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Role of CD20 Monoclonal Antibodies in Previously Untreated Chronic Lymphocytic Leukemia

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Abstract

Monoclonal antibodies (mAbs) directed against the CD20 antigen on B cells have dramatically altered the treatment landscape for patients with chronic lymphocytic leukemia (CLL). Rituximab, a chimeric mouse/human mAb, was the first antibody to be approved for treatment of indolent B cell lymphomas. Although single-agent, standard dose rituximab has limited activity as frontline therapy for patients with CLL, it has synergistic therapeutic activity when combined with chemotherapy. Indeed, chemoimmunotherapy with combined fludarabine (F), cyclophosphamide (C) and rituximab (R) was shown to improve both progression-free and overall survival in a randomized Phase 3 clinical trial compared to FC in previously untreated patients with CLL. In this article, we review important clinical trials that incorporated rituximab with other agents for treatment-naïve patients with CLL. We also highlight second and third generation CD20 mAbs approved or in development for treatment of CLL.

Keywords

CD20; monoclonal antibody; chemoimmunotherapy; rituximab

INTRODUCTION

The advent of monoclonal antibodies (mAbs) has revolutionized the treatment landscape for patients with B cell malignancies. Rituximab, a chimeric mouse/human mAb against the CD20 antigen, is the first and arguably the most important addition to the therapeutic armamentarium against Non-Hodgkin's lymphoma (NHL) and more recently against chronic lymphocytic leukemia (CLL). The addition of rituximab to cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) is considered the standard treatment for patients with aggressive NHL.¹ This review outlines the rationale and clinical studies of rituximab in previously untreated patients with CLL. It also highlights some of the newer mAbs that are approved or in development for treatment of CLL.

CD20 AS A THERAPEUTIC TARGET

CD20 is a transmembrane phosphoprotein expressed on B cells through most stages of development; but is not expressed on stem cells, plasma cells, or other lymphoid lineages.² The precise function of CD20 is not known; although it is thought to be involved in B cell activation, regulation of B cell growth and transmembrane calcium flux.³ CD20 is an appealing target by virtue of not being internalized or down-regulated following binding of mAbs.^{3,4} Upon intravenous administration, rituximab binds to CD20 expressed on B cells, marking these cells for elimination through multiple mechanisms – including direct induction of apoptosis, complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and clearance by the reticuloendothelial system.^{3,4}

SINGLE-AGENT RITUXIMAB

Rituximab was initially tested in Phase 1 clinical trials as single-agent at doses ranging from 125-375 mg/m² intravenous (IV) weekly for 4 weeks. No dose-limiting toxicity was observed at up to 375 mg/m², the dose that was feasible for clinical trial given available drug supply, and therefore this dose was selected for further testing.⁵ In a pivotal Phase 2 study that led to Food and Drug Administration (FDA) approval of rituximab, a dose of 375 mg/m² weekly for 4 weeks was administered to 166 patients with relapsed or refractory indolent NHL.⁶ The overall response rate (ORR) was 60%; however, only 4 (13%) of the 30 patients with CLL responded. Several subsequent clinical trials reported no complete remissions (CR) and an ORR of 10%-35% for standard dose rituximab in relapsed or refractory patients with CLL.⁷⁻⁹

Studies evaluating the efficacy and safety of single-agent rituximab in previously untreated patients with CLL are limited (Table I). In a Phase 2 study of 44 patients with previously untreated CLL, rituximab was administered at a dose of 375 mg/m² IV weekly for 4 consecutive weeks.¹⁰ The ORR was 51%; 4% patients achieved CR. Of the 28 patients with stable or responsive disease, rituximab was continued as maintenance therapy with 4 weekly courses every 6 months for a total of 2 years. This improved the ORR to 58% with a CR rate of 9%. After a median follow-up of 20 months, the median progression-free survival (PFS) was 19 months. In another study, rituximab 375 mg/m² IV weekly for 8 weeks was administered to 31 patients with early stage, treatment-naïve CLL. The ORR was 90%, CR was achieved by 19% patients and the median PFS was 43 months.¹¹

