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REVIEW

Role of rituximab in first-line treatment of chronic lymphocytic leukemia

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Abstract: Chronic lymphocytic leukemia (CLL) is a biologically heterogeneous illness that primarily afflicts the elderly. For many decades, the initial therapy for most patients requiring treatment was limited to single-agent alkylator therapy. Within the last two decades, we have seen remarkable progress in understanding the biology of CLL and the development of more effective treatment strategies that have employed monoclonal antibodies, such as rituximab (anti-CD20). Furthermore, recognition of the synergy between fludarabine, cyclophosphamide, and rituximab (FCR) prompted investigators to explore the clinical activity of FCR in Phase II and III trials in patients with relapsed/refractory or previously untreated CLL. On the basis of these findings, the US Food and Drug Administration (FDA) recently approved rituximab in combination with fludarabine and cyclophosphamide for the treatment of patients with relapsed/refractory or previously untreated CD20-postive CLL. Recent data from a randomized Phase III trial has confirmed improved overall survival with FCR in patients with previously untreated CLL. However, FCR is not for everyone. More tolerable regimens using rituximab for the elderly and less fit patients are being pursued in clinical trials. Recent Phase II trials have explored potentially less myelosuppressive approaches by using lower doses of fludarabine and cyclophosphamide, replacing fludarabine with pentostatin, and combining rituximab with chlorambucil. Furthermore new biomarkers predictive of early disease progression have prompted investigators to explore the benefits of early treatment with rituximab combined with other agents. In addition to the proven utility of rituximab as a frontline agent for CLL, rituximab has a favorable toxicity profile both as a single agent and in combination with chemotherapy. The majority of adverse events are Grade 1 and 2 infusion-related reactions (fevers, chills, and rigors) and occur with the first dose of rituximab. The improved tolerability observed with second and subsequent infusions allows for shorter infusion times. Rituximab's proven activity and favorable toxicity profile has made it an ideal agent for expanding treatment options for patients with CLL, the majority of whom are elderly.

Keywords: rituximab, tolerability, chronic lymphocytic leukemia, fludarabine, pentostatin, chlorambucil, elderly

Introduction

Chronic lymphocytic leukemia (CLL), a monoclonal B cell malignancy, is the most common adult leukemia in the Western hemisphere and primarily affects the elderly. The median age at diagnosis is 72 years, and more than two-thirds of the patients are older than 65 years.[1] The clinical course of CLL is highly variable. Many cases behave indolently for decades and never require treatment, while others die from a rapid progression of the disease within a few years of diagnosis. Because the majority of CLL patients are asymptomatic at presentation and there exists a lack of evidence

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demonstrating a survival advantage with early treatment,[2] most patients do not require treatment until evidence of disease progression.

For many years, the initial therapy for CLL consisted of single-agent alkylators, such as chlorambucil, capable of achieving objective response rates in 60%–70% of patients but low complete remission rates.[2–4] However, advances in understanding the biology of the disease, identification of high-risk biologic features, and the introduction of rituximab (Rituxan®; Genentech, Inc., South San Francisco, CA and Biogen Idec, Inc, Cambridge, MA) have markedly changed the treatment landscape for CLL.

Rituximab is a chimeric human-mouse monoclonal antibody with a high affinity for the CD20 surface antigen, a transmembrane protein that is expressed on pre-B cells and normal differentiated B lymphocytes. Rituximab binds a discontinuous epitope in CD20, comprised of amino acids 170–173 and 182–185, with both strings brought in steric proximity by a disulfide bridge between C(167) and C(183).[5] Binding of rituximab to the CD20 antigen elicits a response that results in rapid and durable depletion of normal and malignant B cells via multiple mechanisms that include antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and direct induction of apoptosis.[6–8] Stem cells and plasma cells are spared because they lack CD20 antigens.[9]

Single-agent rituximab has clinical activity in previously treated and untreated CLL. Discovery of synergistic activity between conventional chemotherapy and rituximab prompted investigators to explore an array of novel combination regimens in previously untreated CLL. The most effective regimen combines rituximab with fludarabine and cyclophosphamide (FCR). The FCR regimen yields high complete remission rates and was the first regimen to improve overall survival significantly in CLL. On the basis of these findings, rituximab received approval by the US FDA for the indication of relapsed/refractory or previously untreated CD20-positive CLL in combination with fludarabine and cyclophosphamide. Treatment with single-agent alkylators is now reserved for elderly patients and biologically unfit patients with comorbidities that prevent them from receiving more myelosuppressive therapy. For this subgroup, investigators are exploring less myelosuppressive regimens involving rituximab.

