Review of Current Treatment Options and Evidence for the Use of

Rituximab

(Rituxan®) in the Treatment of

CHRONIC LYMPHOCYTIC LEUKEMIA

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Executive Summary

The management of chronic lymphocytic leukemia (CLL) has recently undergone a new evolution. Newer molecular prognostic factors have been recognized that appear to be more predictive of outcome than previously utilized clinical indicators. The development of more effective therapies has resulted in a greater number of treatment options and an overall improvement in outcome. Tests assessing for minimal residual disease have enabled a closer examination of the quality of responses to therapy. This has prompted a reevaluation of treatment paradigms and has reopened the debate as to whether certain patients should be considered for earlier intervention and more aggressive therapy.

Historically, management of CLL has been based largely on chemotherapy, with purine analogues and alkylators being the most effective agents. However, maximal treatment with chemotherapy has not been curative, nor has it improved long term survival. Therefore, there is a significant need for additional treatment options in CLL beyond standard purine analogue-based therapies. One recent option, rituximab, has shown considerable promise in the treatment of CLL.

The evidence supporting the use of rituximab in CLL has emerged from numerous phase II studies, including two historical cohort comparisons. Rituximab has demonstrated clinical efficacy as a single agent in CLL; and when combined with chemotherapy appears to offer incremental benefits over chemotherapy alone. The addition of rituximab to chemotherapy has resulted in higher response rates, more durable responses and possibly an improvement in overall survival. Improved outcomes have been reported in patients with previously untreated CLL, as well as in those with relapsed or refractory disease. The combination of rituximab with chemotherapy is quickly becoming the standard of care for patients with CLL requiring treatment.

Chronic Lymphocytic Leukemia (CLL)

Introduction

Chronic lymphocytic leukemia (CLL) is considered to be a disease of the elderly, with a median age at diagnosis of 70 years and a rapidly increasing incidence after 60 years of age.[1-4] Earlier diagnosis of CLL is becoming more common, likely due to advances in molecular biology rather than an actual increase in prevalence.

The management of CLL has recently undergone a new evolution. Newer molecular prognostic factors have been recognized which may allow for higher risk patients to be identified more accurately. Furthermore, clinical trial data suggest that the introduction of new therapeutic options has resulted in an overall improvement in outcome. Several new agents and combinations have been evaluated with encouraging results. In particular, the addition of the monoclonal antibody rituximab to chemotherapy has demonstrated benefits over chemotherapy alone in terms of response rates, quality of response, disease-free survival and possibly overall survival.

In the following pages, clinical aspects of CLL and its management are reviewed, illustrating promising new options and evolving treatment strategies. The data regarding the use of rituximab as monotherapy and in combination with other agents in relapsed/refractory and newly diagnosed CLL will be summarized.

Epidemiology

CLL is the most common adult leukemia in the Western hemisphere. It is estimated to account for 25% of all leukemias, with an annual incidence of 2-3 cases per 100,000. In Canada, 3,900 new cases of leukemia are diagnosed each year, and approximately 1,000 will be CLL.[3,4] However, due to the long natural history of this disease, as many as four times that number of patients may be living with CLL at any given time.

Pathophysiology

CLL is an incurable disease. In approximately 95% of patients, the disease originates in CD20 positive B-cells and results in the clonal accumulation of functionally incompetent lymphocytes.[5,6] In the remaining 5% of patients, T-lymphocytes are affected. CLL is a heterogeneous disorder. Differences between patients in morphology, immunophenotype, cytogenetics and molecular characteristics can be recognized and can translate into varying clinical courses and response to treatment.

CLL is a malignancy of small, morphologically mature but immunologically immature lymphocytes that accumulate in the blood, bone marrow, lymph nodes, spleen, and liver. No pathognomonic genetic mutation or abnormality has been identified in CLL. Rather, the disease is characterized by a variety of different chromosomal deletions or abnormalities that can be detected in 40-50% of cases using conventional chromosome banding. Fluorescence *in situ* hybridization (FISH) is a more sensitive test and can detect molecular abnormalities in up to 80% of cases.[5] The most common genetic aberration identified by FISH is the 13q deletion, which is found in 55% of cases. The next most common aberrations are the 11q deletion, found in18% of cases, trisomy 12 in 16%, and 17p deletion in 7% of cases.[7]

The clinical course of CLL is generally indolent, with a progressive accumulation of malignant lymphocytes within the bone marrow, spleen, and lymphatic tissue. Advancing infiltration within the bone marrow can result in anemia, neutropenia, thrombocytopenia, and immunological dysfunction. Hypogammaglobulinema and agammaglobulinemia are frequently observed and severity increases with the duration and stage of disease.[5,8,9] Significant hypogammaglobulinemia and neutropenia predispose CLL patients to infection, which is a primary cause of morbidity and mortality.