The mechanism accounting for the differential efficacy of single-agent, standard dose rituximab in patients with NHL versus CLL remains to be documented. A high level of CD20 expression is seen on malignant B cells from patients with follicular lymphoma, mantle cell lymphoma and hairy cell leukemia, while leukemic B cells from patients with CLL have significantly lower levels.¹²⁻¹⁵ This may render CLL cells less susceptible to rituximab. Another factor may be the presence of circulating CD20 molecules in patients with CLL. Circulating CD20 representing membrane fragments from CLL cells potentially adversely affects the pharmacokinetics of rituximab. Of note, a higher level of circulating CD20 was associated with significantly shorter survival time in patients with CLL.¹⁶ Higher doses of rituximab may overcome these circulating CD20 binding sites and lead to improved

responses. A dose-intense (weekly doses of 500 mg/m² to 2250 mg/m² for 4 weeks) regimen of single-agent rituximab was tested in 50 relapsed patients with CLL (n=40) or other mature B cell lymphoid malignancies (n=10).¹⁷ No CR's were noted and the ORR was 40%. Among patients with CLL, response was correlated with dose: 22% for patients treated at 500 to 825 mg/m² (n=24), 43% for those treated at 1,000 to 1,500 mg/m² (n=7), and 75% for those treated at the highest dose of 2,250 mg/m² (n=8). In a dose-dense (thrice weekly 375 mg/m² for 4 weeks) regimen of single-agent rituximab administered to 33 patients with CLL, CR was reported in 3% and the ORR was 43%.¹⁸ These studies showed that although single-agent rituximab has significant clinical activity in CLL, it was not sufficient to induce CR or produce durable responses.

CHEMOIMMUNOTHERAPY

Chemoimmunotherapy combines chemotherapy with mAbs, such as rituximab, and represents a significant advance in the treatment of patients with CLL (Table II). Observations that rituximab could sensitize malignant B cells to cytotoxic compounds, such as purine analogs, provided preclinical rationale to combine these agents. In the randomized Phase 2 Cancer and Leukemia Group B (CALGB) 9712 study, 104 patients with previously untreated CLL received fludarabine and either concurrent or sequential rituximab therapy (FR regimen).¹⁹ Both arms received fludarabine 25 mg/m² on days 1-5 every 4 weeks for 6 courses. In the sequential arm, rituximab 375 mg/m² was administered weekly for 4 doses beginning 2 months after the completion of fludarabine. In the concurrent arm, rituximab 375 mg/m² was given on days 1 and 4 of the first course and on day 1 for courses 2-6, followed by the same dose weekly for 4 weeks 2 months after the completion of fludarabine therapy. Superior CR rate (47% vs. 28%) was observed for the concurrent versus sequential arm. With long-term follow-up, the median PFS and OS were not different between the two arms (although not powered to identify differences), and for the combined group were 37 months and 85 months, respectively.²⁰ In a historical comparison with 179 previously untreated patients with CLL who received fludarabine 25 mg/m²/day as a single-agent on the CALGB 9011 study, the rates of CR (20% vs. 38%), ORR (63% vs. 84%), 2-year PFS (45% vs. 67%) and 2-year overall survival (OS, 81% vs. 93%) were superior for patients treated with FR.²¹ In a similar study from Europe, fludarabine 25 mg/m²/day was administered on days 1-5 every 4 weeks for 6 courses in combination with rituximab 375 mg/m² on day 1 of courses 3-6 to 31 patients with CLL (20 previously untreated and 11 relapsed).²² A CR of 25% and ORR of 85% was achieved in those patients who were previously untreated.

The combination of fludarabine with the alkylating agent cyclophosphamide was shown to have *in vitro* synergy against CLL cells.²³ A CR rate of 35% and ORR of 88% was reported for the FC regimen in 34 previously untreated CLL patients at the M.D. Anderson Cancer Center (MDACC).²⁴ Rituximab was added to fludarabine and cyclophosphamide (FCR regimen) and evaluated in 300 previously untreated patients at MDACC.^{25,26} The FCR regimen consisted of rituximab 375 mg/m² on day 1 followed by fludarabine at 25mg/m²/day and cyclophosphamide at 250mg/m²/day on days 2-4 for course 1. Five more courses consisting of rituximab 500 mg/m² on day 1, fludarabine 25 mg/m² and cyclophosphamide 250 mg/m² on days 1-3 were given every 4 weeks to complete a total of