Therapeutic potential of single-agent rituximab in CLL

Previously treated patients

The therapeutic potential of rituximab for CLL was realized from its reported activity (overall response 50%) in previously treated low-grade and follicular B cell lymphomas.[10–12] In a pivotal study, rituximab 375 mg/m^2 given intravenously (IV) as monotherapy to previously treated CLL and small lymphocytic leukemia patients demonstrated limited activity (overall response 13%) and durability.[12,13] The poor performance of rituximab in these studies may have been due to the low expression of CD20 antigens on CLL cells, whereas malignant B cells from patients with follicular lymphoma are more densely populated with CD20 antigens.[14] Secondly, rituximab may bind to circulating CD20-positive cellular debris generated from prior cytotoxic therapies, thus rendering CLL cells less vulnerable to rituximab. However, subsequent studies investigating dose-dense (thrice weekly) rituximab and higher weekly doses of rituximab 500–2250 mg/m^2 in previously treated patients reported modest overall response rates of 43% and 40%, respectively,[15–17] and the latter study revealed a correlation between the dose of rituximab and the clinical response.[16] Additionally, the thrice weekly regimen produced a modest complete remission rate of 3%, but patients harboring a 17p13.1 deletion did not achieve a meaningful response with this regimen. Nonetheless, the activity demonstrated by rituximab in these studies supported the rationale for investigating single-agent rituximab in treatment-naïve CLL, as summarized in Table 1.

Treatment-naïve patients

In a study conducted by Thomas et al,[18] eight weekly doses of rituximab 375 mg/m^2 were given to 21 previously untreated, early-stage (Rai 0–II), asymptomatic CLL patients with beta2-microglobulin (B2M) $\geq$ 2 mg/dL. The overall response rate reported was 90%, and 19% of these patients experienced a complete response. In a different single-agent rituximab study by Hainsworth et al,[19] 44 previously untreated, symptomatic patients with CLL or small lymphocytic leukemia who received four weekly doses of rituximab 375 mg/m^2 achieved overall response and complete response rates of 51% and 4%, respectively. An additional four-week course

Table 1 Single agent rituximab studies in treatment-naïve chronic lymphocytic leukemia

Reference	Doses	N	OR (%)	CR (%)	Median PFS (months)
Thomas et al[18]	4	21	90	19	43
Hainsworth et al[19]	4	44	51	4	19
	8	28[a]	58	9	

Note: [a]Patients who received additional maintenance therapy with rituximab.
Abbreviations: N, evaluable patients; OR, overall response; CR, complete remission; PFS, progression-free survival.

of rituximab given every six months for up to four cycles to 28 patients with stable or responsive disease slightly increased the overall response rate to 58% and the complete response rate to 9%. Median progression-free survival was 19 months, similar to that achieved by single-agent fludarabine in the frontline setting. The promising activity demonstrated by single-agent rituximab in these studies provided the rationale for expanding its role by coadministering rituximab with chemotherapy.

Chemoimmunotherapy with rituximab

Fludarabine–rituximab regimen

Chemoimmunotherapy regimens with rituximab have markedly changed the treatment landscape for previously untreated patients with CLL. This transformation began with the introduction of purine nucleoside analogs, such as fludarabine, demonstrating superior response rates to single-agent alkylator-based therapy in CLL.[20] As monotherapy, fludarabine was associated with high overall response rates and modest complete response rates of 20% to 30%,[20–23] but relapse was inevitable. Being the most active agent for CLL at the time, further exploration of fludarabine-based combinations such as fludarabine and rituximab (FR) was conceived. The proven antileukemic activity of both agents, in vitro synergistic antitumor activity,[24,25] and lack of overlapping toxicity inspired Schultz et al[26] to conduct a Phase II trial to investigate the safety and efficacy of FR in 31 CLL patients, 20 of whom were previously untreated. Fludarabine 25 mg/m^2/day was given IV on days 1–5 every 28 days for four cycles. Beginning with the third cycle, rituximab 375 mg/m^2 was given monthly on day 1 for four doses. The FR regimen yielded an overall response rate of 87% and a complete response rate of 33%, while 85% and 25% of the treatment-naïve patients achieved an overall response and complete response, respectively. The median duration of response was 18.8 months.

