Review Article

Progress in immunotherapy Rituximab

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ABSTRACT

Rituximab is an anti-CD20 chimeric monoclonal antibody that has shown substantial activity. Since its discovery, rituximab has been used with great success in a variety of hematological malignancies. Its success in the management of aggressive lymphomas led to expansion of its use in other conditions such as stem cell transplantation, post-transplant lymphoproliferative disorder, and other non-malignant conditions where B cell activation is thought to be important, such as idiopathic thrombocytopenic purpura and rheumatoid arthritis. The side effects have been remarkably few, particularly, infection is not more common than with chemotherapy alone. This article reviews the structure, mechanism of action, and uses of rituximab as monotherapy or in combination with chemotherapy.

Saudi Med J 2007; Vol. 28 (11): 1635-1644

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Antibodies are produced in response to specific molecules (antigens). The range of antibodies that can be formed is enormous. Each plasma cell secrets antibodies of a single specificity. By fusing a plasma cell with an immortalized myeloma cell line in tissue culture, which generates a hybridoma, a continuously growing cell line produces antibodies of a single class and specificity,[1,2] known as monoclonal antibodies. With the advent of monoclonal antibodies, it became possible to identify and target specific molecules on the surface of tumor cells. The development of genetic techniques has allowed the creation of antibodies by incorporating selected human and murine characteristics. Chimeric antibodies combine the Fab (antigen binding region) of the mouse antibody with the Fc (constant region) of the human antibody. These antibodies can bind effectively to target antigens. Rituximab is a chimeric antibody directed against the CD20 antigen which is a 297 amino acid phosphoprotein (33-35KD) found on the surface of B-cells. The CD20 is highly expressed on the surface of B-cells but not on stem cells, pro B-cells, plasma cells, or other cell types. It is the first monoclonal antibody licensed for treatment of non-Hodgkin's lymphoma (NHL), and it was approved by the Food and Drug Administration (FDA) in the United States in November 1997.[3]

CD20 as target antigen. The CD20 is a transmembrane surface antigen expressed only by B-cell precursors and mature B-cells. It is involved in the regulation of B-lymphocyte growth and differentiation. CD20 is expressed on more than 85% of B-cell in NHL, but not on stem cells or normal mature plasma cells, or other normal tissues, and lost when normal B-cell differentiate into antibody secreting plasma cells.[1,2] CD20 is present on malignant-plasma cells in 20% of patients with multiple myeloma, and up to 50% of patients with plasma cell leukemia, and 75-100% of patients with Waldenström's macroglobulinemia.[3] The CD20 positive cell can totally be eradicated without causing specific toxicity because normal B-cells will re-emerge following differentiation from stem cells. CD20 is not internalized after binding to an antibody, then the anti-CD20 antibody initiates an immune response and apoptosis.

Structure and design. Rituximab is a genetically engineered human/mouse chimeric monoclonal antibody that is specific for CD20 B-cell surface antigen.[4] Rituximab consists of human IgG1, kappa constant region with a variable region isolated from murine anti-CD20 antibody. It consists of 2 heavy chains and 2 light chains with a molecular weight of 145 KD (**Figure 1**). Rituximab has low potential for immunogenicity, as most of the molecules are of human origin.

Mechanism of action. Rituximab effectively reduces the circulating B-cell count in lymphoma patients by complement medicated cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC) and induction of apoptosis. Rituximab binds directly to the C1q complement component, initiating complement mediated lysis of circulated B-cells.[4] Rituximab binds

