

Critical appraisal of the role of rituximab in the treatment of patients with previously untreated or treated chronic lymphocytic leukemia (CLL)

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Abstract: Patients with chronic lymphocytic leukemia (CLL) have benefited from the introduction of targeted therapy for leukemia. Rituximab (a chimeric murine-derived monoclonal antibody that targets CD20 on lymphocytes) was the first monoclonal antibody to affect the natural course of this disease. Several reports have shown modest single-agent activity in patients with CLL. However, the best results come from the combination of this agent with chemotherapy; a significant benefit has been seen with the use of fludarabine, cyclophosphamide, and rituximab (FCR). The addition of rituximab to chemotherapy boosted overall response rates, complete response rates and prolonged progression free survival. Recent data showed an overall survival benefit with FCR. Other combinations including bendamustine and rituximab appear more effective than bendamustine alone, while combining rituximab with other types of agents also appears to improve response rates. This type of relatively nontoxic regimen is being investigated in elderly patients who may not tolerate standard combination chemoimmunotherapies.

Keywords: chronic lymphocytic leukemia, rituxan, bioimmunotherapy

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world. It is estimated that 15,490 patients will be diagnosed and 4,390 will die of CLL in 2009 in the USA.¹ It is considered by the World Health Organization (WHO) to be one of the indolent mature B-cell neoplasms and is classified with small lymphocytic lymphomas.² CLL is diagnosed by the presence of a typical B-cell immunophenotype and co-existence of CD5⁺/CD19⁺/CD20⁺/HLA-DR⁺/CD23⁺/sIg dim.^{3,4} Monoclonal antibodies targeting CD20 have been evaluated in the treatment of CLL due to wide expression of this surface antigen on leukemic cells. CD20 is a calcium channel that interacts with the B-cell immunoglobulin receptor complex (BCR) and is present on both normal and malignant B-cells.⁵ It is important to note that the antigen density of CD20 on CLL cells is significantly less than that seen in lymphoma.

Rituximab

Rituximab is a chimeric murine-derived monoclonal antibody that consists of a human IgG1-k constant regions and variable regions from the murine monoclonal anti-CD20 antibody IDEC-2B8. It recognizes CD20 and activates complement and promotes C3b deposition in close proximity to cell-bound rituximab. Rituximab exerts its actions via several pathways including antibody-dependent cellular activity (ADCC), complement-dependent cytotoxicity (CDC), and through apoptosis via activation of caspase 3.^{6,7}

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Resistance to rituximab in cell lines has been linked to the presence of surface antigens such as CD55 and CD59; which block CDC/ADCC.^{8,9}

Maloney et al evaluated rituximab in a phase I study using a single dose of 10–50 mg/m² in patients with relapsed B-cell non-Hodgkin lymphoma (NHL) and then obtained biopsies two weeks later to confirm binding of the antibody. They showed rapid depletion of B-cells, positive binding to tumor cells, and no dose limiting toxicity.¹⁰ Another phase I study used rituximab weekly \times 4 at doses of 125, 275, and 375 mg/m² in patients with relapsed lymphoma.¹¹ Six of 14 patients (40%) with low grade lymphoma responded and 375 mg/m² was selected for phase II studies.

Single-agent rituximab

McLaughlin et al authored the pivotal phase II study in 1998 using rituximab 375 mg/m² IV weekly \times 4 in patients with relapsed low grade lymphoma; 48% responded, the response rate was only 13% in patients with small lymphocytic lymphoma (SLL).¹² Several other studies reported similar response rates (Table 1).^{13–17} In an attempt to improve the efficacy of rituximab, 2 trials evaluated higher doses or more frequent administration. Doses of 500 mg/m² to 2250 mg/m² IV weekly \times 4 were given to 40 patients with CLL yielding an overall response rate (ORR) of 29% at a dose of 500 mg/m² and 75% at a dose of 2250 mg/m². All responses were partial remission (PR) and time to progression (TTP) was 8 months.¹⁸ Thrice weekly rituximab at 375 mg/m² \times 4 weeks was evaluated by Ohio State University in 33 patients with SLL/CLL; this produced an ORR of 45%; one complete remission (CR) was seen.¹⁹ Rituximab has also been evaluated as frontline therapy for CLL. Hainsworth et al assessed rituximab 375 mg/m² IV weekly \times 4 in untreated patients with CLL with maintenance rituximab for patients who had objective response or stable