Summarized in Table 2, the Cancer and Leukemia Group B (CALGB) also conducted a Phase II study (CALGB 9712) to determine the optimal positioning of rituximab when combined with fludarabine.[27] This Phase II study randomized 104 previously untreated patients with CLL to receive six monthly cycles of IV fludarabine 25 mg/m^2/day for five consecutive days in combination with rituximab, concurrently or sequentially. Patients randomized to the concurrent rituximab arm received rituximab 375 mg/m^2 on days 1 and 4 in cycle 1 and on day 1 in cycles 2–6, followed two months later with four weekly doses of rituximab in patients who demonstrated a response or had stable disease. Similarly, the sequential arm consisted of four weekly doses of rituximab two months after completion of fludarabine for responders or those with stable disease. The concurrent group experienced higher overall response and complete response rates than the sequential group (90% and 47% versus 77% and 28%, respectively) at the expense of higher grade 3 or 4 neutropenia. Despite the higher response rates observed in the concurrent group, a long-term follow-up revealed similar estimated overall survival and progression-free survival between the two groups.[28] In a subsequent analysis, patients treated with FR in the CALGB 9712 trial were retrospectively compared with a similar control group treated with fludarabine alone in the CALGB 9011 trial. The retrospective analysis revealed a significantly higher progression-free survival and overall survival in the FR group (CALGB study 9712) than for single-agent fludarabine. Although this promising evidence was not generated from a randomized study, the suggestion of a survival advantage was enough to change the approach to treating CLL.[29]

Fludarabine–cyclophosphamide–rituximab regimen

Combining fludarabine and cyclophosphamide (FC) provided an additive advantage that translated into improved remission rates, progression-free survival, and treatment-free survival.[30–32] Striving for a complete response rate higher than 50%, investigators at MD Anderson Cancer Center (MDACC) added rituximab to FC and treated 300 previously untreated patients with advanced CLL in a single-center Phase II study.[33] The first cycle of FCR consisted of rituximab 375 mg/m^2 on day 1 and IV fludarabine 25 mg/m^2/day and cyclophosphamide 250 mg/m^2/day on days 2, 3, and 4. In cycles 2–6, the rituximab dose was increased to 500 mg/m^2 on day 1 and fludarabine and cyclophosphamide were administered on days 1, 2, and 3 as well. Courses were repeated every four weeks for a total of six courses, and growth factor support was used at the discretion of the treating physician. The median age of the patients was 57 years and 14% of the patients were over 70 years. The majority of patients had Rai stage I or II disease. After a median follow-up of six years, the overall response rate was 95% and the complete response rate was 72%, the highest response rates reported with any frontline regimen for CLL. The overall survival and failure-free survival rates at six years were 77% and 51%, respectively.[34] Compared with a historic group of patients who received frontline fludarabine-based regimens at MDACC, the complete response rate and overall survival appeared significantly better with FCR. Moreover, 78% of the patients achieving a complete response were also negative for minimal residual disease as assessed by flow cytometry,

Table 2 Studies exploring chemoimmunotherapy with rituximab in treatment-naïve chronic lymphocytic leukemia

Reference	Phase	N	Regimen(s)	OR (%)	CR (%)	Median survival (months)
Single-arm studies						
Keating et al[33] and Tam et al[34]	II	300	Fludarabine + Cyclophosphamide + Rituximab (FCR)	95	72	NR
O'Brien et al[36]	II	65	Fludarabine + Cyclophosphamide + Rituximab × 3 days (FCR3)	94	65	NR
Foon et al[37]	II	50	Fludarabine (↓20%) + Cyclophosphamide (↓40%) + Rituximab × 2 days (FCR-Lite)	100	77	NR
Kay et al[38]	II	64	Pentostatin + Cyclophosphamide + Rituximab (PCR)	91	41	PFS:33
Kay et al[39]	II	33	Pentostatin + Rituximab (PR)	76	27	TFS:16
Bosch et al[40]	II	67	Fludarabine + Cyclophosphamide + Mitoxantrone + Rituximab (FCMR)	93	82	NR
Faderl et al[47]	II	30	Fludarabine + Cyclophosphamide + Mitoxantrone + Rituximab (FCMR)	96	83	NR
Fischer et al[52]	II	117	Bendamustine + Rituximab (BR)	91	33	NR
Randomized Studies						
Byrd et al[27] and Woyach et al[28]	II	104	Fludarabine + Rituximab (FR) (Concurrent)	90	47	OS:84; PFS:32
			Fludarabine + Rituximab (FR) (Sequential)	77	28	OS:91; PFS:40
Hallek et al[35]	III	817	Fludarabine + Cyclophosphamide + Rituximab (FCR)	95	44	PFS:52; OS:NR
			Fludarabine + Cyclophosphamide (FC)	88	22	PFS:33; OS:NR

Abbreviations: N, evaluable patients; OR, overall response; CR, complete remission; OS, overall survival; PFS, progression-free survival; FFS, failure-free survival; TFS, treatment-free survival; NR, not reported or reached.

defined as CD5- and CD19-coexpressing cells less than 1%, with normalization of the kappa:lambda ratio (<3:1 in patients with monotypic kappa and >1:3 in patients with monotypic lambda). Minimal residual disease negativity was associated with superior survival (84% at six years versus 65% by flow cytometry positivity; $P = 0.001$). In addition, patients with some high-risk features and age 70 years or older were associated with inferior response rates. From the long-term follow-up, the rate of serious infections was highest in the first year of remission (10%) and declined rapidly to less than 1.5% per year by the third year. The occurrence of opportunistic infections was limited to the first year.[33] However, the incidence of dose reductions was significantly higher in patients older than 60 years and in patients with Rai stage IV disease.