6 courses. With this regimen, all patients received tumor lysis prophylaxis with hydration on day 1 of course 1 and allopurinol daily for the first 2 weeks of course 1. Antibiotic prophylaxis for herpes viruses and *Pneumocystis jirovecii* was not mandated nor was neutrophil growth factor support. Neutrophil growth factor support and/or FC dose reduction was allowed for elderly (age > 65 yrs) and patients who experience grade 3 neutropenia. The ORR was 95%, with CR in 72%, nodular partial remission (nPR) in 10% and partial remission (PR) in 13%. Six-year OS was 77% and the estimated median time-to-progression among responders was 80 months. Pretreatment characteristics independently associated with inferior response were age ≥ 70 years, serum beta-2 microglobulin (β2M) ≥ twice the upper limit of normal, white cell count ≥ $150 \times 10^9/L$, deletion 17p, and serum lactate dehydrogenase ≥ twice the upper limit of normal. FCR therapy was the strongest independent predictor for survival among all patients who received frontline fludarabine-based therapy at MDACC. Grades 3 and 4 neutropenia occurred in 24% and 28% courses, respectively; and grades 3 and 4 thrombocytopenia occurred in 4% and less than 1% of courses, respectively. Despite the significant incidence of neutropenia, major infections (pneumonia and sepsis) occurred in 2.6% courses, and minor infections (fever of unknown origin, upper respiratory infection, urinary tract infection and cellulitis) occurred in 10% courses. The risk of serious or opportunistic infections was 10% and 4% during the first and second years of remission, respectively.

The CR rate, ORR and PFS in the MDACC Phase 2 FCR study are the best reported in the literature to date and formed the basis of a randomized Phase 3 study, CLL8 trial, performed by the German CLL Study Group (GCLLSG). CLL8 was a randomized open-label, multicenter Phase 3 study comparing FCR (n=409) versus FC (n=408) in previously untreated patients with CLL. Both groups received fludarabine at 25 mg/m^2 and cyclophosphamide at 250 mg/m^2 on days 1-3 every 28 days for a total of 6 courses.²⁷ Rituximab at 375 mg/m^2 on day 1 for the first course and 500 mg/m^2 on day 1 for courses 2-6 was administered to patients randomized to the FCR arm. Prophylactic antibiotics and growth factor support was not mandated in this study. Five percent patients were Binet stage A, 64% Binet B and 32% Binet C. With a median follow-up time of 37 months, the estimated median PFS was 52 months for the FCR arm compared to 33 months for FC (p-value <0.001, hazard ratio 0.56), meeting the primary objective of the trial. The FCR regimen was associated with superior CR rate (44% vs. 22%) and ORR (95 vs. 88%) compared to FC. Importantly, statistically significant improvement in OS was also observed for FCR – with 84% patients alive in the FCR arm compared to 79% in the FC arm at 38 months. Interestingly, the largest benefit was observed in Binet Stage A and B patients. This is in contrast to work from MDACC, demonstrating improved outcomes with FCR including for patients with Rai high-risk disease in historic comparisons. Age, sex, FCR treatment, response to therapy, number of courses received, 17p deletion, increased level of serum thymidine kinase and β2M were independent prognostic factors predicting PFS and OS in the CLL8 trial. Grade 3/4 neutropenia was higher with FCR compared with FC (33.7% vs. 21.0%; p<0.0001); but this was not associated with increased incidence of grade 3/4 infection (18.8% vs. 14.9%; p=0.14).²⁸

Weiss and colleagues developed the PCR regimen consisting of pentostatin 4mg/m², cyclophosphamide 600 mg/m² and rituximab 375 mg/m² all given on day 1 every 21 days for a total of 6 courses.²⁹ All patients routinely received neutrophil growth factor with each course of treatment. A CR rate of 25% and an ORR of 75% was reported in previously treated patients with CLL. Investigators at the Mayo Clinic and Ohio State University evaluated a modified PCR regimen: pentostatin 2 mg/m² with cyclophosphamide 600 mg/m² and rituximab at 375 mg/m² on day 1 every 21 days for up to 6 courses in 64 previously untreated patients with CLL.³⁰ Rituximab 100 mg/m² was given on day 1 and 375 mg/m² on days 3 and 5 for course 1 only. All patients received prophylactic antibiotics and filgrastim with each course of therapy. Forty-one percent patients achieved CR, 22 % nPR with an ORR of 91%. Eighteen patients (28%) were older than 70 years and their CR and ORR rate of 39% and 83% were similar to patients younger than 70. Of the 3 (5%) patients who had 17p deletion by fluorescence *in situ* hybridization (FISH), none achieved CR or nPR. Twenty-six patients (41%) had grade 3 or 4 neutropenia and 13 patients (21%) had grade 3 or 4 thrombocytopenia. Six patients (9%) developed an infection and 4 (6%) had non-neutropenic fever during the study.