Autoimmune-related cytopenias can also be observed in patients with CLL. It has been reported that between 4 and 25% of patients develop autoimmune hemolytic anemia. Immune thrombocytopenia, pure red-cell aplasia, and immune neutropenia have been reported, but less frequently. Autoimmune disorders are more commonly seen in patients with advanced disease who have undergone prior therapy.[5,10,11] These disorders have also been associated with purine analogue therapy.

In 3% to 10% of patients, the disease undergoes a transformation to a more aggressive condition distinct from CLL. The transformation is usually into large-cell lymphoma, which is known as Richter's syndrome. The prognosis for these patients is poor, with a median survival of approximately 6 months. Transformation into prolymphocytic leukemia can also occur, but the disease rarely transforms into acute leukemia.

PREDISPOSING FACTORS

Unlike other leukemias, there is no firm evidence linking environmental or occupational exposure with an increasing incidence of CLL.[12,13] However, a family history of CLL or other lymphoproliferative disorder is a strong risk factor. It is estimated that one in ten patients has such a history.[14,15] There is a 30-fold increased risk in first degree relatives of patients with CLL. It has also been observed that 13.5% of first degree relatives have circulating peripheral blood lymphocytes with the typical CLL immunophenotype. However, it is not known at this time whether individuals with these abnormal cells will eventually develop CLL.[15]

CELLULAR ORIGIN & IGV$_H$ GENE MUTATIONAL STATUS

Recent data indicates that patient prognosis is related to the basic cellular origin of CLL. Previously, it was believed that the normal counterpart of the CLL clone was the CD5$^+$ B-lymphocyte because the CLL cell itself is CD5$^+$. However, the CD5$^+$ lymphocyte does not exhibit mutations of the immunoglobulin variable region (IgV$_H$) gene, while the CLL cell has undergone this mutation in approximately 50% of cases. This more recent finding indicates that there may be two forms of CLL: one arising from a pre-germinal lymphocyte that lacks mutations of the IgV$_H$ gene and the other arising from a cell that has traversed through the germinal center and contains IgV$_H$ gene mutations. Importantly, these two forms have been shown to have significantly different genetic abnormalities and prognoses. Those with IgV$_H$ gene mutations are more likely to have deletions of 13q14 and a good prognosis; while those without IgV$_H$ gene mutations more frequently express trisomy 12 and have a poorer prognosis.[16-18]

DIAGNOSIS

The International Workshop on CLL (IW-CLL) and the National Cancer Institute–sponsored Working Group on CLL (NCI-WG) have outlined specific criteria for diagnosing CLL, as detailed in Table 1.

Table 1. Criteria for Diagnosing CLL

IW-CLL Criteria	NCI-WG Criteriat
• A sustained peripheral blood lymphocyte count greater than 10 x 10^9/L • A bone marrow aspirate showing greater than 30% lymphocytes • Peripheral blood lymphocytes identified as monoclonal B cells Under the IW-CLL criteria, any one of the three above critieria is enough to establish a diagnosis of CLL.[19]	• A peripheral blood lymphocyte count greater than 5 x 10^9/L, with less than 55% of the cells being atypical • The lymphocytes should be monoclonal B lymphocytes expressing B-cell surface antigens (CD19, CD20, CD23), low-density surface immunoglobulin (M or D), and CD5 positivity.[20]

Differential Diagnosis

There are a number of other B-cell malignancies that present with increased circulating lymphocytes and thus would have to be included in the differential diagnosis at presentation. With advances in monoclonal antibody flow cytometry technology, immunophenotyping is currently a routine diagnostic tool used to differentiate CLL from disorders such as prolymphocytic leukemia (PLL), the leukemic phase of non-Hodgkin's lymphoma, and hairy cell leukemia (HCL).[5] In addition, the NCI-WG has specified that a lymphocyte count greater than 5 x 10^9/L may distinguish CLL from small lymphocytic lymphoma.[20]