These favorable results from MDACC prompted the German CLL Study Group (GCLLSG) to conduct a multicenter, international Phase III randomized trial (CLL8) comparing FCR with FC as frontline therapy for CLL.[35] The GCLLSG randomized 817 physically fit CLL patients to receive six monthly cycles of FC or FCR, using the same dosing regimen as the MDACC trial. The median patient age was 61 years and the majority of patients were Binet stage B or C. The interim report included 761 patients evaluable for response, 790 patients evaluable for progression-free survival, and all patients were evaluable for overall survival. After a median follow-up of 37.7 months, FCR yielded a higher overall response rate (95.1% versus 88.4%), higher complete response rate (44.1% versus 21.8%; $P < 0.001$) and longer progression-free survival (51.8 months versus 32.8 months; $P < 0.001$) compared with FC. Likewise, superior overall survival was observed with the FCR arm compared with the FC arm (84.1% and 79.0%; $P = 0.01$). The largest survival benefit after FCR treatment was seen in patients with Binet stages A and B. The FCR regimen was associated with more hemato-

logic adverse events, particularly neutropenia. However, this did not result in an increased infection rate. This was the first randomized trial demonstrating an overall survival advantage with chemoimmunotherapy. Although the MDACC and GCLLSG studies produced similar overall response rates, the complete response rate was lower in the GCLLSG study. The lower complete response rate in CLL8 than in the MDACC trial may be attributed to a difference in patient demographics. The patients in CLL8 were older and a smaller proportion of the patients in CLL8 were Binet stage A.

Improving on the fludarabine–cyclophosphamide–rituximab regimen

FCR-3 regimen

Despite the recent advances in the development of new treatment strategies, there is no evidence yet that these new and effective treatments are curative. Therefore, in an attempt to increase the activity of FCR and based on the dose-response data with rituximab in relapsed CLL patients,[16] investigators at MDACC increased the rituximab dose to three infusions per cycle (FCR-3, Table 2). O'Brien et al[36] treated 65 CLL patients with the FCR-3 regimen, which consisted of three consecutive days of IV fludarabine 25 mg/m^2/day, cyclophosphamide 250 mg/m^2/day, and rituximab 375 mg/m^2 as the first dose and rituximab 500 mg/m^2/day for all subsequent doses every 28 days for six cycles. In short, the trial failed to reveal any additional benefit by adding two additional daily doses of rituximab to FCR.

FCR-Lite regimen

Additionally, there still exists an elderly population (70 years and older) that may not be able to tolerate FCR. As summarized in Table 2, several investigators have explored modifications to the FCR regimen in an attempt to reduce toxicity, while maintaining or improving upon the excellent response rates reported by the MDACC experience. Another approach was to decrease the daily doses of fludarabine and cyclophosphamide by 20% and 40%, respectively, and increase the monthly exposure to rituximab. In a recent Phase II study by Foon et al[37] 50 treatment-naïve patients were treated with six cycles of FCR-Lite every four weeks. FCR-Lite consisted of IV fludarabine 20 mg/m^2/day and IV cyclophosphamide 150 mg/m^2/day for three consecutive days, days 2–4 during cycle 1, and days 1–3 during cycles 2–6. Rituximab 375 mg/m^2 was administered on day 1 of cycle 1 and 500 mg/m^2 was administered on day 14 of cycle 1 and days 1 and 14 in subsequent cycles. Following completion of six cycles, rituximab 500 mg/m^2 was given as maintenance therapy once every three months until relapse. Similar to the MDACC experience, high overall response (100%), complete response (77%), and minimal residual disease negative rates were observed with this regimen. The designed reduction in FC doses and use of granulocyte colony-stimulating factor most likely contributed to the reduction in Grade 3 or 4 neutropenia (13%). However, the impact of this regimen on survival has not been determined.

