PE, anti-CD20-fluorescein isothiocyanate (FITC) (Becton Dickinson), and anti-CD79b-FITC (Serotec, Oxford, United Kingdom). The 2 combinations CD19/CD5/CD20/CD79b and CD19/CD5/CD38/CD20 allowed us to discriminate B-CLL cells from normal B cells in all analyses, as previously described.[27] The threshold of positivity was set at >1% CD19+/CD5+/CD79b-negative (CD79b−) CLL cells.

Interphase Fluorescence In Situ Hybridization

Separate hybridizations were performed for loci on chromosomes 11, 12, 13, and 17. For chromosomes

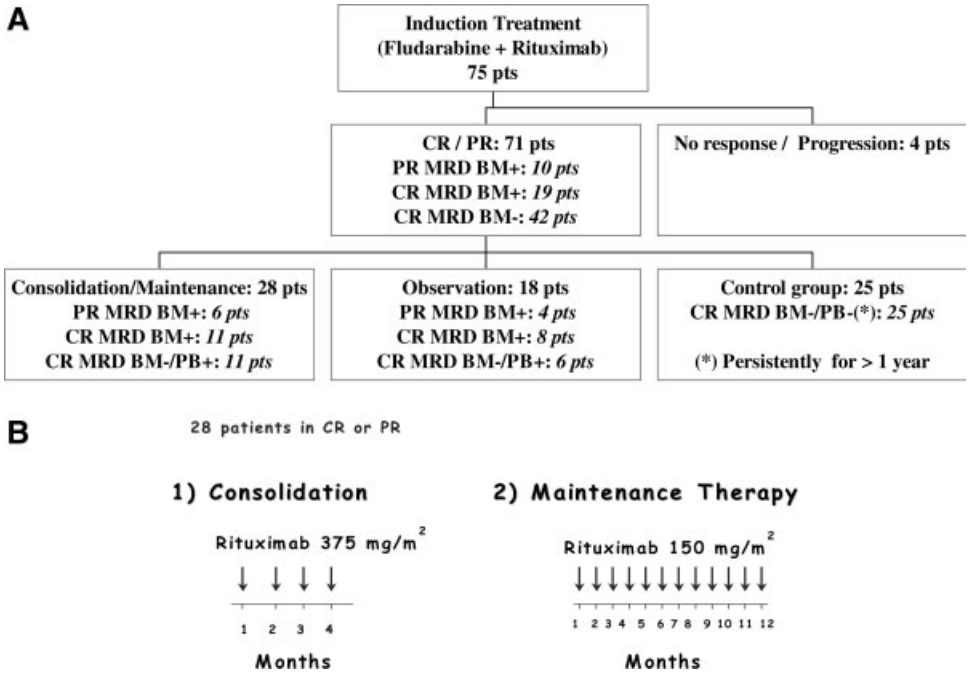

FIGURE 1. (A) Flow chart of patient groups. Chronic lymphocytic leukemia populations (induction, consolidation/maintenance, observation, minimal residual disease [MRD] status) are detailed; CR indicates complete remission; PR, partial remission; pts, patients; BM, bone marrow; +, positive; PB, peripheral blood; −, negative. (B) Rituximab consolidation and maintenance scheme. Twenty-eight MRD-positive patients in clinical CR or PR underwent consolidation/maintenance therapy with 4 monthly cycles of rituximab at a dose of 375 mg/m^2 followed by 12 monthly doses of rituximab at a dose of 150 mg/m^2.

11 (q23), 13, and 17, commercial probes (ATM-2, Rb-1, and p53, respectively) were used (Vysis Inc., London, United Kingdom). An α satellite DNA probe CEP12, which was labeled directly with SpectrumGreen, was used to detect aneuploidy of chromosome 12. LSIp53 labeled with SpectrumOrange (Vysis Inc.) was used to evaluate chromosome deletion at 17p13.1. The procedure used has been described previously.[29]

IgV$_H$ Mutation Analysis

Total RNA was extracted and reverse-transcribed as reported previously.[30] The resulting combinational DNAs, which were checked for first-strand synthesis, were amplified by using a mixture of sense primers annealing either to the *V$_H$1* through *V$_H$6* leader sequences or to the 5′ end of *V$_H$1* through *V$_H$6 FR1*, as reported.[31,32] *V$_H$* gene sequences that deviated >2% from the corresponding germline gene were defined as mutated. The procedure that we used was described previously.[13]