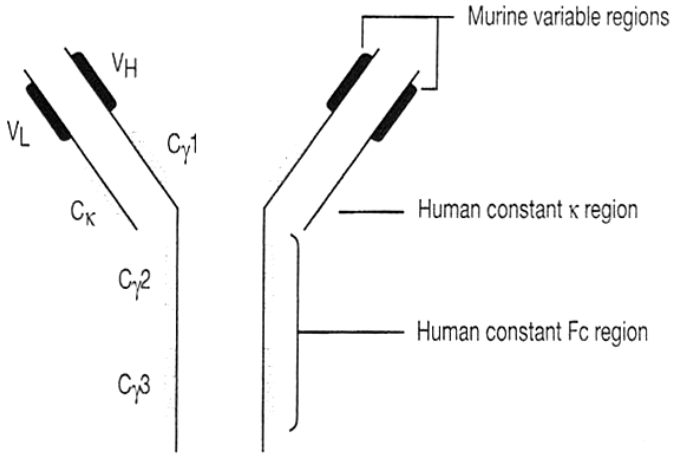

Figure 1 - Photograph showing 2 heavy chains and 2 light chains with a molecular weight of 145 KD.

strongly to the FC receptor on macrophages and natural killer cells inducing ADCC.[5] Rituximab induces apoptosis.[5] Recent studies suggest that complement-dependant cytotoxicity may be more important than ADCC.[6] Rituximab may be effective in patients who have failed to respond to chemotherapy or who have relapsed after chemotherapy. Regarding toxicity, no significant toxicological effects were observed at various doses and schedules. Only B-cell depletion was observed, and time to recovery is 3 months, with partial recovery most commonly occurring after 4-8 weeks.

Pharmacokinetics of rituximab. Rituximab was found on lymphoid cells in the thymus, the white bulb of the spleen and most B-lymphocytes in peripheral blood and lymph nodes. Serum levels of rituximab have been shown to be dose-dependent over the dose range of 100-500 mg/m^2 given as an intravenous infusion.[7] Peripheral B-lymphocyte count is reduced by approximately 90% within 3 days of a single infusion of rituximab 250 or 500 mg/m^2. The half-life is 60 hours after first infusion, and 174 hours after fourth infusion. Clearance decreases following multiple infusions from 38.2 ml/hour following one single infusion to 9.2 ml/hour after the fourth dose, and a terminal elimination half life of rituximab increases from 3.2 days following a single infusion to 8.6 days following the fourth dose, confirming that progressive accumulation occurs.[8]

Safety and tolerability. More than 300,000 patients worldwide have been treated with rituximab. It is a well-tolerated treatment. Rituximab administration is not associated with severe hematological or other adverse events, commonly seen with chemotherapy. Patients may experience an infusion related reaction, such as fever, and chills during the first 2 hours of the first infusion, these decrease substantially with subsequent infusions. Other side effects include, dyspnea, often with bronchospasm, and hypoxia, flushing, angioedema, nausea, urticaria, and rash, headache, throat irritation, rhinitis, vomiting, and tumor pain. In 10% of patients, these events are accompanied by hypotension. Tumor lysis syndrome has also been reported following rituximab administration.[9] Despite profound B-cell depletion, the incidence of infection is not increased compared with chemotherapy alone. The Group d'Etude Lymphoma de l'Adult (GELA)-Lymphome Non Hodgkinien study 98-5(LNH98-5). study, showed no additional toxicity with chemotherapy.[10]

Uses of rituximab. Non-Hodgkin's lymphoma (NHL) is a composite lymphoid malignancy with an increased annual rate of 4-7% over the last 20 years in both the USA and Europe.[11] Low grade NHL accounts for approximately 40% of the incidence of NHL in the USA. While patients with intermediate and high grade are potentially curable with combination chemotherapy, low grade NHL is still considered to be essentially incurable with standard therapy. Patients respond to treatment, but it follows a course of recurrent relapse and shorter remission. The median survival for low-grade lymphoma is 6.2 years and 5 years from time of first relapse.