Pentostatin–cyclophosphamide–rituximab regimen

Also in pursuit of a less myelosuppressive regimen without sacrificing antileukemic activity, investigators at the Mayo Clinic and The Ohio State University replaced fludarabine in the FCR regimen with pentostatin (PCR). In a single-arm Phase II study,[38] 65 symptomatic and previously untreated CLL patients were treated with pentostatin 2 mg/m^2, cyclophosphamide 600 mg/m^2, and rituximab 375 mg/m^2 for one day every 21 days for six cycles. Of note, rituximab 100 mg/m^2 was given on day 1 and 375 mg/m^2 on days 3 and 5 during the initial cycle. Support from granulocyte colony-stimulating growth factor was provided. Sixty-four patients were evaluable in this study and 34 (53%) were Rai stages 3 or 4. The median age of the patients was 63 years, and 18% of the patients were 70 years or older. The overall response rate reported with PCR was 91%, the complete response rate was 41%, and median progression-free survival was 32.6 months. Similar to the FCR experience, patients who achieved minimal residual disease negativity by two-color flow cytometry had significantly longer survival, demonstrating the clinical benefit of acheiving MRD negative status. Grade 3 or 4 neutropenia and infections occurred in 16% and 2% of the cycles, respectively. The initial expectation with the PCR regimen was that the infectious complications would be less than with the FCR regimen. However, a randomized community-based trial in previously untreated or minimally pretreated patients reported a better complete response rate with FCR with a comparable overall response rate, cytopenias, and infectious complications.

Pentostatin–rituximab regimen

In an effort to maintain the response observed using PCR, and improve upon the tolerability of the pentostatin-based regimen, Kay et al[39] removed the cyclophosphamide and treated a small cohort of treatment-naïve patients (n = 33) with a higher dose of pentostatin 4 mg/m^2 and the same dose and schedule of rituximab as used in the PCR regimen. This regimen was well tolerated, with only 12% experiencing grade 3 hematologic

events and 15% with grade 3 or higher nonhematologic toxicity. The overall response rate was 76% with 9 patients achieving a complete response. Compared to the previously reported PCR, the PR regimen yielded inferior overall response and complete response rates and shorter treatment-free survival.

Fludarabine–cyclophosphamide–mitoxantrone–rituximab regimen

The addition of rituximab to combination chemotherapy consisting of fludarabine, cyclophosphamide, and mitoxantrone (FCM) was explored in a recent Phase II study.[40] Justification for this approach evolved from preclinical evidence demonstrating synergism between these agents,[42,43] efficacy of FCM in previously treated and untreated CLL patients,[41,44,45] and efficacy associated with rituximab-based chemoimmunotherapy in previous studies. In a multicenter Phase II trial, 77 previously untreated CLL patients (median age, 60 years) with active disease defined by National Cancer Institute-Working Group[46] received combination therapy consisting of rituximab 375 mg/m^2 in cycle 1, and 500 mg/m^2 in subsequent cycles, IV fludarabine 25 mg/m^2 for three days, IV cyclophosphamide 200 mg/m^2 for three days, and IV mitoxantrone 6 mg/m^2 on day 1 for a total of six cycles, followed by maintenance therapy with rituximab every three months in responders. With assistance from granulocyte colony-stimulating growth factors, 88% of the patients completed the full course of therapy. A high overall response of 93% and complete response of 82% were achieved, and 46% of the patients achieving a complete response were negative for minimal residual disease. Subgroup analysis of toxicity showed more severe neutropenic episodes and infectious episodes in patients aged 60–70 years.

In a second Phase II study, mitoxantrone was added to FCR and used as frontline therapy in a small cohort of 30 patients, aged less than 70 years and with a B2M $\leq$ twice the upper limit of normal.[47] Compared with a historic group of patients treated with FCR, similar overall response, complete response, and minimal residual disease-negative rates were achieved (96%, 83%, and 67% by flow cytometry, respectively). Thus, the benefit for adding mitoxantrone to the FCR regimen is not so evident from these studies.

Bendamustine–rituximab regimen

With the availability of new cytotoxic agents having activity in CLL, more opportunities to explore the benefit of adding rituximab are possible. Bendamustine (Treanda®; Cephalon, Inc, West Chester, PA) is an alkylating agent with cytotoxic properties similar to those of the purine nucleoside analogs. Single-agent bendamustine was approved in 2008 by the FDA for the treatment of CLL and rituximab-refractory non-Hodgkin's lymphoma. The efficacy of bendamustine as monotherapy for CLL has been demonstrated in several Phase I and II trials.[48,49] Based on in vitro evidence demonstrating synergy between bendamustine and rituximab in CLL cell lines,[50] and encouraging evidence of this combination in the relapsed and/or refractory CLL setting,[51] the GCLLSG investigated the bendamustine–rituximab (BR) regimen in treatment-naïve CLL patients. In a Phase II study,[52] BR was administered to 117 previously untreated CLL patients. The median age was 64 years, and nearly half the patients were Binet stage C. The BR regimen consisted of bendamustine 90 mg/m^2/day IV for two consecutive days every 28 days for six cycles and rituximab 375 mg/m^2 for the first cycle and 500 mg/m^2 for cycles 2–6. The overall response rate was 91%, with 33% (36 of 110 patients) of the patients experiencing a complete response. Although the response rate was lower than for FCR, fewer neutropenic events and infectious complications were observed. These promising findings prompted the GCLLSG to conduct an ongoing Phase III trial comparing BR with FCR in previously untreated CLL patients.[53]