disease. This yielded a 9% CR and a 58% ORR; progression free survival (PFS) was 18.6 months.²⁰ Thomas et al gave rituximab 375 mg/m² IV weekly \times 8 weeks to patients with previously untreated CLL with high beta-2 microglobulin but no standard indication for treatment; this provided an ORR of 90% and a CR rate of 19%.²¹

Rituximab and chemotherapy

The Cancer and Leukemia Group B (CALGB) 9712 randomized 104 chemonaive patients to concurrent vs sequential fludarabine and rituximab (FR) therapy. Fludarabine was given at 25 mg/m² IV days 1–5 every 28 days for 6 cycles while rituximab was given at 375 mg/m² IV on day 1 of each cycle (an additional dose on cycle one day 4 was given to ensure adequate saturation of CD20 binding sites).²² Sequential rituximab 375 mg/m² weekly \times 4 was given 2 months after the end of fludarabine or FR if patients had at least stable disease or better per National Cancer Institute (NCI) criteria. Ninety percent ORR including 47% CR was achieved in the concurrent arm compared to 77% ORR and 28% CR in the sequential arm. However, in a recent update with a long-term follow up, there was no difference in PFS or overall survival (OS) between concurrent vs sequential arms; with an estimated 17% of responders still in remission 8 years later.²³ The Europeans showed similar results in a multi-center phase II study using FR.²⁴ A retrospective comparison between CALGB 9712 and 9011 (single-agent fludarabine [F]) showed a superior ORR with FR vs F (84% vs 63%), as well as a superior 2-year PFS (67% vs 45%) and 2-year OS (93% vs 81%).²⁵

MD Anderson Cancer Center (MDACC) evaluated fludarabine, cyclophosphamide and rituximab in both treated and untreated patients (Table 2). Fludarabine 25 mg/m², cyclophosphamide 250 mg/m² on days 2–4 of cycle one and on days 1–3 of cycles 2–6, and rituximab 375 mg/m² on day 1 of cycle one and 500 mg/m² on day 1 of cycles 2–6 (fludarabine, cyclophosphamide, and rituximab [FCR]) were given every 28 days for a total of 6 cycles to 177 patients with relapsed CLL.²⁶ The ORR was 73% with 25% CR, 16% nodular PR and 32% PR. Thirty-two percent of complete responders achieved molecular remission in the bone marrow. FCR was given to 300 patients with previously untreated CLL producing a 94% ORR, including a 72% CR rate, nodular partial remission in 10%, and partial remission due to cytopenia and residual disease in 7% and 6% respectively.^{27,28} Median time to progression was 80 months with a 6-year OS of 77%, and responses predicted longer survival.

Lamanna et al used another purine analogue, pentostatin, in patients with relapsed CLL. Pentostatin 4 mg/m²,

Table 1 Rituximab single agent trials

Ref	Prior Rx	N	Doses (W)	ORR%	PFS/TTP
McLaughlin ¹²	Y	30	4	13	ND
Nguyen ¹³	Y	12	4	0	ND
Winkler ¹⁴	Y	9	4	11	ND
Ladetto ¹⁵	Y	7	4	0	ND
Huhn ¹⁶	Y	28	4	25	TTP 16 w
Itala ¹⁷	Y	24	4	35	TTP 12.5 w
O'Brien ¹⁸	Y	40	4 (500–2250)	36	TTP 8 m
Byrd ¹⁹	Y	33	4 (thrice/w)	45	PFS 9 m
Hainsworth ²⁰	N	44	4	51	18.6 m
Thomas ^{21,*}	N	21	8	21	ND

Note: *No standard indication for therapy, high risk features

Abbreviations: Ref, reference; Prior Rx, prior therapy; N, number of patients; ORR, overall response rate; PFS, progression free survival; TTP, time to progression; ND, not given; W, weekly.