Treatment Plan

For induction treatment, all patients received daily fludarabine (25 mg/m^2) delivered intravenously on days 1 through 5 of 28-day intervals for a total of 6 cycles. Then, patients with stable disease or better, as defined by the NCI criteria, received 4 weekly doses of rituximab (375 mg/m^2), as reported previously.[13] Patients were observed for approximately 1 month and then were restaged completely to determine their overall response according to the NCI 1996 criteria.[28] At this point, we performed a careful immunophenotypic BM evaluation in which samples with CD19+/CD5+/CD79b− cells >1% were considered positive for MRD. Furthermore, we continued monitoring the immunophenotype of the peripheral blood lymphocytes (PBLs) every month for 1 year in MRD BM-negative patients; and, when CD19+/CD5+/CD79b− (MRD) PBLs were >1000/mL, a new BM immunophenotypic analysis was performed. Based on this threshold in peripheral blood, all of these patients also had MRD-positive BM (>1%).

Therefore, both of these patient subsets (n = 28 patients), 1 subset (n = 17 patients) in clinical CR or PR who were MRD-positive after induction and another subset (n = 11 patients) in CR who were MRD-negative after induction but who became MRD-positive within 1 year, underwent consolidation/maintenance therapy with 4 monthly cycles of rituximab at 375 mg/m^2 followed by 12 monthly doses of rituximab at 150 mg/m^2 (Fig. 1A,B). In

detail, inclusion in the consolidation/maintenance treatment group for each patient was determined either immediately after induction in BM MRD-positive patients or soon after the detection of MRD in BM in BM MRD-negative patients within 1 year. Eighteen patients with clinical and biologic characteristics similar to those of the consolidation/maintenance group underwent observation only. The complete outline of the study is provided in Figure 1A.

Assessment and Management of Toxicity During Consolidation/Maintenance Treatment

Hematologic toxicity was graded according to the modified NCI criteria for CLL,[28] whereas nonhematologic toxicity was graded according to the NCI Common Toxicity Criteria. Infusion toxicity was assessed according to the criteria described previously by Byrd et al.[12] Patients who experienced grade 3 or 4 neutropenia were supported with growth factors (granulocyte–colony-stimulating factor) and remained without treatment until this parameter recovered within 50% of the baseline value. Thereafter, they received the same original dose. There were no dose reductions for rituximab therapy because of hematologic toxicity. At the onset of fever, chills, rigors, or other infusional reactions, patients had their infusion discontinued; and, after the resolution of symptoms, the rituximab infusion was restarted at a rate of 50 mg per hour and then escalated as tolerated to 200 mg per hour. Patients who developed an infection were observed without further CLL treatment until the infection had resolved, but no dose reductions were implemented.

Patient Monitoring and Response Evaluation

Patients were assessed for induction response 4 weeks after the last rituximab infusion with a detailed clinical and instrumental evaluation, as reported previously.[13] These same evaluations were performed at the end of consolidation/maintenance therapy. Criteria for response used the revised 1996 NCI Working Group guidelines.[28] A CR or PR had to be maintained for at least 8 weeks. PFS was defined as the time from the end of induction treatment in the subgroup of patients who achieved a CR or PR, encompassing the time of consolidation/maintenance treatment, until the last follow-up, the time at which disease progression occurred, or the time of death from any cause. Similarly, OS was measured from the end of induction treatment to either the last time the patient was seen alive or the date of death.

TABLE 1
Patient Characteristics (n = 75)

Characteristic	No. of patients (%)
Median age, y (range)	60 (37–74)
Men	39 (52)
Rai stage	
Low	7 (9)
Intermediate	65 (87)
High	3 (4)
B symptoms	23 (31)
Time since first diagnosis to treatment, y	
≤1	21 (28)
2–5	39 (52)
>5	15 (20)
Bone marrow infiltration pattern	
Nodular	6 (9)
Mixed	14 (20)
Diffuse	50 (71)

Sample Size Determination

With a 2-sided significance level (α) of 5% and an 80% power 1-β, in total, 46 patients would be eligible to receive consolidation and maintenance therapy, and 12 events would be enough to reveal a 55% increase in 5-year PFS after inclusion in the study (from 32% to 87% in the consolidation/maintenance group).[33]

Statistical Analysis

For the efficacy analysis, patients had to have received at least 1 cycle of therapy. Rate comparisons between patient subgroups were performed by using the 2-tailed Fisher exact test. Event-related data (survival and PFS) were estimated by using the Kaplan-Meier product limit method. Prognostic subgroups were compared by using the log-rank test. Statistical analysis and Kaplan-Meier curves were performed using Statistica software (version 6.0 for Windows).