Novel rituximab combinations for high-risk and elderly patients

Fludarabine–cyclophosphamide alemtuzumab–rituximab regimen

A subgroup analysis of patients with high-risk features in the FCR trial by MDACC revealed lower complete response rates, and shorter time to progression and overall survival.[34] With this in mind and the positive data from a previous study using cyclophosphamide, fludarabine, alemtuzumab, and rituximab (CFAR) in relapsed/refractory patients, investigators at MDACC explored the CFAR regimen in 60 previously untreated high-risk patients who had a 17p deletion or B2M level higher than twice the upper limit of normal (Table 3).[54,55] Frontline CFAR consisted of 200 mg/m^2 of IV cyclophosphamide and 20 mg/m^2 of IV fludarabine on days 3, 4 and 5, 30 mg of alemtuzumab IV on days 1, 3, and 5, and 375–500 mg/m^2 rituximab on day 2. Courses were repeated every 28 days for a total of six courses. CFAR achieved a 92% overall response and 70% complete response rate in this high-risk group. Notably, 52% (8/14) of the patients with a 17p deletion attained a complete response, but experienced a shorter time to progression compared with all evaluable patients (18 months versus 38 months, respectively). Similar rates of Grade 3/4 cytopenias and infections were seen with these patients when compared with a historic high-risk group

Table 3 Studies exploring novel combinations with rituximab in treatment-naïve high-risk or elderly chronic lymphocytic leukemia

Reference	Patient characteristics	N	Regimen(s)	OR (%)	CR (%)	Median survival (months)
Wierda et al[54] and Parikh et al[55]	Median age: 59 yrs Median B2M: 5.1 mg/L Rai stage III–IV: 51%	59	Cyclophosphamide + Fludarabine + Alemtuzumab + Rituximab (CFAR)	72	92	NR
Zent et al[62]	Median age: 61 yrs Rai stage 0–II: 100% 11q- or 17p-: 57% UM IgV_H + ZAP-70+ ± CD38+: 43%	30	Rituximab + Alemtuzumab (RA)	90	37	NR
Hillmen et al[63]	Median age: 70.5 yrs Binet C: 52%	47	Chlorambucil + Rituximab (CHl-R)	84	NR	NR
Castro et al[64]	Median age: 65 yrs Age > 70: 29% 11q- or 17p-: 14% UM IgVH: 53% ZAP-70+: CD38+: 32%	28	HDMP + Rituximab (HDMP-R)	96	32	PFS:30

Abbreviations: N, evaluable patients; B2M, beta2-microglobulin; UM, unmutated; IgVH, immunoglobulin heavy chain genes; ZAP-70, zeta chain-associated protein kinase 70 kDa; ZAP-70+, ≥ 20% expression; CD38+, ≥ 30% expression; –, indicates deletion; HDMP, high-dose methylprednisolone; OR, overall response; CR, complete remission; PFS, progression-free survival; NR, not reported or reached; yrs, years.

of patients who received FCR. The data suggest that CFAR is a highly active regimen as frontline therapy for high-risk CLL and deserves further exploration in the setting of a clinical trial to determine the long-term effects of this combination.