A randomized Phase 2 study to compare the rates of infection between PCR (21-day course: pentostatin 4mg/m², cyclophosphamide 600mg/m², rituximab 375mg/m² all on day 1) and FCR (28-day course: fludarabine 20mg/m²/day on days 1–5, cyclophosphamide 600mg/m² on day 1, rituximab 375mg/m² day 1) as frontline therapy was conducted in 184 previously untreated patients with CLL.³¹ Neutrophil growth factor and antibiotic prophylaxis were not routine on this study. Infection rates and hospitalizations were similar between PCR and FCR. CR was achieved in 17% patients treated with FCR versus 7% in patients treated with PCR, while the ORR in FCR was 57% compared to 45% in PCR. The reason for these low CR rates is unclear; however, the doses and schedules of treatment and supportive measures in this study were different from previous Phase 2 and 3 trials.

CHEMOIMMUNOTHERAPY WITH MODIFICATIONS OF THE FCR REGIMEN

Several Phase 2 trials examined modifications to the FCR regimen in an effort to improve tolerability and maintain durable responses. In a trial using the dose-intense schedule of rituximab, fludarabine 25mg/m²/day, cyclophosphamide 250 mg/m²/day and rituximab 500mg/m² were all given on days 1-3 every 4 weeks for 6 courses in 65 previously untreated patients (FCR3 regimen). The CR rate and ORR of 65% and 94%, respectively, were not significantly better than those reported by MDACC for standard FCR.³² FCR-lite is another modification where reduced dose fludarabine and cyclophosphamide was administered to 50 previously untreated patients with CLL.³³ Fludarabine 20 mg/m² and cyclophosphamide 150 mg/m² both given on days 2-4 of the first course and days 1-3 during courses 2 through 6. Rituximab 375 mg/m² was given on day 1 then 500 mg/m² on day 14 for course 1. Rituximab 500 mg/m² was given on day 1 with an additional dose of 500 mg/m² on day 14 for courses 2 though 6. Maintenance rituximab was also administered to all patients at a dose of 500 mg/m² every 3 months until relapse. A CR rate of 77% and ORR of 100% was reported. Grade 3/4 neutropenia was associated with 13% courses, which is lower than that for frontline FCR-based regimen. However, 81% patients in this study were Rai intermediate risk and only 4 patients were older than 70 years of age, which might

contribute to the excellent responses observed. Also, only 3 patients (6%) had 17p deletion by FISH, none of whom achieved a CR or nPR. In another modification, mitoxantrone was added to the FCR regimen (FCMR regimen) in 30 previously untreated patients with CLL who were less than 70 years of age with a $\beta 2M$ less than twice normal³⁴. Mitoxantrone 6mg/m² was given on day 2 of course 1 and then day 1 of courses 2–6, with standard doses and schedule for FCR. All patients routinely received pegfilgrastim with each course. CR was achieved in 83%, nPR in 10%, and PR in 3%, giving an ORR of 96%. With a median follow up of 38.5 months (range 17– 45+ months), the estimated median PFS was not reached. However, these results were not significantly different from standard FCR in historic comparison. Grade 3/4 neutropenia and thrombocytopenia was observed in 67% and 7% patients, respectively. In a similar study, FCMR was administered in a slightly different schedule to 72 patients (age <70 years) with previously untreated CLL.³⁵ Fludarabine 25mg/m²/day and cyclophosphamide 200 mg/m²/day were given on days 1-3 with rituximab 500 mg/m² (375 mg/m² on day 1 for course 1) and mitoxantrone 6 mg/m² was given on day 1. Courses were every 4 weeks for 6 total courses. All patients routinely received neutrophil growth factor support with each course. All patients who achieved a response received maintenance with rituximab 375 mg/m² every 3 months for 2 years. The CR rate was 82% and PR 11%, giving an ORR of 93%. Grade 3/4 neutropenia and thrombocytopenia were observed in 13% and 2% patients, respectively. Major and minor infections were reported in 8% and 5% courses, respectively. Advanced clinical stage, 17p deletion and an increased $\beta 2M$ correlated with a lower CR rate.