1. Indolent type of Non-Hodgkin's lymphoma. The indolent type of NHL follows a chronic relapsing and remitting course and remains incurable with chemotherapy. Thus, while the disease is responsive to conventional chemotherapy, no chemotherapy regimens affect overall survival. Many patients with asymptomatic diseases may not be treated until their disease progresses, without any detrimental effect on survival.[12] One of the

first clinical trials of rituximab plus chemotherapy was a phase II study,[13] which used rituximab plus Cytoxan, Hydroxydaunorubicin (Adriamycin), Oncovin (Vincristine), Prednisone/Prednisolone (CHOP) chemotherapy, which showed complete response (CR) in 58%, partial response in 42%, and median time to progression was more than 7 years. Subsequently, 2 large prospective phase III randomized trials[14] have confirmed that the addition of rituximab to chemotherapy yielded major improvement in overall response, CR rate, and progression free survival. In a randomized study,[14] 321 patients with advanced follicular lymphoma were treated with 8 cycles of Cyclophosphamide, Vincristine and prednisolone (CVP) plus rituximab (R-CVP). The primary end point was time to treatment failure. At a median follow-up of 18 months, time to treatment failure was significantly longer for patients treated with rituximab plus CVP compared with CVP alone (26 months versus 7 months, $p<0.0001$, (**Table 1**). Patients with low-grade NHL treated with rituximab plus CHOP chemotherapy showed prolonged clinical and molecular remission. In one study,[15] 9 years follow-up of 38 patients previously untreated were included. The overall response rate was 100%, and 87% of patients achieved a complete response. The median time to progression was 82.3 months. Marcus et al[16] showed that the overall and complete response rate were 81%, and 41% in the R-CVP arm versus 57% and 10% in the CVP arm ($p=0.0001$). In the median follow-up of 30 months, median time to treatment failure was 27 months in patients receiving R-CVP, and 7 month in patients receiving CVP alone. The addition of rituximab to CVP significantly improved the clinical outcome in patients with previously untreated advanced follicular lymphoma, without increased toxicity. Another large randomized trial conducted by the German Low Grade Study Group,[17] compared rituximab plus CHOP with CHOP alone in previously untreated follicular lymphoma patients, which showed increased time to treatment failure, improvement in progression free survival and overall survival in the rituximab plus CHOP arm.[17] Rituximab in combination with various chemotherapy regimens has also been evaluated in several phase II studies, rituximab with Chlorambucil in the treatment of newly diagnosed or refractory/relapsed follicular lymphoma, which showed complete response rate of 63%.[18] The combination of rituximab, 9 doses, and 3 cycles of CHOP or CVP as the first line treatment of 86 patients with follicular lymphoma to minimize chemotherapy-related toxicity, showed complete response rate in 57% with less hematological toxicity. At a median follow-up of 15 months, 87% remained progression-free.[19] A randomized study compared 2 combinations of chemotherapy followed by Rituximab in patients with previously untreated follicular lymphoma,[20] a total of 159 patients were randomized to receive 6 cycles of fludarabine and mitoxantrone (FM), or CHOP. The overall survival rate was 94% in both arms. In those patients who had complete or partial response, but were still bcl-2 positive, 4 cycles of rituximab were given as consolidation treatment, which increased the molecular response rate to 59% in the FM and rituximab arm, versus 34% in the FM arm, and 40% in the CHOP-R versus 20% in the CHOP arm.[20] In a randomized comparison of concurrent treatment with rituximab plus fludarabine, mitoxantrone, dexamethasone with sequential treatment with Fludarabine, Mitoxantrone, Dexamethasone (FND) followed by rituximab, the 5-year failure free-survival was significantly higher in the concurrent arm compared with the sequential arm (70.4% versus 44.2%).[21]

Bcl-2 clearance. Follicular lymphomas are characterized by over-expression of the anti-apoptotic protein Bcl-2 as a result of translocation t(14;18). Increased levels of Bcl-2 protect the lymphoma cells from a range of apoptotic signals, suggesting a model whereby cells do not have a proliferative advantage as such, but accumulate through lack of cell death. The

Table 1 - Rituximab plus CVP is superior to CVP alone in multiple clinical end points. In patients with low-grade lymphoma.