Alemtuzumab–rituximab monoclonal antibody regimen

Alemtuzumab (CAMPATH 1H; Genzyme, Cambridge, MA) is a humanized monoclonal antibody directed at the CD52 antigen that is expressed on both B lymphocytes and T lymphocytes. Several studies have demonstrated the effectiveness of single-agent alemtuzumab in the salvage setting[56,57] and as initial[58,59] therapy for CLL. Alemtuzumab has remarkable activity in CLL patients resistant to purine nucleoside analogs and harboring the p53 mutation which confers a poor prognosis.[60,61] Alemtuzumab is effective at clearing the bone marrow of disease, but has limited activity in clearing the bulky lymphadenopathy that can be accomplished with rituximab. By virtue of these complementary actions, a novel early treatment approach using these monoclonal antibodies in combination for CLL patients with high-risk features was explored in a single-center Phase II study. Investigators at the Mayo Clinic, Rochester, set out to determine if chemo-naive CLL patients harboring at least one of the high-risk biologic abnormalities, deletions 17p13, 11q22, or a combination of unmutated IgV_H and CD38 or ZAP70 positivity, would benefit from early intervention with alemtuzumab and rituximab rather than delaying therapy until disease progression.[62] Shown in Table 3, this Phase II single-center study treated 30 early-stage (Rai 0–II) high-risk patients who lacked an indication for treatment by National Cancer Institute-Working Group 1996 criteria,[46] with rituximab and alemtuzumab (RA) in combination. This monoclonal antibody combination consisted of a gradual dose escalation of alemtuzumab from 3 mg to 10 mg then 30 mg by subcutaneous injection daily over three days during the first week, followed by alemtuzumab 30 mg thrice weekly for four weeks. Beginning the second week of therapy, rituximab 375 mg/m^2 was administered weekly for a total of four weeks. All patients received prophylaxis for *Pneumocystis jirovecii* pneumonia, herpes simplex, and varicella zoster virus, and were monitored for cytomegalovirus reactivation. Twenty-seven patients (90%) responded to therapy and 11 patients (37%) experienced a complete response. Five patients with a complete response achieved minimal residual disease negativity by immunohistochemical analysis and flow cytometry. The median duration of response in the 27 responders was 14.4 months and the patients who achieved remissions with minimal residual disease negativity experienced the best response durations. Compared with a historic cohort of untreated patients who were similar in age, clinical Rai stage, and risk features, the median time from diagnosis to treatment was longer for patients who received RA than for the untreated control group (4.4 years and 1.9 years, respectively; $P = 0.001$). Interestingly, the patients who progressed were tested for clonal selection or

evolution and found to have no evidence of new aggressive clones, which suggests that early treatment with monoclonal antibodies may not contribute to disease resistance or limit future treatment options.

Chlorambucil–rituximab regimen

Despite the advancements in the treatment of CLL, an elderly population unable to tolerate intensive cytotoxic therapy still exists and chlorambucil remains a viable treatment option. To improve upon the response rates demonstrated by chlorambucil alone in previous trials,[2–4] Hillman et al[63] recently treated 100 elderly patients (median age 70.5 years) with a combination regimen consisting of chlorambucil and rituximab. Chlorambucil 10 mg/m^2/day was administered orally for the first seven days of each month with rituximab 375 mg/m^2 on the first day of course 1 followed by rituximab 500 mg/m^2 on the first day of each subsequent course. Each course was repeated every 28 days for six total courses and responding patients were permitted to continue six additional cycles of chlorambucil monotherapy. Summarized in Table 3, the interim intent-to-treat analysis on 50 patients, 47 of whom were evaluable for response, revealed an overall response rate of 84%. Compared with a similar group who received chlorambucil alone in the LRF CLL4 trial, the patients treated with chlorambucil and rituximab demonstrated a 17.3% higher overall response rate. Grade 3 or 4 neutropenia was still observed in 40% of the patients.

High-dose methylprednisolone–rituximab regimen

Frontline use of rituximab in combination with high-dose methylprednisolone 1 gm/m^2 has also been studied in an elderly population with a median age of 65 years. The administration of both agents for three consecutive days for three cycles every four weeks has been reported to have an overall response and complete response rate of 96% and 32%, respectively, in a trial involving 28 patients.[64] Two patients achieved MRD-negative bone marrows by four-color flow cytometry. Regardless of MRD status, patients who achieved a CR experienced a longer median PFS than those who did not acheive a CR (40.3 months and 23.9 months, respectively). Interestingly, patients with high-risk features such as elevated ZAP-70 expression and CD38, unmutated IGVH, unfavorable cytogenetics, and bulky lymphadenopathy achieved similar response rates as those who did not have these disease features. All patients older than 70 years responded and three patients achieved a complete response. Hyperglycemia, fatigue, sinusitis, and dyspepsia were the most common adverse events. Of particular interest is the encouraging clinical activity of this regimen and its favorable toxicity profile that makes it a potential treatment option for older patients not eligible for more aggressive approaches.

Tolerability of rituximab

Early Phase I and II studies using rituximab in CD20-positive B cell lymphomas established the activity and toxicity potential of rituximab.[12,65] In a pivotal trial consisting of single-agent rituximab in indolent lymphomas, rituximab was shown to be generally well tolerated.[11] The majority (96%) of adverse events were Grade 1 or 2 infusion-related reactions and occurred during the first infusion. However, over half of the patients remained free of adverse events with the second and subsequent infusions[11] The majority of infusion-related reactions consisted of fever, chills, and rigors, and less commonly, hypotension and bronchospasm. Some studies suggest that cytokine release may be partially responsible for infusion-related reactions and a high number of circulating lymphocytes is predictive of an infusion-related reaction.[66–68] However, subsequent studies have shown no correlation between high circulating lymphocytes and infusion-related reactions.[14,19] Regardless, management of infusion-related reactions begins with prevention. Premedicating rituximab with acetaminophen and an antihistamine will reduce the incidence and severity of infusion-related reactions. For patients who appear to be at high risk for infusion-related reactions, a steroid such as hydrocortisone can be administered in addition to the other premedications.