Table 2 Nucleoside analogues/rituximab trials

Ref	Regimen	Rituxan	Prior Rx	Phase	N	ORR% (CR%)	PFS/TTP
Keating ²⁸	FCR	500 q 4w	No	II	300	95 (72)	TTP 80 m
Kay ³⁰	PCR	375 q 3w	No	II	64	91 (41)	PFS 32.6 m
Hallek ³¹	FCR	500 q 3w	No	III	817	95 (52)	PFS 43 m
	FC					88 (27)	PFS 32 m
Byrd ²²	FR con	375 q 4w	No	II	104	90 (47)	NR
	FR seq					77 (28)	NR
Schulz ²⁴	FR	375 q 4w	No	II	20	85 (25)	ND
		(cycle 3)	Yes		11	91 (45)	
Wierda ²⁶	FCR	500 q 3w	Yes	II	177	73 (25)	PFS 28 m
Lamanna ²⁹	PCR	375 q 3w	Yes	II	46	75 (25)	TTP 25 m
Robak ³²	FCR	500 q 3w	Yes	III	552	70 (24)	PFS 30.6 m
	FC					50 (13)	PFS 20.6 m

Abbreviations: Ref, reference; Prior Rx, prior therapy; N, number of patients; ORR, overall response rate; CR, complete remission; PFS, progression free survival; TTP, time to progression; ND, not given; W, weekly; M, month; Con, concurrent; Seq, sequential; NR, not reached.

cyclophosphamide 600 mg/m² (starting cycle one) and rituximab 375 mg/m² (starting cycle two) (PCR) were given IV on day 1 every 3 weeks \times 6 to 46 patients with relapsed CLL yielding an ORR of 75% including 25% CR.²⁹ This regimen was assessed in the front-line setting by Kay et al in 64 previously untreated patients with CLL who received the combination of pentostatin 2 mg/m², cyclophosphamide 600 mg/m² and rituximab 375 mg/m² IV on day 1 every 3 weeks \times 6 cycles.³⁰ Responses occurred in 91% including 41% CR, 22% nodular PR, and 28% PR. This regimen was equally effective in young versus older patients and in those with del 11q22; 0/3 patients with 17p del reached CR.

Two phase III studies evaluated the comparison of fludarabine and cyclophosphamide (FC) to FCR in a randomized trial.^{31,32} The German CLL Study Group (GCLLSG8) compared FCR to FC in 817 previously untreated patients.³¹ Patients were randomized to receive FC (fludarabine 25 mg/m² IV, and cyclophosphamide 250 mg/m² IV days 1–3) or FCR (rituximab 375 mg/m² IV day 0 in cycle 1 and 500 mg/m² day 1 of subsequent cycles) every 28 days \times 6. ORR was 95% vs 88%, CR rate 52% vs 27% for FCR vs FC respectively. There was improved PFS ($P < 0.0001$ vs 0.18). Recently Hallek et al updated their results and showed an OS benefit at 37 months for FCR (84%) vs FC (79%) ($P = 0.01$); only patients in Binet stages A and B had a superior OS after FCR therapy.³³ In the REACH study, 552 relapsed patients were randomized to FC vs FCR every 28 days for 6 cycles resulting in a 10-month gain in PFS with FCR.³²

MDACC added alemtuzumab to the FCR combination in patients with heavily pretreated CLL.³⁴ Alemtuzumab 30 mg IV was given on days 1, 3, 5, rituximab 375–500 mg/m² IV day 2, fludarabine 25 mg/m² IV days 3–5, and cyclophosphamide 250 mg/m² days 3–5 every 28 days for 6 cycles. Of

the 74 patients who completed treatment, 24% achieved CR, 2.7% nodular PR, and 37.8% PR (ORR 65%). In patients with 17p del, 44% responded to cyclophosphamide, fludarabine, alemtuzumab, and rituximab (CFAR), while 19% of patients treated previously with FCR achieved CR on this regimen. Median survival for all patients was 19 months but more than 35 months for patients achieving CR. This regimen was given by the same group to patients with previously untreated CLL with high risk features (beta-2 microglobulin >4) in a phase II study.³⁵ The initial report on the first 21 patients showed 71% CR, 5% nodular PR, PR in 19%. This has been recently updated with no significant change in response rates.³⁶ Therefore, it seems that CFAR does not provide better results than FCR as front-line therapy in these high risk patients.