RITUXIMAB COMBINATION THERAPY IN HIGH-RISK PATIENTS

Alemtuzumab is the mAb that targets CD52, an antigen ubiquitously expressed by lymphocytes. The rationale for combining rituximab and alemtuzumab is as follows³⁶: 1) both mAbs target different targets on the surface of the malignant B cells; 2) rituximab is effective at depleting the lymph nodes of CLL cells compared to alemtuzumab, which is highly effective at depleting CLL cells from blood and bone marrow; 3) potential for dose-reduction of both antibodies to maximize efficacy and reduce toxicity and 4) potentially enhance activity for patients with high-risk features, particularly 17p deletion or *TP53* gene mutation.

Rosen and colleagues studied the combination of alemtuzumab (given to 3 cohorts of patients in escalating doses of 3mg, 10 mg and 30 mg IV thrice a week for weeks 2-5) and rituximab 375 mg/m² weekly for 4 weeks in 14 previously treated patients with CLL.³⁷ One patient attained PR and all other patients had stable disease lasting a median of 101.5 days. There were no treatment related deaths and no patient had reactivation of CMV. Investigators at the Mayo Clinic administered alemtuzumab and rituximab to 30 previously untreated patients who did not meet indications for therapy by National Cancer Institute-Working Group (NCI-WG) criteria, but had 1 or more of the following high-risk features: 17p deletion or *TP53* gene mutation, 11q22–, or a combination of unmutated immunoglobulin heavy chain (*IGHV*) and CD38+/- Zeta-chain-associated protein kinase 70(ZAP70)+.³⁸ Patients received subcutaneous (SC) alemtuzumab with dose escalation (3 mg, 10 mg, 30 mg) over the first 3 days and then 30 mg SC per day thrice weekly for the next 4 weeks. Rituximab 375 mg/m² was started on day 8 and was repeated weekly for a

total of 4 doses. All patients routinely received prophylaxis for herpes simplex, *Pneumocystis jirovecii* and cytomegalovirus. Thirty-seven percent patients achieved CR and the ORR was 90%. The median duration of response was 14.4 months. Grade 3/4 neutropenia was noted in 17% patients and no patient developed grade 3/4 thrombocytopenia.

Alemtuzumab 30 mg IV on days 1, 3 and 5 combined with fludarabine (20 mg/m²/day on days 3-5), cyclophosphamide (200 mg/m²/day on days 3-5) and rituximab (375 mg/m² on day 2) (CFAR regimen) was administered every 4 weeks for a total of 6 courses to 60 previously untreated high-risk ($\beta 2M > 4\text{mg/L}$) patients younger than 70 years with CLL at MDACC.^{39,40} Pegfilgrastim was routinely given for all courses and all patients also received prophylaxis for herpes simplex, *Pneumocystis jirovecii* and cytomegalovirus. The CR rate was 72%, nPR was 3%, PR was 18% and an ORR of 92% was reported. After a median follow-up of 25 months, the estimated median PFS of 38 months was not significantly different from a historic comparison with high-risk patients treated with FCR. Grade 3/4 neutropenia and thrombocytopenia were observed in 33% and 13% patients, respectively, which were not significantly different from historic high-risk patients treated with FCR at MDACC. Interestingly, of the 14 (23%) patients with 17p deletion by FISH, 8 (57%) achieved CR, the highest rate reported for any FCR-based frontline treatment to date. However, the median PFS of 15 months for this subgroup was not superior to historic patients with 17p deletion treated with FCR-based regimens.