Combining rituximab with chemotherapy has been extensively studied in CLL. Several randomized studies have shown that the pattern of adverse events of rituximab-based chemoimmunotherapy is broadly similar to the comparator regimen with the exception of higher Grade 3 or 4 neutropenia that was not associated with an increased risk of infection.[27,35]

Tumor lysis syndrome is also a well documented toxicity which generally affects patients with high tumor burden and/or high circulating tumor cells and occurs within the first 24 hours of the first treatment with rituximab.[68,69] This syndrome can manifest as renal failure, hyperuricemia, hyperkalemia, and/or hyperphosphatemia, and patients require close monitoring, adequate hydration, correction of electrolyte abnormalities, and dialysis as needed. Prior to starting treatment, patients should be well hydrated, receive prophylaxis with allopurinol or rasburicase, and be observed closely.

Other rare but serious toxicities include hypersensitivity pneumonia, mucocutaneous reactions, and hepatitis B virus reactivation. Case reports of fatal reactivation of hepatitis B virus in patients with B cell malignancies following rituximab

therapy have been described.[70–72] Therefore, patients should be screened for hepatitis B virus prior to starting rituximab, and rituximab should be discontinued in patients who develop viral hepatitis. Prophylaxis against hepatitis B virus reactivation should be considered for those with evidence of past exposure. Although rare, a recent report describes 57 cases of progressive multifocal leukoencephalopathy following therapy with rituximab.[73] Multifocal leukoencephalopathy is a demyelinating disease of the central nervous system caused by the reactivation of JC polyoma virus. The mechanism behind the reactivation of virus following rituximab therapy is not known. Currently, there is no satisfactory treatment for the disease and most cases are fatal.

Tolerability of rapid infusion rituximab

Due to the potential for infusion-related reactions as described earlier, rituximab was approved to be administered via slow IV infusion (rate escalation schedule) over 5–6 hours for the initial infusion and over 3–4 hours for subsequent infusions as tolerated. The approved administration time is inconvenient for patients and incurs high resource demands for infusion centers. Therefore, over the past five years, many institutions have evaluated the safety and tolerability of a shorter infusion time, ie, 60- or 90-minute infusions, for second and subsequent rituximab cycles. Studies administering rituximab over a total of 90 minutes (20% of the dose given over 30 minutes and the remaining 80% over 60 minutes, with standard premedications with or without corticosteroids) elicited only Grade 1 infusion-related toxicities and no Grade 3 or 4 toxicity.[74,75] Studies that evaluated the safety and tolerability of a 60-minute rituximab infusion (total dose given over 60 minutes, with standard premedications and corticosteroids) also proved to be safe and tolerable, with no Grade 3 or 4 toxicities.[76,77] In addition to the published studies, a large multicenter Phase III trial evaluating the safety of a 90-minute rituximab infusion in patients with previously untreated diffuse large B cell or follicular non-Hodgkin's lymphoma is ongoing. The results of these studies suggest that rapid infusion of rituximab at 375 mg/m^2 is safe and tolerable. While many institutions may have adopted the rapid infusion of rituximab into their current practice, several questions regarding the indications and timing for rapid infusions, eligible patient populations, and the impact on response rates have not been fully answered.

Conclusion

The introduction of rituximab greatly expands the treatment options for patients diagnosed with CLL. Single-agent rituximab showed modest activity in previously treated and untreated CLL, but is a therapeutic option for patients unfit for fludarabine-based regimens because of advanced age and/or poor performance status. Several large randomized trials comparing fludarabine with FC demonstrated significantly improved response rates, progression-free survival, and treatment-free survival with FC,[29–31] but a demonstrable improvement in overall survival remained elusive until Keating et al combined rituximab with FC (ie, FCR). Using the FCR regimen, the GCLLSG demonstrated an overall survival advantage over FC and established a new standard of care for patients under the age of 65 years. However, similar success in biologic unfit patients remains to be a challenge.[33]

Additionally, we have also seen important progress in understanding the biology of CLL. This progress has been seen in the development of clinical staging systems and identification of complex genetic aberrations indicative of high-risk disease using immunologic, cytogenetic, and molecular techniques. While the watch and wait paradigm is still the standard, the benefit of early intervention in high-risk patients needs further exploration, especially in patients with 17p deletions. Patients harboring a 17p deletion are particularly hard to treat, given the poor responses and poor survival observed with single-agent rituximab and FCR. Overall, rituximab has contributed markedly to improved outcomes in CLL and has changed the way CLL is viewed and treated.

Disclosure

The authors report no conflicts of interest in this work.

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