Clinical Features

With the use of routine blood testing, the number of CLL patients who are asymptomatic at diagnosis has increased to approximately 40%, many of whom may remain asymptomatic for long periods of time.[5] The remaining 60% of patients present with various symptoms. The most common chief complaint at presentation is low grade fatigue. Patients may present with enlarged lymph nodes or infection, although bacterial infections are more common in patients with advanced-stage disease. In addition, infections secondary to opportunistic viruses such as herpes zoster may also occur. An exaggerated skin reaction to a bee sting or an insect bite can be seen (Well's syndrome) and splenomegaly may be observed, but massive splenomegaly is usually only seen with advanced disease. Splenic infarction is rare. General skin involvement occurs in fewer than 5% of cases.[5]

Laboratory Findings

Lymphocytosis consisting of mature lymphocytes in the peripheral blood and bone marrow is by definition universally present in CLL. Absolute lymphocyte counts generally range from 5 x 10^9/L to 500 x 10^9/L. Marrow infiltration by lymphocytes varies from 30% to 100% and cellularity can be either normal or increased. In general, the lymphocyte count usually increases over time in patients with CLL. In most cases the lymphocytes appear small and mature. However, variations in cellular morphology can be seen, with some lymphocytes being larger or atypical, whereas others may appear plasmacytoid, cleaved or prolymphocytic.[5] The French-American-British (FAB) classification system divides patients into three groups based on the percentage of abnormal cells, as detailed in Table 2.[21] Patients can also be classified based on lymphoid infiltration of the bone marrow. There are three types of lymphoid infiltration of the marrow: nodular, interstitial, and diffuse. Sometimes a mixture of the first two patterns is seen. Marrow infiltration can indicate disease progression and prognosis, as shown in Table 2.[5]

Table 2. Other Laboratory Findings in CLL

The French-American-British (FAB) classification system[21]	
Typical CLL	>90% of cells are small
CLL/PLL	11% to 54% of cells are prolymphocytes
Atypical CLL	Heterogeneous morphology; ≥10% prolymphocytes
Marrow Infiltration[5]	
Diffuse Infiltration	Usually advanced disease; worse prognosis
Nodular or Interstitial Infiltration (Non-diffuse Infiltration)	Associated with less advanced disease; better outcome
Other Laboratory Findings[5]	
Anemia (hemoglobin less than 11 g/dL)	Frequently seen with disease progression, but occurs in only a minority of patients at the time of initial diagnosis.
Thrombocytopenia (platelet count less than 100 x 10^9/L)	

Abbreviations: CLL=chronic lymphocytic leukemia; PLL= prolymphocytic leukemia

CLINICAL STAGING

Table 3 is a summary of the three major staging systems used for the classification of CLL: the Rai system, the Modified Rai system, and the Binet staging system. The Binet system is more commonly used in Europe.

Table 3. Common Staging Systems for Classification of CLL

System	Stage	Definition
Rai Staging System	0	Lymphocytosis only
	I	Lymphocytosis and lymphadenopathy
	II	Lymphocytosis and spleen or liver enlargement
	III	Lymphocytosis and anemia (hemoglobin <11g/dL)
	IV	Lymphocytosis and thrombocytopenia (platelet count <100 x 10^9/L)
Modified Rai Staging System	Low risk of progression	Rai stage 0
	Intermediate risk of progression	Rai stage I or II
	High risk of progression	Rai stage III or IV
Binet Staging System	A	Lymphocytosis, with enlargement of <3 lymphoid areas (cervical, axillary, zinguinal, liver); no anemia or thrombocytopenia
	B	Lymphocytosis, with enlargement of ≥3 lymphoid areas
	C	Lymphocytosis and either anemia (hemoglobin <10 g/dL) or thrombocytopenia (platelet count <100 x 10^9/L), or both

Rai & Binet Staging Systems

The original Rai system was published in 1975 and consists of stages 0-IV. It is based on the presence of lymphadenopathy, organomegaly, and cytopenias.[22] It was later modified from the five tier system to a three-tier system that categorizes patients as having a low, intermediate, or high risk of disease progression. The Binet system is also a three tier system with A, B, and C categories. It was based on a retrospective analysis of disease burden that drew a correlation between the number of nodal groups involved and bone marrow failure with disease progression.[23]

Although both the Rai and Binet staging systems give a general indication of prognosis, it is difficult to make comparisons between clinical trials using different staging systems. Moreover, survival within each stage can vary significantly, particularly in those patients with Binet stage A and Rai stage 0. In these groups as many as 30% of patients have a "smoldering" CLL which progresses slowly. Conversely, others have more aggressive disease that may require earlier intervention. The median survival of patients with Rai stage 0 exceeds 12 years and may reach 20 years, with a 10-year overall survival rate of 70% to 75%. Patients with Rai stage I and II have median survival rates of 8 to 10 years and 5 to 8 years respectively; whereas recent data show a median survival of 5 years and longer in Rai stage III and IV patients.[24]

Thus, clinical staging alone is inadequate to predict outcome. Significant research in the basic science of CLL has focused on the development of newer prognostic factors and biologic markers in an attempt to refine prognostication. The objective of this work is to allow for a more rational approach to treatment by identifying patients with potentially poorer outcomes who may benefit from earlier intervention.