COMBINATION OF RITUXIMAB WITH OTHER AGENTS

A number of new agents have been used in combination with rituximab to improve responses in patients with treatment-naïve CLL. Bendamustine combined with rituximab has synergy *in vitro*.⁴¹ The GCLLSG conducted a Phase 2 study in 117 previously untreated patients with CLL who received bendamustine 90 mg/m² on days 1 and 2 with rituximab 375 mg/m² on day 1 (500 mg/m² for subsequent courses) (BR regimen) every 4 weeks for a total of 6 courses.⁴² The CR rate was 33% and ORR was 91%. Grade 3/4 neutropenia and thrombocytopenia occurred in 7% and 6% of all courses, respectively. With a median follow-up of 18 months, the PFS was not reached. However, none of the 7 patients with 17p deletion achieved CR. In an effort to develop a less myelosuppressive regimen with potential for activity in high-risk patients like those with 17p deletion, high-dose methylprednisolone 1 gm IV daily for 3 days was combined with either rituximab given weekly for 12 weeks at 375mg/m² or at 750mg/m² in 9 doses over 12 weeks to 46 chemotherapy-naïve patients with CLL.⁴³ ORR was 96% with CR achieved by 32% of patients. No patient developed grade 3/4 neutropenia or thrombocytopenia. Rituximab (375 mg/m² for the first course followed by 500 mg/m² for courses 2-6) was combined with an older agent, chlorambucil 10 mg/m²/day given orally on days 1-7 to 50 treatment-naïve patients with CLL.⁴⁴ Six more courses of chlorambucil were permitted in patients with continuing clinical response at the end of the first 6 courses of therapy. The median age of patients was over 70 years. In an early interim analysis, the ORR was 84%, which was significantly better than historic single-agent chlorambucil. Grade 3/4 neutropenia occurred in 40% patients.

TOLERABILITY OF RITUXIMAB

With over 1 million patients worldwide having received rituximab for various diagnoses, the safety profile is well established. The most common toxicities associated with rituximab include infusion-related reactions (IRR), myelosuppression and infectious complications.⁴⁵ Premedication consisting of acetaminophen and an antihistamine should be administered before each infusion of rituximab. Corticosteroid premedication should be given for the first infusion. High-dose methylprednisolone (60-80mg IV) should be given for patients at high risk for IRR's like those with high (e.g. ALC >100K/ μ L) leukemia count or large bulk of disease with slow first infusion. Leukapheresis should be considered prior to first infusion for patients with ALC $\geq$ 200K/ μ L. These authors do not split first dose but prefer to give high-dose corticosteroids and slow infusion with first dose. Most IRR's are typically mild (grade 1-2) and include flu-like symptoms such as fever, chills and rigors. Less common reactions include hypotension, bronchospasm, pruritus and rash. These are easily treated with a reduction in the rate of infusion and administration of antihistamines and corticosteroids. The frequency and severity of IRR's decrease with subsequent infusions of rituximab. Some patients (<0.1%) experience severe IRR, either a cytokine release syndrome or a true hypersensitivity reaction to rituximab. For these patients, it is imperative to stop the infusion immediately followed by supportive care measures such as systemic corticosteroids, oxygen, intravenous fluids and vasopressors. For these patients, corticosteroids may be less effective at attenuating symptoms and if rechallenge results in severe reaction (grade >3) they should not be subsequently challenged with rituximab.

In the pivotal study of rituximab monotherapy, grade 3/4 thrombocytopenia and neutropenia were reported in only 1.7% and 4.2% of patients, respectively; severe anemia was reported in 1.1% of patients.⁶ Although the rates of grade 3/4 hematologic toxicity are higher in combination therapy with purine analogs or other traditional chemotherapy agents, these have not translated into a higher rate of infectious complications.⁴⁵ Human anti-chimeric antibodies rarely develop following administration of rituximab, but have no apparent impact on clinical outcome or toxicity. Reactivation of hepatitis B virus (HBV) can be associated with rituximab and other immunosuppressive treatments for CLL; therefore all patients should be screened for hepatitis B surface antigen (HBsAg), anti-hepatitis B surface (HBsAb) and core antibody (HBcAb) prior to therapy.⁴⁶ In HBsAg⁺ patients, baseline quantitative PCR for HBV DNA should be obtained. Therapy for HBV is recommended for all individuals with acute and active HBV infection (HBsAg⁺, HBsAb⁻, HBcAb⁻) and rituximab should not be given. Patients with chronic HBV infection (HBsAg⁻, HBsAb⁻, HBcAb⁺) should receive prophylactic lamivudine or equivalent during rituximab therapy. Prior HBV vaccination (HBsAg⁻, HBsAb⁺/HBcAb⁻) in previously uninfected individuals is not a risk for HBV reactivation. Pure red cell aplasia, hemolytic anemia, Stevens-Johnson syndrome, prolonged pancytopenia and marrow hyperplasia have rarely been reported during post-marketing surveillance. Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease of the central nervous system that results from reactivation of latent JC polyoma virus. Fifty-seven cases of PML were described in a recent study by the Research on Adverse Drug Events and Reports project in human immunodeficiency virus negative patients who received rituximab.⁴⁷ The median

time from last rituximab dose to the diagnosis of PML was 5.5 months and the case fatality rate was 90%.