RESULTS

Patient Characteristics

The pretreatment features of the patients are summarized in Table 1. The median age was 60 years (range, 37–74 years). All patients met the guidelines criteria for having CLL.[27] According to Rai staging criteria,[1] 7 patients (9%) had low-risk CLL (stage 0), 65 patients (87%) had intermediate-risk CLL (stage I or II), and 3 patients (4%) had high-risk CLL (stage III or IV). More than half of the patients (55%) had a good ECOG performance status (0). The time from diagnosis to treatment ranged from 1 to 10 years. Only 23 of 75 patients (31%) exhibited B symptoms.

TABLE 2
Induction Treatment Results

Treatment	No. of patients (%)			
	Total no.	CR	PR	SD or PD
Fludarabine induction	75	49 (65)	21 (28)	5 (7)*
Rituximab induction	73	61 (83)	10 (14)	2 (3)

CR indicates complete response; PR, partial response; SD, stable disease; PD, progressive disease.
* Three patients presented with SD and therefore underwent rituximab induction; 71 patients in CR or PR after induction with fludarabine plus rituximab entered the consolidation and maintenance protocol.

Toxicity

Focusing on the consolidation and maintenance program, the 3 most frequent side effects were infusion-related toxicity, myelosuppression, and infections. Four of 28 patients who received rituximab in consolidation/maintenance experienced a mild infusion-related symptom complex consisting of fever, chills, and rigors. There were 7 opportunistic infections, including 3 dermatomal herpes zoster infections and 4 localized herpes simplex infections, but no pulmonary toxicity was observed. Hematologic toxicity included only neutropenia (grade 1 and/or 2 in 8 patients, grade 3 and/or 4 in 4 patients) during rituximab treatment.

Response to Treatment and Treatment Outcome

All patients who were enrolled on this study (n = 75 patients) were evaluable for response. The response induction rate included a first induction CR rate of 65% after fludarabine that jumped to 83% after rituximab. Seventy-one patients in CR or PR after induction entered the protocol consolidation and maintenance study (Table 2). MRD analysis performed on BM by flow cytometry after induction therapy was positive (>1% CD19+/CD5+/CD79b− CLL cells) in 29 of 71 patients (41%). All patients in PR (n = 10) were positive for MRD, and 19 of 61 patients in CR (31%) were positive for MRD (Fig. 1A).

Twenty-eight patients (22 in CR and 6 in PR) who either had CD5+/CD19+/CD79b− MRD BM cells >1% (n = 17 patients) immediately after induction treatment or initially were MRD BM-negative but who presented with MRD in PBLs >1000/mL (n = 11 patients) within 1 year after the completion of induction treatment underwent consolidation/maintenance therapy with rituximab. Eighteen patients (14 in CR and 4 in PR) who presented with similar biologic and clinical features underwent observation only, including 12 patients with MRD-positive BM after induction and 6 patients with MRD in PBLs >1000/mL within 1 year (Table 3). In addition, 25 patients in CR with persistently MRD-negative BM (>1 year) were observed only and compared with the subset of consolidated patients in terms of PFS.

TABLE 3
Consolidated (n = 28) Versus Observed (n = 18) Patient Characteristics

Characteristic	No. of patients	
	Consolidated	Observed
Median age (range), y	58 (44–70)	62 (48–76)
Men	13	11
Rai stage		
Low	0	2
Intermediate	27	16
High	1	0
B-symptoms	7	7
Date first patient enrolled	July 2000	March 2000
Date last patient enrolled	January 2005	February 2005
Median time (MRD-BM+), mo*	1	1
Median time (MRD-PB+), mo*	3.6	3.5
Median follow up (range)†	31.5 (11–72)	20.5 (3–62)

MRD indicates minimal residual disease; BM, bone marrow; +, positive, PB, peripheral blood.
* Between the end of induction and the beginning of consolidation or observation.
† From the beginning of consolidation/maintenance or observation.

Outcome data relative to PFS and OS are shown in Figures 2A and 2B. The estimated 5-year PFS after the end of induction treatment was 73% (Fig. 2A).

After a median of 26 months of follow-up from the beginning of consolidation/maintenance or observation (March 2000), until the end of June 2006, 13 of 71 patients (18%) experienced disease recurrence. Among the 46 patients who were enrolled in the consolidation/maintenance or observation study, 3 patients died. One patient died in CR of a fulminant B hepatitis, and 2 other patients died of progressive CLL after receipt of the prescribed protocol therapy.