PROGNOSTIC MARKERS

There is a considerable variation in survival in CLL, with some patients having a prolonged survival without treatment, whereas others experience a more rapid downhill course. Although the Rai and Binet staging systems are simple and reliable prognostic tools, there is considerable variation in outcome within each stage. Additional prognostic markers can be used in conjunction with staging to predict outcome more accurately. As shown in Table 4, some of these markers are routinely available, whereas others are experimental or available only at specialized centers.[25]

Table 4. Markers of Poor Prognosis in CLL

Routinely Available Markers
Advanced Rai or Binet Stage
Peripheral lymphocyte doubling time of <12 months
Lymphocyte count >50 x 10^9/L
Immunophenotyping: CD38+
High B2-microglobulin level
Diffuse marrow histology
Investigational Markers
Lack of IgV_H gene mutation
Expression of ZAP-70 protein
FISH studies showing del 11q, del 17p (loss of p53)

Adapted from Johnston, 2004[25]

Age & Sex

The median age at presentation of patients diagnosed with CLL is in the mid-sixties. Approximately 10% of patients with CLL are under 50 years of age, and have similar clinical features, stage at presentation and survival to those older than 50 years of age. However, the proportion of deaths due to CLL is greater and the risk of Richter's transformation is five-fold higher in the younger group, indicating that a subgroup of younger patients may have more aggressive disease.[26]

The male to female ratio for CLL is 2:1. Women are more likely to have early-stage disease and have a better prognosis than men regardless of stage and age.[27]

Molecular Genetics, Cytogenetics & ZAP-70 Expression

Significant advances have been made in the understanding of CLL biology over the last few years. These advances have helped to highlight novel molecular markers.

Overall, CLL patients can be divided into two groups based on their IgV_H gene mutational status. Patients with mutated IgV_H genes have a better prognosis (median survival 25 years), while those with unmutated IgV_H genes have a poorer prognosis (median survival 8 years).[25,28]

Routine cytogenetics or interphase FISH can detect genetic aberrations that can provide important prognostic information in CLL. Deletions of 17p and 11q have been correlated with outcome. Abnormalities in the p53 pathway caused by chromosomal deletions at 17p13 predict for more aggressive disease with patients surviving an average of only 3 years.[28] Similarly, patients with an 11q deletion have a poorer overall prognosis. Patients with 13q deletions have a more favorable outcome.

Recent research has shown that over-expression of the protein tyrosine kinase ZAP-70 may be a surrogate marker for IgV_H gene mutational status. ZAP-70 over-expression is highly correlated with an unmutated IgV_H phenotype and predicts for a poorer outcome in CLL.[29-31] Outcomes associated with some of these investigational areas are summarized in Table 5.

Table 5. Investigational Markers and Outcomes in CLL

Technique	Outcome Association		
	Favorable	Neutral	Unfavorable
DNA Sequencing: IgV_H gene	mutated		unmutated
Flow Cytometry: ZAP70 (>20% leukemic cells)	Negative		Positive
Flow Cytometry: CD38+	Negative		Positive
Interphase Cytogenetics (FISH)	13q- (sole abnormality)	Normal	+12
		11q-	17p-

Adapted from NCCN Clinical Practice Guidelines in Oncology, 2005[32]

Thus, CLL is a heterogeneous entity with subsets of patients having a more aggressive disease course and worse prognosis. Early identification of these patients will be pivotal in the future refinement of CLL management.

Clinical CLL Management

In general, CLL is an indolent disease and has been incurable with standard therapy. Patients can present with varied clinical courses, many surviving for long periods without need for definitive therapy, while others display a more rapidly progressive course despite intensive treatment. Because of the older age of the patients affected, the low rates of responses achieved with standard chemotherapy, and the lack of improvement in overall survival (OS) with aggressive therapy, the management of CLL has been largely palliative. Previous studies have demonstrated that the treatment of early stage CLL in asymptomatic patients was of no benefit.[33-35] Consequently, treatment has been initiated later in the course of disease for palliative control of symptoms and control of disease progression. However, with the development of more effective therapy and better prognostic tools, this practice is being reevaluated.