End point	Rituximab + CVP	CVP	*P* value
ORR (%)	80.9	57.2	<0.0001
CR (%)	40.7	10.0	<0.0001
Median time to treatment (months)	26.0	7.0	<0.0001
Median time to progression (months)	27.0	15.0	<0.0001

ORR - Overall response rate, CR - complete response, CVP - combination of cyclophosphamide, vincristine, and prednisone,

Bcl-2 over-expression will also reduce the ability of chemotherapeutic agents to induce apoptosis and this explains why it is notoriously difficult to cure patients of follicular lymphomas. Seventy percent of patients with follicular lymphoma have t(14;18) chromosomal translocations that result in rearrangement of the Bcl-2 gene and over-expression of the Bcl-2 protein. The importance of Bcl-2 clearance by rituximab as part of immunochemotherapy has been demonstrated by a study of 128 patients with previously untreated follicular lymphoma who were Bcl-2 positive.[22] They received CHOP, and in those who achieved clinical response but were still Bcl-2 positive, they received 4 doses of once weekly rituximab. At the 12th week, 59% of Bcl-2 positive converted to Bcl-2 negative. At 44 weeks, 63% remained negative.

First-line rituximab as monotherapy for indolent NHL. Fifty patients with follicular NHL with lesions less than 7 cm, stage II -IV, received 4 doses of rituximab once weekly by intravenous injection, the objective response rate was 73%, (complete response, 26% and partial response, 47%).[23] Rituximab as monotherapy can be given in 4 weeks, it is well tolerated and not associated with the toxicity of the standard chemotherapy regimens. In asymptomatic patients with indolent NHL, watchful waiting is a commonly used option, because early treatment with chemotherapy does not improve survival.[11] Rituximab may have the potential to delay the onset of chemotherapy treatment and this improves the quality of life for patients with previously untreated indolent NHL. A phase II study[24] shows that rituximab is an effective and well-tolerated treatment for patients with previously untreated indolent NHL and is a viable alternative to watchful waiting. Thirty-seven patients were included in this study, the overall response rate was 61%, and the median time to progression was 20 months.[24]

Retreatment with rituximab as monotherapy. Fifty-eight patients with relapse or refractory low-grade NHL who had previously responded to rituximab, but subsequently progressed, received 4 doses of rituximab on retreatment with 40% achieving further response. The median time to progression in responders was 17.8 months, and median duration of response 16.3 months. This study shows that re-treatment with rituximab is feasible and effective.[25] A retrospective study[9] evaluated retreatment of indolent NHL with rituximab, as monotherapy or in combination with chemotherapy. The overall response rate to retreatment was 93% and the response duration was longer than the following the first treatment with rituximab.

Rituximab as maintenance therapy in indolent NHL. The Swiss Group for Clinical Cancer Research conducted a randomized trial to evaluate maintenance therapy in indolent NHL.[26] In this study, 202 patients with relapsed/refractory or previously untreated indolent NHL were treated with 4 doses of rituximab weekly, followed by one dose every 2 months up to 8 months. In previously untreated patients, the response rate was 67% compared with 46% in relapsed or refractory patients. In indolent NHL, the event free survival and duration of response with rituximab as a monotherapy may be improved with maintenance therapy for either previously untreated or relapsed patients. In the Hainsworth et al study,[27] patients were treated with a standard of 4-doses and then 4 x 375 mg/m^2 every 6 months for a maximum of 4 courses, or until disease progression. Following maintenance, the overall response rate was increased to 73%, the complete response rate was increased from 7-47%. In a phase III study by the German Low Grade Study Group,[28] patients with relapsed or refractory indolent or mantle cell lymphoma, received fludarabine, cyclophosphamide, and mitoxantrone (FCM), with or without rituximab for 4 doses, then the responding patients were given maintenance therapy of 4 weekly doses at months 3 and 9, at the end of chemotherapy, both progression free and overall survival were significantly higher in the rituximab plus chemotherapy compared with chemotherapy alone, and the overall response rate of 94% versus 75% (p=0.047).