It is interesting to note that MRD-positive patients who underwent consolidation therapy (n = 28 patients) had a significantly longer response duration (87% vs 32% at 5 years; $P = .001$) (Fig. 3A) compared with the subset of patients who were not consolidated and were positive for MRD in BM or PBLs (n = 18 patients). It also is worth noting that patients with persistently MRD-negative BM (>1 year; n = 25 patients) had a response duration similar to that of the consolidated patients (Fig. 3B).

Clinical and Biologic Features Predicting Response and Outcome

Thirty-five patients (47%) were identified who had ZAP-70 expression >20% (range, 4–70%), and 23 of 75 patients (31%) presented CD38 values >20%

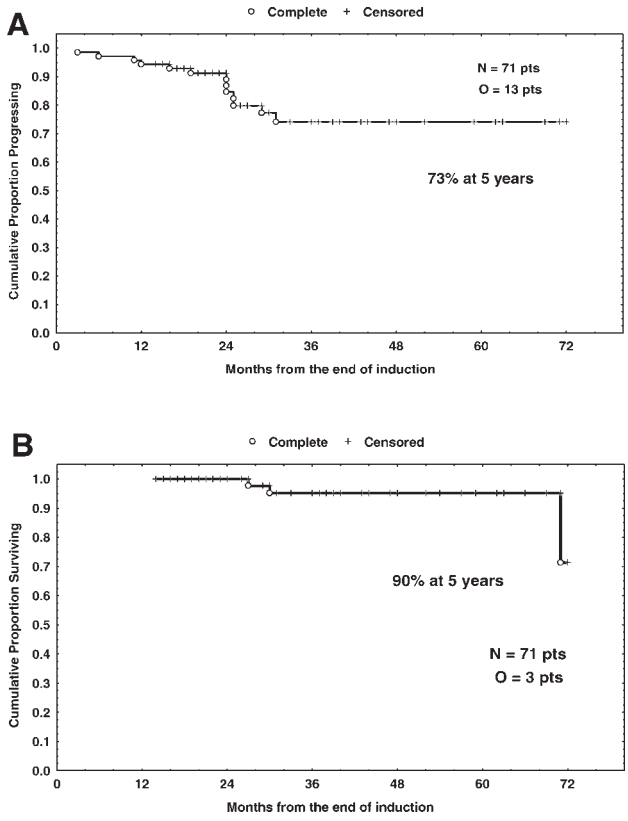

FIGURE 2. (A) Kaplan-Meier analysis of progression-free survival (PFS). The proportion of 71 patients who responded to treatment and remained in complete or partial remission are shown. The estimated 5-year PFS rate from the end of induction was 73%. Thirteen of 71 patients (18%) experienced disease recurrence. O, observed events. (B) Kaplan-Meier analysis of overall survival from the end of induction for 71 patients with chronic lymphocytic leukemia. At the time of last follow-up, 3 patients had died either of progressive disease or of opportunistic infections. O indicates observed events.

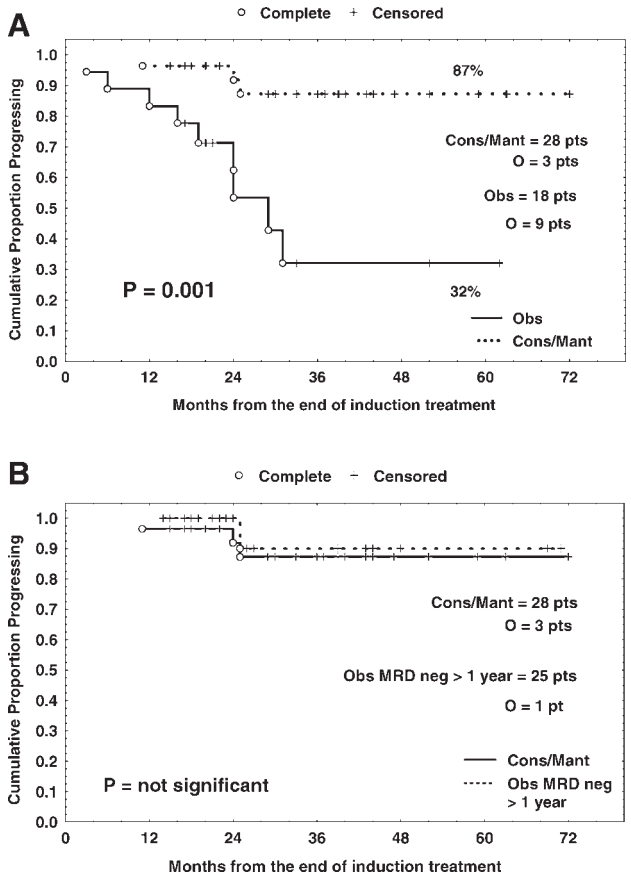

FIGURE 3. (A) Response duration by consolidation (Cons) and maintenance (Mant) in patients (pts) who were positive for minimal residual disease (MRD). Twenty-eight MRD-positive consolidated (Cons) patients had a longer response duration compared with 18 MRD-positive nonconsolidated patients ($P = .001$). Obs indicates observed patients; O, observed events. (B) Response duration in consolidated MRD-positive patients versus observed persistently MRD-negative (neg) patients. Twenty-five MRD-negative unconsolidated patients demonstrated a similar response duration compared with 28 MRD-positive consolidated patients.