NEXT GENERATION CD20 ANTIBODIES

Given the immense therapeutic success of rituximab in patients with NHL and other lymphoid malignancies (including CLL), there has been an explosion in the field of newer mAbs directed against CD20. Based on mechanism of action, anti-CD20 mAbs can be divided into 2 types: type 1 mAbs, such as rituximab, in which the primary mechanism of action is a combination of CDC and ADCC, in contrast to type 2 mAbs, such as GA101, in which the primary mechanism of action is direct induction of apoptosis and little CDC or ADCC.⁴⁸ The newer antibodies are fully human or humanized IgG1 molecules to decrease immunogenicity and potentially side effects, including anaphylactic reactions.^{49,50} A complete listing of the newer mAbs with their mechanisms of action *in vitro* is shown in Table III.

Ofatumumab (HuMax CD20) is a fully human mAb that binds to a distinct epitope of the CD20 antigen. This type 1 mAb exhibits greater complement binding and *in vitro* is more efficient at CDC than rituximab. In a multi-center Phase 2 study, fludarabine 25 mg/m²/day on days 1-3 and cyclophosphamide 250 mg/m²/day on days 1-3 were administered with either 500 mg ofatumumab (n=31) or 1000 mg ofatumumab (n=30) on day 1 in previously untreated patients with CLL (OF-C regimen).⁵¹ This treatment schedule was repeated every 4 weeks for a planned total of 6 courses. The CR rate was 32% and 50%, and ORR was 77% and 73%, respectively for the low-dose and high-dose ofatumumab groups. Overall, neutropenia was reported in 48% and thrombocytopenia in 15% patients. The rates of CR and ORR for this multicenter, international study were lower compared to the Phase 2 MDACC FCR report, but comparable to those achieved by patients randomized to the FCR arm in the CLL8 trial reported by the GCLLSG. The median follow-up of 8 months for patients treated with the OF-C regimen precludes a meaningful comparison of survival data between these treatment regimens. The optimal dose of ofatumumab in chemoimmunotherapy regimens remains an area for investigation. Ofatumumab is currently being investigated in combination with a number of other agents in CLL. Trials that are currently actively being conducted in the frontline setting include a combination of pentostatin, cyclophosphamide and ofatumumab. Trials being pursued in the relapsed setting include a combination of ofatumumab with lenalidomide, and ofatumumab with bendamustine.

GA101 is a novel CD20 antibody that has significant activity in relapsed/refractory CLL and NHL.^{52,53} Veltuzumab is another humanized anti-CD20 antibody that, despite targeting the same epitope as rituximab, has shown clinical activity against NHL at much lower doses.⁵⁴ Ocrelizumab is a humanized version of the murine 2H7 antibody with more potent ADCC *in vitro*;⁵⁵ however it has not been tested in a clinical trial yet.

CONCLUSION

The introduction of mAbs targeting CD20 has ushered a new era in the management of previously untreated patients with CLL. The addition of rituximab, the first CD20 mAb, to chemotherapy was shown to provide a survival advantage compared to cytotoxic chemotherapy alone in the management of previously untreated CLL. Future developments will focus on the testing of newer CD20 antibodies in combination with chemotherapy and other biological agents for both relapsed and frontline patients. It is hoped that these novel mAbs will improve response rates and prolong survival with minimal toxicity.