Rituximab was also investigated in combination with bendamustine, a novel alkylating agent which contains a benzimidazole ring and is only partially cross-resistant with other alkylating agents *in vitro*. Eighty-one patients with relapsed CLL were treated in a phase II multi-center study using bendamustine 70 mg/m² IV on days 1–2 combined with rituximab 375 mg/m² for cycle one and 500 mg/m² for cycles 2–6 every 28 days. The ORR was 77% including 14.5% CR and 62.9% PR.³⁷ Among the genetics subgroups, ORR was 92.3% in 11q⁻, 100% in 12⁺ and 44.4% in patients with 17p del. Bendamustine combined with rituximab (BR) proved to be effective in 117 previously untreated patients with CLL yielding an ORR of 90.9% (including 32.7% CR).³⁸

In summary, FCR appear to be the most effective front-line regimen in CLL based on both single institutional and phase III randomized trial data. The MDACC data indicated a CR rate of 72% and an ORR of 95%. The German randomized trial (of FCR vs FC) also produced a 95% ORR in the FCR arm. The CR rate dropped to 44%–50% (including

uncertain CR), not surprising when single institutional data are reproduced in a large multicenter randomized trial. However, this is still the highest CR rate reported in patients with CLL. However some investigators have questioned whether, given the marked synergy seen when rituximab is added to chemotherapy, the alkylating agent is necessary. In other words would FR be as effective as FCR? The PFS with FR appears to be inferior to that reported with FCR. However, this question will be more definitively answered in the current Intergroup trial comparing FCR to FR with or without lenalidomide maintenance (NCT00602459). While the CR rates with BR, in a smaller study, were inferior to those seen with FCR, the Germans are conducting a randomized study in frontline CLL of BR vs FCR (GCLLSG10, NCT 00769522). PCR also appeared inferior to FCR; of interest is that FCR is clearly less well tolerated in patients over the age of 70 whereas PCR was as safe and effective in elderly patients.

Rituximab and non-chemotherapy agents

Alemtuzumab is a humanized monoclonal antibody that targets the CD52 antigen. CD52 is a 21–28 kD cell surface glycopeptide expressed on virtually all human lymphocytes, monocytes, macrophages, a small subset of granulocytes, but not erythrocytes, platelets or bone marrow stem cells. It has been FDA approved in both previously untreated and fludarabine refractory patients with CLL.^{39,40} Rituximab has been combined with alemtuzumab in several studies. Faderl et al gave rituximab 375 mg/m² weekly \times 4 with alemtuzumab using a loading-dose schedule of 3 mg, 10 mg, and 30 mg on 3 consecutive days during week 1 and 30 mg on day 3, 5 of weeks 2–4 with a repeat course depending on response and toxicity.⁴¹ Twenty of 32 (63%) patients with relapsed CLL achieved response, including 6% CR. Median TTP was 6 months and median OS was 11 months; 15% developed symptomatic CMV antigenemia requiring therapy. Mayo Clinic assessed this combination in 30 previously untreated patients with high risk features (del 17p13, del 11q22) and lacking a standard indication for therapy (early therapy) giving alemtuzumab 30 mg weekly \times 4 and rituximab 375 mg weekly \times 4; this yielded an ORR of 90% including 37% CR.⁴²