(range, 1–69%). Fifty-three patients were analyzed by interphase fluorescence in situ hybridization: In that analysis, 26 patients (49%) had a normal karyotype, 15 patients (28%) had deletion (del) 13q abnormality, and 12 patients (23%) had an "intermediate/high-risk" karyotype encompassing trisomy 12 (5 patients), del 11q (5 patients), and del 17p (2 patients). Forty-seven patients were analyzed for IgV_H mutations: In that analysis, 19 of 27 patients with IgV_H mutations <2% presented with ZAP-70 protein >20% ($P = .0002$). Moreover, the strict relation between CD38 and IgV_H mutational status was confirmed (15 of 17 patients with IgV_H mutations <2% had CD38 values >20%; $P = .00002$).

A significantly shorter PFS from the end of induction was observed among ZAP-70+ patients (40% vs 96% at 5 years; $P = .00002$) (Fig. 4A), CD38+ patients (30% vs 88% at 5 years; $P = .001$) (Fig. 4B), unmutated patients (0% vs 92% at 2.1 years; $P = .0003$) (Fig. 4C), and within the "intermediate/high-risk" (trisomy 12, or del 11q, or del 17p) cytogenetic subset (0% vs 88% at 2.1 years; $P = .02$) (Fig. 4D). It is noteworthy that, within the ZAP-70+ subset (n = 35 patients), considering only the MRD-positive patients who were in CR or PR (n = 23 patients), patients in the consolidated group (n = 12) had a significantly longer response duration (69% vs 0% at 2.6 years; $P = .007$) (Fig. 5) compared with patients in the observation-only group (n = 11).

DISCUSSION

In this Phase II study, we confirmed that induction immunochemotherapy consisting of 6 cycles of flu-