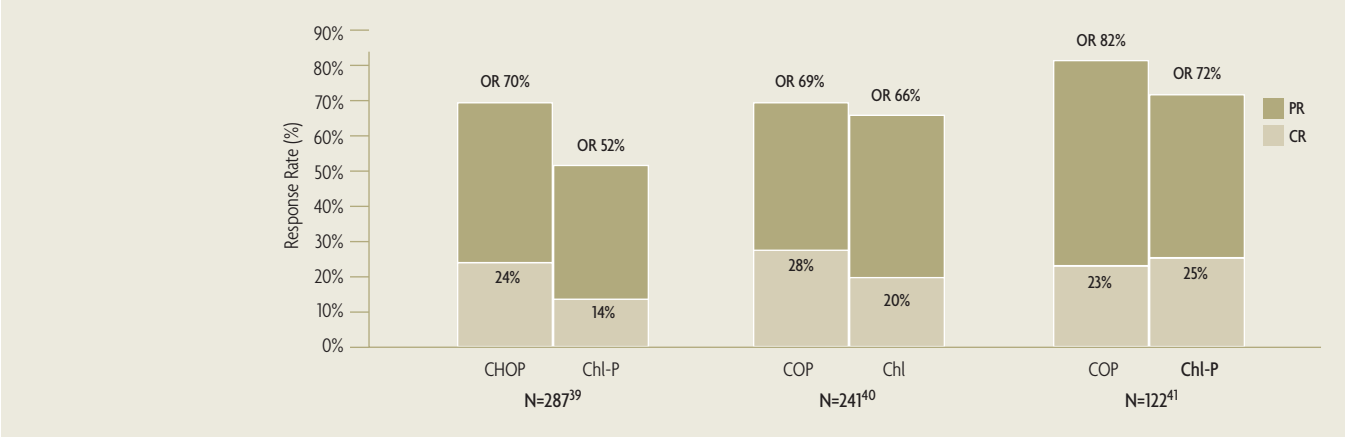
Historical Approach to the Management of CLL: Alkylating Agents

Historically, therapy for CLL has relied on chemotherapy, with alkylator-based regimens being the mainstay of treatment. Chlorambucil, an oral alkylator, has been used frequently because of its ease of administration and favorable toxicity profile. Treatment with chlorambucil is usually continued for many months until optimum response is achieved. Response rates have been reported to range from 30% to 70%; however, complete responses typically do not exceed 10% to 15%.[36,37]

Various alkylator-based combinations have been investigated, primarily in patients with advanced-stage disease. As shown in Figure 1, common alkylator-based combination regimens include COP or CVP (cyclophosphamide, vincristine, prednisone), CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), and chlorambucil combined with prednisone.

Myelosuppression is the primary toxicity associated with alkylator use. An increased number of secondary cancers (eg, skin, colon, or breast) and acute leukemias have also been reported. Alkylator-based combination chemotherapy, particularly with the inclusion of an anthracycline, is more toxic and has not been shown to significantly prolong survival when compared with single-agent chlorambucil in previously untreated patients.[38-40]