Increasing response to rituximab by addition of interferon-alpha in indolent NHL. Interferon-alpha has significant single-agent activity in NHL and also has immunomodulatory effects including up regulation of CD20 expression.[29] The ability of interferon to augment the efficacy of rituximab in partial responders was studied, a total of 125 patients with indolent NHL (untreated or in first relapse, including those with low CD20 expression) were given 4 cycles of single-agent rituximab. Sixty-six patients with partial or minor response were randomized to retreatment with single agent rituximab or rituximab plus interferon. The results are shown in **Table 2**. In summary, 8 cycles of rituximab plus chemotherapy represents a new standard first-line treatment of indolent lymphomas. Rituximab plus chemotherapy improve response rate, time to treatment failure, and progression free survival compared with chemotherapy alone. Rituximab maintenance therapy prolongs the duration of remission and helps prevent relapse.

2. Use of rituximab in aggressive NHL. Diffuse large B-cell lymphoma is the most frequent, representing 40% of all lymphomas. For more than 25 years, CHOP has been the standard treatment of aggressive NHL, however, <50% of patients were cured. Eight cycles of rituximab and CHOP are now the standard first line treatment for patients between 60-80 years with

aggressive NHL (previously untreated) based on The Groupe d'Etude des lymphomas de L'dulte (GELA. LNH-98.5) study.[30] Rituximab is given in 375 mg/m^2 on day one in each of 8 cycles of chemotherapy, the results are shown in **Table 3**. Subsequent analysis at 3 and 4 years follow-up confirmed that the significant survival advantage for rituximab and CHOP was maintained. The addition of rituximab to CHOP increases the number of patients being cured of their disease. According to the International Prognostic Index (IPI), rituximab and CHOP significantly improved response rate, event free survival, and overall survival rate compared with CHOP alone in low-risk as well as high-risk patients.[31] Based on the GELA-LNH 98.5 trial, the British-Colombia Cancer Agency implemented a new policy recommending that 8 doses of rituximab and CHOP should be given to all newly diagnosed patients with aggressive NHL.[32]

The Eastern Cooperative Oncology Group (ECOG) 4494 trial.[33] This trial began in 1994 where only 4 or 5 doses of rituximab were combined with 6 or 8 cycles of CHOP in elderly patients with aggressive NHL, then rituximab was given to responders every 6 months for 2 years compared with nothing. In the maintenance versus observation analysis of all patients, there was a statistically significant improvement in time to treatment failure favoring Rituximab maintenance over observation (p=0.004). A major reason for the difference in outcome between the ECOG 4494 and the GELA LNH-98.5 trial is the difference in number of rituximab cycles administered (4-5 in the ECOG study compared with 8 in the GELA study), however, the GELA regimen gave superior results compared with ECOG 4494, as the rituximab concentration in the GELA trial was 200 µg/ml, in contrast to 50 µg/ml in the ECOG study.[34]

The rituximab international trial (MInT)[35] shows rituximab plus chemotherapy significantly improved the outcome in young low-risk patients with aggressive NHL compared with chemotherapy alone. It improves complete response rate, time to treatment failure (TTF) and overall survival, an estimated 84% of patients in the rituximab plus chemotherapy group were failure free, compared with 62.5% in the chemotherapy-alone group. The GELA trial showed that rituximab did not add any significant toxicity to CHOP, the same results were confirmed in the ECOG and MInT trial.

Rituximab in relapsed aggressive NHL. High dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT) is the standard of care for relapsed aggressive NHL. Several studies demonstrate that the addition of rituximab to second line chemotherapy can improve complete response rate. In a phase II study[36] of 36 patients with relapsed aggressive NHL, the addition of rituximab to ifosfamide and carboplatin and etoposide doubled the complete response rate compared to chemotherapy alone (complete response 53% versus 27%). In another study,[37] 80% of patients who either relapsed or had refractory aggressive B-cell NHL responded to rituximab with paclitaxel and topotecan,

Table 2 - Response rate to Rituximab plus Interferon compared with Rituximab alone in patients with indolent non-Hodgkin's lymphoma.