Lenalidomide is an immunomodulatory drug that is a more potent analog of thalidomide and has shown activity in relapsed patients with CLL, including activity in patients with high-risk cytogenetics.^{43–45} Ferrajoli et al recently added rituximab 375 mg/m² weekly \times 4 then every 4 weeks on cycles 3–12 of lenalidomide 10 mg starting day 9 on

cycle one and then daily every 28 days for 12 cycles.⁴⁶ After 6 cycles of therapy 25 of 37 patients achieved response (68%) including 6 patients with nodular PR, 19 PR, 6 SD; 6 patients failed to respond. Patients with poor cytogenetics features did equally well. This compares favorably to single agent lenalidomide with superior ORRs and less tumor flare reaction.⁴⁴

Rituximab has also been combined with high dose methylprednisolone. Glucocorticoids kill lymphoid cells by a p53 independent mechanism and appear to be active in patients with 17p deletions.⁴⁷ They also reduce bulky lymphadenopathy and cause less myelosuppression than chemotherapy. Bowen et al reported data from a retrospective study wherein 37 previously treated patients with CLL were given 1 g/m² of methylprednisolone daily \times 5 and rituximab 375 mg/m² weekly \times 4 with repeat of each cycle up to three.⁴⁸ Seventy-eight percent had an objective response, including 5 of 9 patients with del 17p; however, 29% developed infectious complications before completing one month of therapy. Several other studies are summarized in Table 3. James et al tested this combination in 28 previously untreated patients using methylprednisolone 1 g/m² daily \times 3 every 28 days with weekly rituximab (375 mg/m² \times 12 or 750 mg/m² weekly \times 7).⁴⁹ The ORR was 96% including 32% CR with better response if patients had less prominent splenomegaly and lower beta-2 microglobulin. This combination is proven to be effective but at the cost of a significant infection rate.

Finally, rituximab was evaluated in combination with human granulocyte macrophage colony stimulating factor (GM-CSF); the rationale was the increased surface expression of CD20 seen on some CLL cells with the use of GM-CSF *in vitro*.⁵⁰ Ferrajoli et al gave GM-CSF 250 μ g SC thrice weekly \times 8 and rituximab 375 mg/m² weekly \times 4 to 118 patients with CLL (3 different groups; elderly previously untreated, high risk with elevated beta-2 microglobulin and no standard indication for therapy, relapsed patients) achieving 65% ORR including 9% CR with minimal side effects including mild GM-CSF injection site erythema. These results compare favorably to historical control data using rituximab alone.⁵¹

Special conditions Complications

It is known that some patients will experience fever, chills, nausea, vomiting, hypotension and dyspnea predominantly with their first infusion of rituximab. This is known as ‘cytokine release syndrome’ and is associated with more

Table 3 Rituximab trials using non-chemotherapy agents

Ref	Regimen	Dose	Prior Rx	N	ORR% (CR%)	PFS/TTP
Faderl ⁴¹	Rituximab	qw × 4	Yes	32	63 (6)	TTP 6 m
	Alemtuzumab	30 2/w × 4				
Nabhan ⁵⁸	Rituximab	qw × 4	Yes	11	9 (0)	ND
	Alemtuzumab	3, 10, 30 3/w × 4				
Zent ⁴²	Rituximab	qw × 4	No	30	90 (37)	TTP 12.5 m
	Alemtuzumab	30 3/w × 4				
Frankfurt ⁵⁹	Rituximab	qw × 8	No	20	90 (75)	NR
	Alemtuzumab	30 3/w × 17				
Ferrajoli ⁴⁶	Rituximab	qw × 4 then qm × 10	Yes	37	68 (0)	ND
	Lenalidomide	10 mg qd × 12m				
Bowen ⁴⁸	Rituximab	qw × 4	Yes	37	78 (22)	PFS 12 m
	Methylprednisolone	1 gm qd × 5				
Dungearwalla ⁶⁰	Rituximab	qw × 6	Yes	14	93 (14)	PFS 7 m
	Methylprednisolone	1 gm qd × 5				
Castro ⁶¹	Rituximab	qw × 4	Yes	14	93 (36)	TTP 15 m
	Methylprednisolone	1 gm qd × 5				
James ⁴⁹	Rituximab	375 qw × 12 or 750 qw × 9	No	28	96 (32)	NR
	Methylprednisolone	1 gm qd × 3				