Figure 1. Alkylating Agent-based Therapy in Previously Untreated CLL

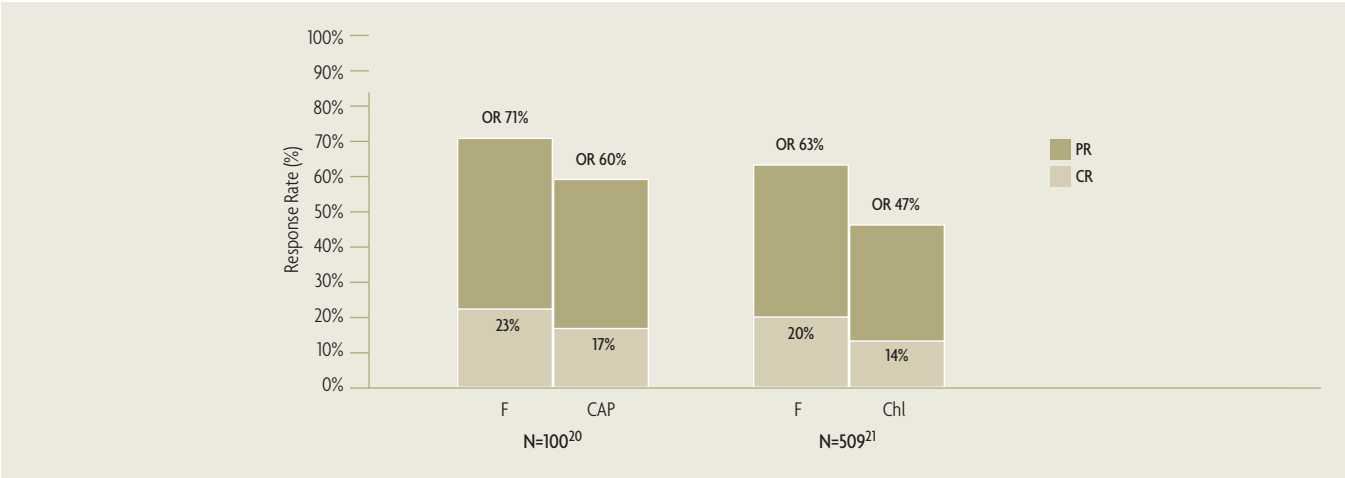


Adapted from: The French Cooperative Group on Chronic Lymphocytic Leukemia, 1990[39]; The French Cooperative Group on Chronic Lymphocytic Leukemia, 1994[40]; Raphel et al., 1991[41]
Abbreviations: C, cyclophosphamide; H, doxorubicin; O, vincristine; P, prednisone; Chl, chloramubucil; OR, Overall Response; PR, Partial Response; CR, Complete Remission

Current Approach to CLL Management

The therapeutic landscape of CLL is changing rapidly. More effective agents have become available, offering a greater number of treatment options with better efficacy.[42] As shown in Figure 2, several studies have demonstrated that purine analogues produce a higher response rate and a longer disease-free interval compared with alkylator-based treatment. This observation has led to the routine use of purine analogues, particularly fludarabine, in the front-line therapy of CLL.[42-46]

Figure 2. Fludarabine vs Alkylating Agent-based Therapy in Previously Untreated CLL



Adapted from: Johnson et al., 1996;[47] Rai et al., 2000.[48]
Abbreviations: F, fludarabine; C, cyclophosphamide; H, doxorubicin; O, vincristine; P, prednisone; Chl, chloramubucil; OR, Overall Response; PR, Partial Response; CR, Complete Remission

Traditional chemotherapy with alkylators and/or purine analogues has achieved complete remissions in 30% to 40% of patients. More recently, the combination of chemotherapy and monoclonal antibodies such as rituximab (chemo-immunotherapy) has almost doubled the complete remission rate to 60% to 70%.[42]

Some first-line treatment combinations included in the 2005 National Comprehensive Cancer Network (NCCN) Practice Guidelines in Oncology are summarized in Table 6.

Table 6. Selected CLL First-Line Therapy Combinations

Fludarabine ± rituximab[49]
Chlorambucil (pulse or continuous) ± prednisone[48]
Cyclophosphamide ± prednisone
CVP (cyclophosphamide, vincristine, prednisone)
FC (fludarabine, cyclophosphamide) + rituximab

Adapted from NCCN Clinical Practice Guidelines in Oncology, 2005[32]

Initiating First-Line Treatment

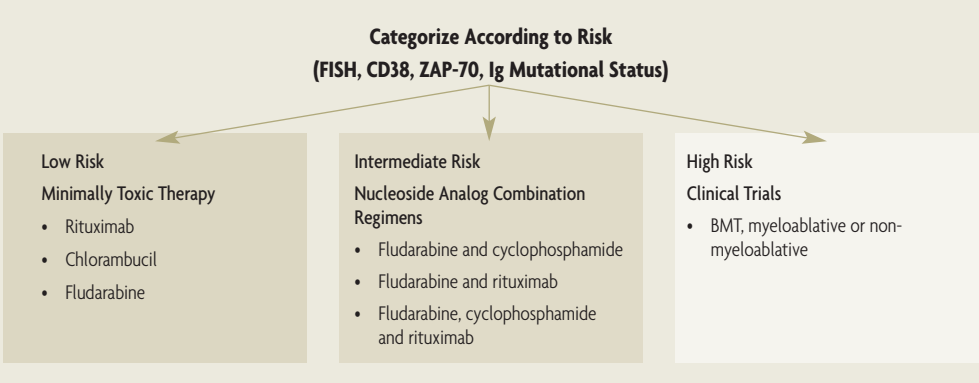
In clinical practice, CLL treatment is often not initiated until patients have an indication for treatment. The established indications for initiating therapy include; the development of B-symptoms (fevers, night sweats, or weight loss), progressive enlargement of lymph nodes or hepatosplenomegaly, obstructive adenopathy, thrombocytopenia and anemia. Other indications include immune cytopenias not responsive to steroids, and rapid lymphocyte doubling time.[6]