Parameter	Rituximab + Interferon	Rituximab alone
Overall response rate	99	82
Complete response	54	24

Table 3 - Response rate, event free and overall survival to treatment in the GELA LNH-98.5 trial.

Response rate	CHOP alone (n=197)	Rituximab plus CHOP (n=202)	*P*-value
ORR	63	75	<0.005
2 year EFS	38	57	<0.001
OS	57	70	<0.007

ORR=Overall response rate, CR= complete response, EFS= event free survival, CHOP - Cytoxan, Hydroxydaunorubicin (Adriamycin), Oncovin (Vincristine), Prednisone/ Prednisolone

with 60% achieving a complete response. The response to combination therapy was 80% and this was better than the response to prior therapy 65%. Horwitz et al,[38] administered rituximab as consolidation therapy to prevent relapse in patients receiving ASCT for relapsed aggressive NHL. Two courses of 4 doses of rituximab were given, the first dose at day 42, and the second dose on day 180. The 2-year overall survival was 80%, and the event-free survival was 83%. Feugier et al,[39] showed that a combination of rituximab with CHOP leads to significant improvement of outcome in elderly patients with diffuse B-cell lymphoma with significant survival benefit maintained during a 5-year follow-up. In summary, 8 cycles of rituximab plus CHOP represents the first advance in therapy for aggressive lymphoma in almost 25 years and is now the standard therapy.

3. Mantle cell lymphoma. Mantle cell lymphoma (MCL), which accounts for 5-8% of all NHL has one of the poorest prognoses, with a median survival of less than 3 years and is rarely cured by conventional chemotherapy. Two prospective randomized studies by the German Low Grade Lymphoma Study Group have compared rituximab plus chemotherapy with chemotherapy alone in MCL. In the first study,[40] 62 patients with previously untreated MCL were included, the overall response rate was higher for rituximab with CHOP than for CHOP alone (90% versus 75%) (p=0.004). This study requires longer follow-up to determine the effects of rituximab plus CHOP on survival. In the other study,[28] 48 patients with relapsed or refractory MCL were included, the overall response rate was superior in the rituximab plus FCM arm compared with FCM alone (62% versus 43%) and a significant overall survival advantage was observed (p=0.0058). In the Toronto study,[41] where 20 patients with stage III/IV who prospectively received HDT/ASCT with rituximab were compared with those of 40 matched historical controls with MCL who received chemotherapy alone, rituximab was given as a single infusion prior to stem cell collection, and as 2, 4 weeks courses, at weeks 8 and 24 post ASCT. Seventeen out of 20 patients who received rituximab plus ASCT/high dose chemotherapy (HDT), remained alive and in remission at a medium follow-up of 30 months from diagnosis. Overall and progression free survival at 3 years were significantly higher for patients with rituximab and HDT/ASCT, compared with those who received conventional chemotherapy alone, the overall response rate was 88% versus 65%, (p=0.052). In the Milan study,[42] 28 patients with untreated MCL, received complex regimen in 2 phases (a purging phase and a myeloablative phase) that comprised 4 steps, a high dose chemotherapy sequence of cyclophosphamide, cytarabine, melphalan and mitoxantrone + melphalan, and 6 infusions of rituximab 375 mg/m^2 with a stem cell infusion for hematological support. All 28 patients achieved complete clinical remission, and 20 patients who are by polymerase chain reaction (PCR), Bcl-2 positive at baseline achieved a complete clinical remission, and complete molecular response. At 4.5 years, the projected overall survival was 89%, and event free survival was 79% compared with 18% event free survival and 42% overall survival for historical controls treated with anthracycline containing regimens. Both the Milan and Toronto studies indicate rituximab in combination with HDT/ASCT represents an important advance in treatment. In summary, patients with MCL have poor prognosis with conventional chemotherapy. However, the combination of rituximab with chemotherapy or HDT/ASCT, or both, appears to represents an important advance in treatment.