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Table I

Single-Agent Rituximab in Previously Untreated CLL

Reference	N	Rituximab Dose (mg/m ²)	CR	ORR	Median PFS
Hainsworth ¹⁰	44	Induction: 375 weekly $\times$ 4	4	51	19 months
	28	Post-induction: 375 weekly $\times$ 4; q6 mos $\times$ 4	9	58	
Thomas ¹¹	31	375 weekly $\times$ 8	19	90	43 months

Abbreviations: CR: complete remission; ORR: overall response rate; PFS: progression-free survival

Table II

Chemoimmunotherapy Regimens in Previously Untreated CLL

Reference	N	Regimen	Dose (mg/m ²)	CR (%)	OR R (%)	Median PFS/FFS/OS (months) or %
Byrd ¹⁹ and Woyack ²⁰	104	Concurrent FR	F 25 (d1-5), R 375 (d1, d4 course 1 then R 375 d1 courses 2-6); q4wks × 6 courses; responders given R 375 × 4 as post-induction	47	90	84 (OS) and 32 (PFS)
		Sequential FR	F 25 (d1-5); q4wks × 6 courses; followed by R 375 weekly × 4 courses	28	77	91 (OS) and 40 (PFS)
Keating ²⁵ and Tam ²⁶	300	FCR	F 25, C 250 (d1-3), R 375 (d0 course 1 then R 500 d1 courses 2-6); q4wks × 6 courses	70	95	57% (6-year FFS)
		FCR	F 25, C 250 (d1-3), R 375 (d0 course 1 then R 500 d1 courses 2-6); q4wks × 6 courses	44	95	52 (PFS)
Hallek ²⁷	817	FC	F 25, C 250 (d1-3); q4wks × 6 courses	22	88	33 (PFS)
Foon ³³	50	FCR-Lite	R 375 (d1), R 500 (d14) F 20, C 150 (d2-4) - course 1; R 500 (d1, d14) F 20, C 150 (d1-3) - courses 2-6; followed by R 500 q3mos until relapse	77	100	22 (DR)
Kay ³⁰	64	PCR	P 2, C 600, R 375 (d1); q3wks × 6 courses (first course R three times weekly)	41	91	33 (PFS)
O'Brien ³²	65	FCR3	F 25, C 250, R 500 (d1-3); q4wks × 6 courses (R 375 course 1)	65	94	Not reported
Faderl ³⁴	30	FCMR	F 25, C 250 (d1-3), M 6 (d1), R 375 (d0 course 1 then R 500 d1 courses 2-6); q4wks × 6 courses	83	96	Median PFS NR at 39
Bosch ³⁵	71	FCMR	F 25, C 250 (d1-3), M 6 (d1), R 375 (d1 course 1 then R 500 d1 courses 2-6); q4wks × 6 courses	82	93	Not reported
Zent ³⁸	30	RA	R 375, A 30mg flat dose (d1, d3, d5) weekly × 4 (initial dose escalation for A in week 1)	37	90	14.4 (DR)
Wierda ⁴⁰ and Parikh ³⁹	60	CFAR	C 250, F 25 (d3-5), A 30 flat dose (d1, d3, d5), R 500 (d2); q4wks × 6 courses (R 375 course 1)	70	92	38 (PFS)
Fischer ⁴²	117	BR	B 90 (d1-2), R 375 on day 1 q 4 wks × 6	33	91	NR at 18 months
Hillmen ⁴⁴	50	ChR	Ch 10 (d1-7) po, R 375 (d1 course 1 then R 500 d1 courses 2-6)	Not reported	84	Not reported

Abbreviations: CR: complete remission; ORR: overall response rate; PFS: progression-free survival; OS: overall survival; F: fludarabine; C: cyclophosphamide; R: rituximab; P: pentostatin; B: bendamustine; M: mitoxantrone; A: alemtuzumab; Ch: Chlorambucil; PFS: progression-free survival; DR: duration of response; NR: not reached

Doses are in mg/m² except as noted for alemtuzumab (flat dose)

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Table IIIMechanism of Action of CD20 Monoclonal Antibodies *in vitro*

Name of antibody	Type	Property	ADCC	CDC	Direct effects	Phase of development
Rituximab ⁵⁶	I	Chimeric IgG1	++	++	+	Approved 1997 for NHL Phase 3
Ofatumumab ⁵⁷	I	Human IgG1	++	++++	+	Approved 2009 for CLL Phase 3
Veltuzumab ⁵⁴	I	Humanized IgG1	++	++	+	Phase 1/2
Ocrelizumab ⁵⁵	I	Humanized IgG1	+++	+/-	+	Phase 3
GA101 ⁵²	II	Humanized IgG1	++++ +	-	++++	Phase 1/2

Adapted in part from Maloney D. Hematology 2007; from the ASH 2007 ASH Education Book

Abbreviations: ADCC: antibody-dependent cell-mediated cytotoxicity; CDC: complement-dependent cytotoxicity