Risk-Adaptive Strategies

The Rai or Binet classifications are commonly used to stage patients with CLL.[22,23] These clinical staging systems have been shown to correlate with survival, however there remains significant variability in outcome within individual stage groups. The identification of newer prognostic markers with better predictive capacity (such as IgV_H gene mutational status or expression of ZAP-70) may allow for the development of risk-adaptive strategies. However, these markers remain investigational and are not currently available for routine clinical use. Also, it remains to be determined whether earlier initiation of therapy or more aggressive therapy in poor prognosis patients as determined by these molecular indicators will translate into improved outcomes.

Identifying patients who may benefit from earlier or more aggressive initial management may prove to be critical in changing long term outcome and improving survival in CLL. In recent clinical trials, newer combinations such as chemo-immunotherapy have demonstrated higher complete response (CR) rates and the ability to achieve molecular remissions. These studies have also indicated an improvement in remission duration and possibly overall survival.[46] Investigators have proposed risk-adapted management strategies incorporating the newly recognized biomarkers and more effective treatment strategies. One such attempt at risk stratification is depicted in Figure 3.[50] However, validation of these approaches should be performed within the context of well designed clinical trials.

Figure 3. Proposed Risk-Stratified Treatment of CLL



Adapted from: Finn et al, 2004

Determining Response to Therapy

Response to therapy or overall response (OR) in CLL is most often determined using the National Cancer Institute Working Group response criteria defining complete response (CR), partial response (PR), stable disease (SD), and progressive disease. These response criteria are listed in Table 7.[20] However, more sensitive evaluation techniques have since become available, and recent clinical trials have frequently reported nodular PRs (meets all the criteria for CR but with a few remaining lymphoid nodules in bone marrow) and molecular complete remissions.

Table 7. NCI Working Group Criteria for Response in CLL

Complete Response(CR)	Absence of lymphadenopathy, hepatosplenomegaly, and constitutional symptoms; normalization of CBC (neutrophils >1,500/µL, platelets >100,000/µL, hemoglobin >11g/dL, lymphocytes <4,000/µL); bone marrow biopsy shows normal cellularity; lymphocytes <30%; nodules and infiltrates in the bone marrow are permitted. Duration of response >2 months.
Partial Response (PR)	At least 50% reduction in absolute blood lymphocyte count and in lymphadenopathy and/or 50% reduction in splenomegaly or hepatomegaly; neutrophils >1,500/µL or 50% improvement over baseline; platelets >100,000/µL or 50% improvement over baseline; hemoglobin >11g/dL (not supported by transfusions) or 50% over baseline. Duration of response: >2 months.
Stable Disease (SD)	No complete or partial response; or no progression.
Progressive Disease (PD)	At least one of the following: >50% increase in size of at least two lymph nodes, or new palpable lymph nodes; ≥50% increase in hepatomegaly or splenomegaly, or appearance if previously absent; transformation to a more aggressive histology (Richter or PLL); >50% increase of absolute peripheral blood lymphocyte count.

Eliminating Minimal Residual Disease (MRD)

The availability of more effective treatments for CLL is prompting a reconsideration of therapeutic goals. Recent investigations have focused not only on eliminating visible disease, but on eradicating the malignant clone.[46] However, the standard response definitions do not reflect this level of sensitivity of assessment. For example, the NCI-WG definition of CR is based on <30% lymphocytes in the marrow, which does not preclude the presence of minimal residual disease (MRD). A more sensitive method of evaluating the response to CLL therapy is the assessment of MRD by either polymerase chain reaction or four-color flow cytometry.[43]

Recently, researchers have reported that patients who achieve a negative MRD status have longer disease-free survival and possibly longer overall survival than patients with detectable disease post treatment.[51] The improved quality of response as measured by the attainment of disease eradication at a molecular level appears to have therapeutic benefit.[46,52] To date, the routine use of purine analogues has resulted in increased CR rates and prolonged disease-free survival. Unfortunately, almost all patients have detectable MRD following therapy and will eventually experience a relapse. Therefore, clinicians are in need of new treatment modalities targeted at the eradication of MRD, which may lead to improved long-term outcomes. The addition of monoclonal antibodies such as rituximab to chemotherapy has been a step forward in achieving this goal.