4. Rituximab in chronic lymphocytic leukemia. Studies in chronic lymphocytic leukemia (CLL) indicate that rituximab can be effective in reducing the lymphocyte count and induce remission in this disease. Typically, standard single agent, rituximab, 375 mg/m^2 for 4 doses yields a response rate of up to 50%, predominantly partial response.[43] The response rate in indolent NHL appears to be higher than those achieved in relapse of CLL, this may be due to lower expression of CD20 antigen in CLL cells, more rapid clearance of the antibody by high numbers of circulating cells, or a combination of both. In addition, the expression of complement-inhibitory antigens CD55 and CD59 on CLL cells may decrease the effectiveness of rituximab in inducing complement mediated cytotoxicity.[44] Using doses of rituximab up to 2,250 mg/m^2 once weekly achieved a response rate up to 80%.[45] Byrd et al[46] showed increased activity of rituximab in CLL, with an overall response rate of up to 83% in previously untreated patients. In CLL, fludarabine, and fludarabine containing regimens are the most commonly used chemotherapy agents. In a randomized phase II study, the efficacy and safety of administering Rituximab and Fludarabine either concurrently or sequentially to patients with previously untreated CLL was evaluated. Fludarabine was given as 25 mg/m^2 on day 1-5 every 4 weeks for 6 cycles with rituximab as concurrent arm or as sequential arm.[47] Rituximab was initially administered on day one and 4 of the first cycle, and on day one of cycles 2-6. After 2 months, all patients who had complete or partial response, received consolidation therapy with rituximab weekly for 4 weeks. The overall (90%) and complete response rate (47%) were high in the concurrent arm (90%) and the overall response rate was 77% and complete response rate was 26% in the sequential arm. The combination of rituximab with fludarabine and cyclophosphamide (FC) appears to be one of the most active regimens available in both previously untreated

and relapsed CLL.[48] In chemoimmunotherapy regimens of fludarabine, cyclophosphamide, and rituximab (FCR) as initial therapy for CLL, time to treatment failure analyses showed that 69% of patients were failure free at 4 years (95%; CI 57%-81%).[49] Similarly, chemoimmunotherapy with FCR in relapsed or refractory CLL is an active regimen. Molecular remission was achieved in a third of patients achieving complete response.[50] In summary, the combination of rituximab with FC appears to be one of the most active regimens available in both previously untreated and relapsed CLL, achieving high rate of complete response and prolonged disease free survival.

5. Rituximab with stem cell transplantation. Stem cell transplantation is being increasingly used in a variety of malignant and non-malignant disorders. It is generally reserved for young patients with relapsed disease, however, the age is being gradually extended to include elderly patients as less myeloablative regimens become popular. Relapse following transplantation is generally due to a contamination of stem cells by malignant cells, or residual tumor cells remaining after bone marrow transplant. A more effective method of CD20 positive cell removal is by administering the anti CD20 antibody to the patients before or during the process of stem cell mobilization, or both.[51] Rituximab has no effect on CD34 positive stem cells. The stem cell yield is maintained after rituximab treatment and demonstrates at least equivalent yields of CD34 positive stem cells, which are required for repopulation of bone marrow after transplant.[52] Rituximab clearance of Bcl-2 positive cells from the blood, and the bone marrow of patients with follicular lymphoma, who have received ASCT, are established as a predictive factor for increased survival.[51] The European Group for Blood and Marrow Transplantation (EBMT) is currently conducting a large, randomized international study, on the clinical efficacy of Rituximab in follicular NHL.[53] Patients are randomized to one of 4 arms, purging, purging with maintenance, maintenance without purging, and no purging or maintenance. Ninety percent complete response rate was reported in patients with relapsed follicular lymphoma who received both rituximab purging and maintenance treatment.[53] A 30-month overall survival of 88% and event free survival of 83% was reported in 35 patients with aggressive NHL who received rituximab purging and maintenance in conjugation with high doses of chemotherapy and ASCT.[38] In summary, rituximab has been used with ASCT, both to purge the stem cell harvest of residual lymphoma cells and to prevent relapse through post-transplant maintenance.