Abbreviations: Ref, reference; Prior Rx, prior therapy; N, number of patients; ORR, overall response rate; CR, complete remission; PFS, progression free survival; TTP, time to progression; ND, not given; NR, not reached; qw, weekly; qm, monthly; qd, daily.

intense cellular expressions of CD20. Standard premedication with acetaminophen and diphenhydramine is warranted; many people also use steroids, at least before the first dose of rituximab. Thus, the initial infusion of rituximab is usually started slowly with a gradual escalation of the rate. Tumor lysis syndrome has been described with rituximab. Most investigators use allopurinol as well as hydration in patients with high volume disease. Progressive multifocal leukoencephalopathy (PML) is a demyelinating disorder of the brain caused by the reactivation of latent JC polyoma virus. Rituximab has been linked to PML in several diseases (especially SLE) and has been found to be associated with malignancy in 52/57 reported cases; 24.6% were CLL.⁵² Those patients usually also received chemotherapy agents and seven patients had had prior hematopoietic stem cell transplant. The median time to diagnosis was 5.5 months from the last dose of rituximab and the median number of rituximab doses was 6. The case-fatality rate was 90%. Another viral reactivation concern is with patients who have had HBV infection. Several reports have documented HBV reactivation with the use of rituximab in patients with NHL and CLL, even with negative HBs Ag.^{53,54}

Elderly

Most published trials of CLL have reported a median age of less than 70, which does not reflect the general population. Data with FCR suggest that patients >70 years old are less likely to complete six cycles, less likely to achieve CR, and tend to have more complications.²⁸ Kay et al reported

a subanalysis of previously untreated patients older than 70 years receiving PCR.⁵⁵ Elderly patients had more delayed cycles (28% vs 7%), but there was no significant difference in the total number of cycles given, need for dose reductions, grade 3–4 hematologic, infectious, or other toxicities. In addition there was no significant difference in ORR, CR or even PFS between both younger and older patients.

Maintenance

Rituximab maintenance has been shown to prolong PFS in patients with NHL. Del Poeta et al gave FR (F 25 mg/m² daily × 5, every 28 days × 6, and then R 375 mg/m² weekly × 4) to 75 previously untreated CLL patients; responders with positive minimal residual disease were given four monthly cycles of rituximab 375 mg/m² followed by 12 monthly low dose (150 mg/m²) rituximab maintenance.⁵⁶ Twenty-eight patients who received maintenance therapy had longer response duration (85% vs 32% at 5 years; *P* = 0.001) compared to 18 patients who did not. However; no explanation was given for why the observation group did not receive maintenance, which makes the comparison specious. Foon et al introduced the FCR-Lite regimen with dose reduction of fludarabine and cyclophosphamide, rituximab twice each cycle, and rituximab 500 mg/m² as maintenance every 3 months until relapse. Rituxan 375 mg/m² was given on day 1 of cycle 1 then 500 mg on day 1 of cycle 2–6 and day 14 of cycle 1–6. Fludarabine 20 mg/m² and cyclophosphamide 150 mg/m² were given for 3 days with each cycle. Fifty previously untreated CLL patients achieved 100% ORR (including 79% CR) with a

median duration of complete response of 22.3 + months and no relapse yet seen in complete responders.⁵⁷ Grade 3–4 neutropenia was noted in 13% of the cycles which compares favorably to historical data using standard FCR. Further evaluation of this regimen is warranted. There are, as yet, no data from randomized trials of maintenance rituximab in CLL to establish the benefit of this approach.

Conclusion

In conclusion, rituximab has improved the management of CLL and has become a critical agent in most combination regimens. It increases CR and ORRs, and prolongs PFS. FCR appears to be the best front-line regimen with a survival benefit seen over chemotherapy alone. Non-chemotherapy-based combinations with rituximab may offer safe and effective options for patients who cannot tolerate chemoimmunotherapy. Consolidation and maintenance therapies remain interesting areas of research but randomized trials are needed.

Disclosures

The authors report no conflicts of interest in this work.

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