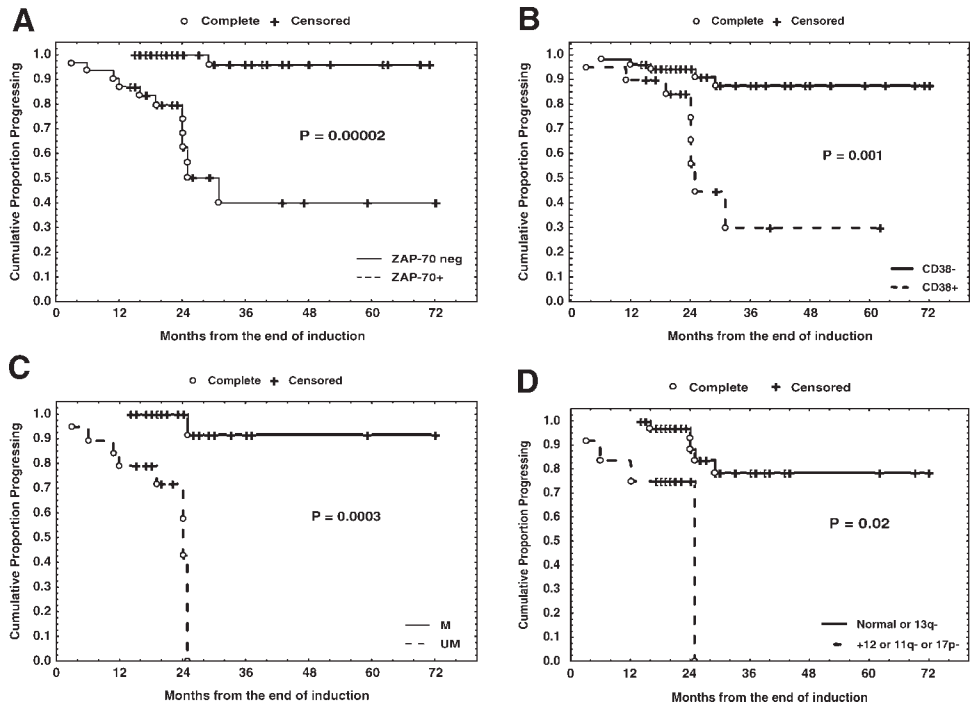

FIGURE 4. These progression-free survival (PFS) curves were based on ZAP-70 (A), CD38 (B), immunoglobulin heavy-chain variable-region (*IgVH*) mutational status (C), and cytogenetics by fluorescence in situ hybridization (D). PFS from the end of induction (indicated in months) was significantly shorter in patients who had ZAP-70 levels >20% (ZAP-70-positive [ZAP-70+] vs ZAP-70-negative [neg]), CD38 levels >20% (CD38+ vs CD38-negative [CD38−]), *IgVH* unmutated status (UM) (vs mutated [M]), and "intermediate/high-risk" cytogenetic status (trisomy 12, deletion 11q, deletion 17p).

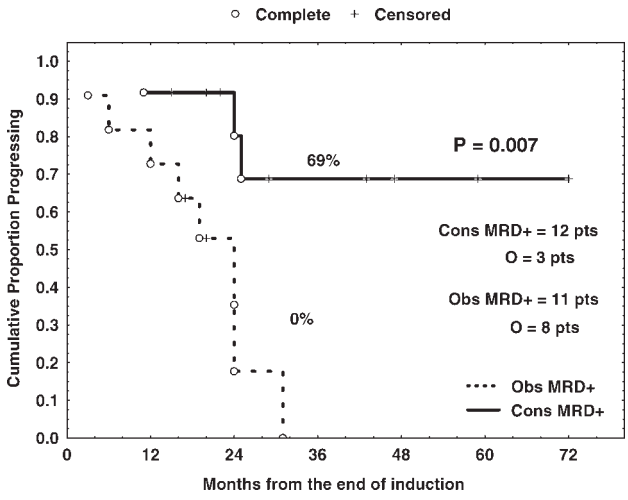

FIGURE 5. Duration of response within the ZAP-70-positive (+) patient subset. Minimal residual disease (MRD)-positive consolidated (Cons) patients had a significantly longer response duration compared with MRD-positive unconsolidated patients. Obs, observed patients; O, observed events.

darabine followed by 4 cycles of rituximab was very effective in patients with untreated B-CLL, and we demonstrated that consolidation/maintenance therapy with rituximab prolonged the response duration in MRD-positive patients. Rituximab dramatically increased response rates. After fludarabine therapy, 65% of patients achieved CR. This CR rate jumped to 83% after rituximab treatment. This very high CR rate was achieved because nearly all of our patients (65 of 75 patients) had intermediate Rai stage disease. In fact, the treatment of this progressive subset before the appearance of anemia or thrombocytopenia allowed us to obtain very good results in terms of both response and response duration. It is noteworthy that PFS outcomes also were impressive after rituximab, and the consolidation/maintenance approach using the same drug resulted in an improved 5-year PFS rate of 73%. Moreover, this treatment regimen is feasible and can be administered safely on an outpatient basis. The risk of opportunistic infections may increase at first by combining fludarabine and rituximab in induction and then prolonging rituximab infusions in the consolidation and maintenance therapy, because fludarabine leads to profound depletion of T cells, whereas rituximab causes prolonged depletion of B lymphocytes.[34,35] During consolidation/maintenance, most of these infections were viral, and they often were localized. Grade 3 and/or 4 hematologic toxicity was observed almost exclusively for neutropenia. None-

theless, this excess neutropenia did not predispose patients to an excess number of neutropenic fever episodes or life-threatening infections.

Moreover, there was no event of autoimmune hemolytic anemia, most likely because the transient B-cell depletion caused by rituximab may avoid the development of fludarabine-associated hemolytic anemia. One concern regarding the use of rituximab in patients with CLL, particularly in consolidation/maintenance, may be infusion-related toxicity. Studies have demonstrated that rituximab can cause severe infusion-related toxicity in a minority of patients and that a high number of circulating B-CLL cells may predispose patients to this problem.[36] In our study, only 4 patients had a mild infusion-related symptom complex, which consisted of fever, chills, and rigors, during consolidation/maintenance with rituximab. Most likely, the low infusion toxicity observed with rituximab treatment during consolidation/maintenance was because of the lower leukocyte counts.