Other B-cell malignancies. Rituximab has shown efficacy in a variety of CD20 positive malignancies other than NHL and CLL, including post transplant lymphoproliferative disorders (PTLD), hairy cell leukemia, HIV associated lymphoma, Waldenström's macroglobulinemia and multiple myeloma. Post transplant lymphoproliferative disorder is a severe complication in patients undergoing immuno-suppressive therapy following organ transplantation. Rituximab has been shown to be effective as a first and second line therapy in PTLD. A complete response rate of 55.3% was achieved following first, and 50% following second-line treatment in 25 patients with PTLD, with an overall response rate of 64%.[54] The European PTLD study group is investigating sequential treatment with 4 cycles of rituximab followed by 4 cycles of CHOP and GCSF. Human immunodeficiency associated lymphoma is a major cause of death. CD20 was expressed in 90% of HIV associated lymphomas, and adding rituximab to CHOP show an overall response rate of 75%, with a 69% complete response.[55] In Waldenström's macroglobulinemia, the preliminary results suggest rituximab in combination with fludarabine is highly active with an 85.7% response rate.[56] In Hodgkin's disease, only 20% of malignant cells express CD20, rituximab shows efficacy in lymphocyte-predominant Hodgkin's disease (LPHD), with a response rate of 75-100%.[57] In a pilot study, Younes et al,[58] demonstrated responses in patients with recurrent classical Hodgkin's disease with malignant cells that were not CD20+ positive who were treated with 6 weekly doses of single agent rituximab. They hypothesized that elimination of B-cells from the tumor may deprive the malignant lymphoid cells of important growth signals. Based on this result, an initial phase II trial combining rituximab with ABVD in patients with newly diagnosed classical Hodgkin's disease, an estimated event-free survival of 83% was achieved after a median follow-up of 21 months.[59] Normal plasma cells do not express CD20, however, the malignant plasma cells of multiple myeloma frequently express CD20. It was demonstrated that gamma interferon induces CD20 expression and facilitates the binding of rituximab to malignant plasma cells.[60] Hairy cell leukemia is a chronic CD20 positive B cell malignancy. In a pilot study, rituximab, in patients who relapsed or have refractory hairy cell leukemia, yielded a response rate of 80%.[61,62] In summary, rituximab is active in CLL.

6. Non-malignant disorders. Rituximab has also been used in cold agglutinin disease,[63] idiopathic thrombocytopenic purpura (ITP),[64] autoimmune hemolytic anemia,[65] rheumatoid arthritis, and antineutrophil cytoplasmic antibodies (ANCA) associated vasculitis. In ITP, patients who respond to rituximab have more profound B-cell depletion in responders compared with non-responders. Early

responders show rapid depletion of anti-platelets antibody compared with delayed fall in late responders.[66] Seventy-two percent of responders have an increased platelets count from 20,000 to 30,000/μL, and complete response was maintained in 28% of patients. Patients who relapsed can respond to second dose with same magnitude and duration as the first response.[67] The dose used is 375 mg/m^2 once weekly for up to 4 consecutive weeks unless there was an early complete response. Four patients out of 11 show CR, which is very promising. The use of rituximab has been evaluated in the treatment of rheumatoid arthritis patients who are refractory to disease modifying anti-rheumatic drugs. The response level was significantly higher than in patients receiving methotrexate alone, indicating that rituximab has a promising effect.[68,69] Rituximab was given as 1000 mg (day 1, day 15) as 2 doses.

In conclusion, rituximab is now standard in combination with CHOP in the management of aggressive B-cell lymphomas. It is assuming increasing importance in the management of follicular lymphoma and other indolent lymphomas. Rituximab appears to be effective in both naïve and relapsing patients. Maintenance rituximab in lymphomas needs larger phase III studies to become standard. The role of rituximab is expanding to include autoimmune diseases and bone marrow transplantation patients. The toxicity of rituximab is tolerable and does not appear to be higher than that of chemotherapy alone, in particular. The incidence of infection does not appear to be higher than in patients who are receiving chemotherapy alone.

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