The employment of an MRD assay by flow cytometry allowed us to define patients in CR as positive or negative for MRD. That is important, because patients in CR based on NCI criteria who are positive for MRD may develop recurrent disease more easily. To avoid this risk, consolidation/maintenance therapy with rituximab was proposed by our group. Twenty-eight MRD-positive patients who were in CR or PR before the consolidated treatment scheme had a significantly increased 5-year PFS rate compared with unconsolidated MRD-positive patients (Fig. 3A). Therefore, rituximab consolidation/maintenance therapy was crucial to prolonging PFS in MRD-positive patients, allowing them to reach the same response duration as that achieved by persistently MRD-negative patients (Fig. 3B). In data from the literature on patients with follicular lymphoma, a >2-fold longer duration of remission was achieved with rituximab maintenance compared with observation in a multicenter, Phase III trial.[16] Moreover, a histologic subgroup analysis from a randomized Phase II trial of rituximab revealed longer PFS with the maintenance regimen than with the retreatment regimen in patients with either follicular NHL (median, 31 months vs 13 months; n = 62 patients) or B-cell CLL (median, 34 months vs 6 months; n = 28 patients).[17] Moreover, the use of lower doses of rituximab during the maintenance phase could reduce the loss of CD20 antigen from the surface of circulating B cells.[37] It is believed that this process of antigenic modulation occurs through "shaving off" of the rituximab-CD20 complexes by monocytes or macrophages in a reaction mediated by Fcγ receptors.[37] In fact, results from a pilot study in this patient population suggest that the loss of CD20 antigen can be reduced and the efficacy of rituximab can be improved with the use of low doses of rituximab that still provide adequate targeting and clearance of circulating CD20+ B lymphocytes.[38] The efficacy of our maintenance approach may be explained on the basis of this recent experience.[38]

Various clinical features, including age, Rai stage, and serum β_2-microglobulin, have been associated with lower response and poor long-term treatment outcomes after alkylator-based and purine analog-based therapy for CLL.[39] Recent studies examining molecular aberrations (ie, *p53* mutations), unfavorable cytogenetics, CD38 expression, somatic *VH* gene mutational status, and ZAP-70 expression have demonstrated that all of these factors are important determinants for treatment outcome in patients with CLL.[40] It is interesting to note that, in our study, higher ZAP-70 levels, CD38 overexpression, and *IgVH* unmutated status identified subsets of patients who had a poor prognosis with regard to PFS measured from the end of induction treatment (Fig. 4). Thus, these markers may be used prospectively to identify subsets of patients who have a low likelihood of improved outcomes with a rituximab consolidation and maintenance approach. Conversely, an MRD assay performed by flow cytometry may allow us to identify MRD-positive patients who should be treated with a consolidation/maintenance immunotherapy approach, because they are at the greatest risk of disease recurrence. Furthermore, within the ZAP-70+ patients, considering only MRD-positive patients, consolidated patients had a better course than unconsolidated patients. Therefore, consolidation and maintenance therapy with rituximab significantly improves the clinical outcome of patients with B-CLL.[41] Our experience should encourage the use of consolidation/maintenance treatment with rituximab in CLL. In fact, the ultimate objective in CLL therapy is not only to achieve high CR rates but also (and mainly) to avoid disease recurrence, thus prolonging remission duration and, finally, OS. Improved OS may be expected in patients who receive rituximab maintenance therapy because of the effect it has on B-cell depletion; at 1-year follow-up, the level of circulating B lymphocytes remained low in patients who received rituximab maintenance, whereas it rose above the baseline value in patients who received no further rituximab treatment.[16] In conclusion, our study demonstrated that a consolidation and maintenance therapy with rituximab further prolongs the response duration in MRD-positive patients with B-CLL.

REFERENCES

1. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukaemia. *Blood*. 1975;46:219–234.
2. Binet JL, Leporrier M, Dighiero G, et al. A clinical staging system for chronic lymphocytic leukaemia. *Cancer*. 1977; 40:855–864.
3. Keating MJ, Kantarjian H, Talpaz M, et al. Fludarabine: a new agent with major activity against chronic lymphocytic leukaemia. *Blood*. 1989;74:19–25.
4. Keating MJ, Kantarjian H, O'Brien S, et al. Fludarabine: a new agent with marked cytoreductive activity in untreated chronic lymphocytic leukaemia. *J Clin Oncol*. 1991;9:44–49.
5. 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 leukaemia. *Lancet*. 1996;347:1432–1438.
6. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukaemia. *N Engl J Med*. 2000;343: 1750–1757.
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. 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.
9. 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.
10. Di Gaetano N, Xiao Y, Erba E, et al. Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line resistant to the cytotoxic activity of either drug alone. *Br J Haematol*. 2001;114:800–809.
11. Schulz H, Klein SK, Rehwald U, et al. Phase II study of a combined immunochemotherapy using rituximab and fludarabine in patients with chronic lymphocytic leukemia. *Blood*. 2002;100:3115–3120.
12. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase II 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.
13. Del Poeta G, Del Principe MI, Irno Consalvo MA, et al. The addition of rituximab to fludarabine improves clinical outcome in untreated patients with ZAP-70 negative chronic lymphocytic leukemia. *Cancer*. 2005;104:2743–2752.
14. 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.
15. 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.
16. Ghielmini M, Schmitz SF, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly × 4 schedule. *Blood*. 2004;103:4416–4423.
17. Hainsworth JD, Lichty S, Shaffer DW, et al. Maximizing therapeutic benefit of rituximab maintenance therapy versus re-treatment at progression in patients with indolent non-Hodgkin's lymphoma—a randomized phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol*. 2005;23:1088–1095.
18. Hainsworth JD, Litchy S, Barton JH, et al. Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol*. 2003;21: 1746–1751.
19. Van Oers MHJ, Klasa R, Marcus RE, et al. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. *Blood*. 2006;1086:3295–3301.
20. Hochster HS, Weller E, Gascoyne RD, et al. Maintenance rituximab after CVP results in superior clinical outcome in advanced follicular lymphoma (FL): results of the E1496 phase III trial from the Eastern Cooperative Oncology Group and the Cancer and Leukemia Group B. [abstract]. *Blood*. 2005;106:106a.
21. Forstpointner R, Unterhalt M, Dreyling M, et al. Maintenance therapy with rituximab leads to a significant prolongation of response after salvage therapy with a combination of rituximab, fludarabine, cyclophosphamide, and mitoxantrone (R-FCM) in patients with recurring and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low Grade Lymphoma Study Group (GLSG). *Blood*. 2006;108:4003–4008.
22. Damle RN, Wasil T, Fais F, et al. IgV gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94:1840–1847.
23. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated IgV(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94:1848–1854.
24. Del Poeta G, Maurillo L, Venditti A, et al. Clinical significance of CD38 expression in chronic lymphocytic leukemia. *Blood*. 2001;98:2633–2639.
25. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*. 2003;348: 1764–1775.
26. Del Principe MI, Del Poeta G, Buccisano F, et al. Clinical significance of ZAP-70 protein expression in B-cell chronic lymphocytic leukemia. *Blood*. 2006;108:853–861.
27. Rawstron AC, Kennedy B, Evans PAS, et al. Quantitation of minimal disease levels in chronic lymphocytic leukaemia using a sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy. *Blood*. 2001;98:29–35.
28. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, Rai KR. National Cancer Institute Sponsored Working Group Guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*. 1996;29:4990–4997.
29. Del Principe MI, Del Poeta G, Venditti A, et al. Clinical significance of soluble p53 protein in B-cell chronic lymphocytic leukemia. *Haematologica*. 2004;89:1468–1475.

30. Gattei V, Degan M, Gloghini A, et al. CD30 ligand is frequently expressed in human hematopoietic malignancies of myeloid and lymphoid origin. *Blood*. 1997;89:2048–2059.
31. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *J Clin Invest*. 1998;102:1515–1525.
32. Gurrieri C, McGuire P, Zan H, et al. Chronic lymphocytic leukemia B cells undergo somatic hypermutation and intraclonal V_HDJ_H gene diversification. *J Exp Med*. 2002; 196:629–639.
33. Freedman LS. Tables of the number of patients required in clinical trials using the log rank test. *Stat Med*. 1982;1:121–129.
34. 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.
35. Byrd JC, Houdle-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.
36. 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.
37. Kennedy AD, Beum PV, Solga MD, et al. Rituximab infusion promotes rapid complement depletion and acute CD20 loss in chronic lymphocytic leukemia. *J Immunol*. 2004; 172:3280–3288.
38. Williams ME, Densmore JJ, Pawluczkowycz AW, et al. Thrice-weekly low-dose rituximab decreases CD20 loss via shaving and promotes enhanced targeting in chronic lymphocytic leukemia. *J Immunol*. 2006;177:7435–7443.
39. Cheson BD, Zwiebel J. Chronic lymphocytic leukemia: staging and prognostic factors. *Semin Oncol*. 1998;25:42–59.
40. Shanafelt TD, Geyer SM, Kay NE. Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. *Blood*. 2004;103:1202–1210.
41. Del Poeta G, Del Principe MI, Maurillo L, et al. Consolidation and maintenance immunotherapy with rituximab improves progression free survival within ZAP-70 positive chronic lymphocytic leukemia (CLL). [abstract]. *Blood*. 2006; 108:799a.