Options in Relapsed and Refractory CLL

When relapse occurs following treatment, retreatment with the same drugs (such as chlorambucil or fludarabine) can induce new remissions, but overall outcomes begin to deteriorate. The choice of therapy for relapsed disease is guided by prior treatments received and responses achieved. Fludarabine is often used as a treatment option for relapse after first-line treatment or for alkylator-refractory disease. Patients who become refractory to fludarabine have poor prognoses with limited effective treatment options. Therefore, there is an unmet need for innovative therapies for relapsed or refractory disease.[6,46,53]

Some common second-line treatment combinations included in the 2005 NCCN Practice Guidelines in Oncology are summarized in Table 8. These new treatment options include rituximab, a monoclonal antibody that is currently being evaluated in clinical trials.

Table 8. Selected Second-Line CLL Therapy Combinations

Alemtuzumab[46]
PC (pentostatin, cyclophosphamide)[46] ± rituximab
Chemotherapy as in first line ± rituximab or alemtuzumab

Adapted from NCCN Clinical Practice Guidelines in Oncology, 2005[32]

Rituximab: Rationale for Use in CLL

Advances in prognostic factors and the detection of MRD have facilitated the prediction of outcomes and optimization of therapy of CLL. However, CLL remains incurable and more effective treatments are required. As a class, monoclonal antibodies have dramatically changed the therapeutic landscape for patients with lymphoproliferative disorders and have become an attractive option for CLL therapy. Monoclonal antibodies offer selectivity due to the tumor target expression on the surface of CLL cells, as well as a different mechanism of action compared to that of traditional chemotherapeutic agents.[54]

Because CLL is a CD20-positive disease, the chimeric monoclonal antibody rituximab, which targets CD20 and has proven effective in the treatment of non-Hodgkin's lymphoma, has been evaluated in CLL. Similar to other indolent lymphoproliferative disorders where rituximab has proven beneficial, CLL and small lymphocytic lymphoma (SLL) have long natural histories and long disease-free intervals. Although rituximab is not currently indicated for use in patients with CLL, a significant body of efficacy and safety data has been reported.

Initial investigations of rituximab in CLL focused on its use as monotherapy, with promising responses observed. Based on this initial demonstration of efficacy and its distinct mechanism of action compared with traditional chemotherapy, rituximab has been evaluated in combination with other agents in CLL. Supporting this strategy of chemo-immunotherapy, in vitro data have suggested that rituximab combined with a number of chemotherapeutic agents including purine analogues and alkylating agents can markedly increase overall cytotoxic effects.[46,55]

The clinical trials summarized in the following sections demonstrate that rituximab is safe and effective as a single agent and as part of combination therapy in the management of CLL. Its utility has been demonstrated in the relapsed/refractory setting and in front-line therapy of CLL.

Rituximab Monotherapy

Rituximab Monotherapy for Relapsed/Refractory CLL

The use of standard-dose (375 mg/m^2) single agent rituximab in patients with relapsed/refractory CLL has yielded variable results. Although responses were reported in up to 30% of patients, these were largely partial responses and some reports suggested poorer efficacy in CLL than in other indolent lymphomas.[56,57] These initial reports of rituximab monotherapy in previously treated patients with CLL are summarized in Table 9.

Table 9. Standard-Dose Rituximab Monotherapy in Relapsed/Refractory CLL

Author	N	Response (%)	Response Duration (months)
McLaughlin[57]	30	13	NA
Winkler[58]	10	10	NA
Nguyen[59]	12	0	0
Foran[60]	29	14	NA
Huhn[61]	28	25	4.5
Itala[62]	24	35	3.0

SELECTED STANDARD-DOSE RITUXIMAB MONOTHERAPY REPORTS

Huhn and colleagues reported results of a phase II trial of single agent rituximab in 28 patients with relapsed/refractory or prolymphocytic leukemia (Binet B and C stages).[61] Patients received four weekly intravenous infusions of 375 mg/m^2 rituximab. Partial responses were observed in 7 patients (25%), with a median response duration of 20 weeks. The authors concluded that rituximab was clinically active; however the duration of response was short and suggested that combination with other agents was warranted.

Similarly, Itala and colleagues assessed the utility of standard-dose rituximab (375 mg/m^2 given once weekly for four doses) in 24 heavily pretreated patients with CLL.[62] The overall response rate was 35% (all responses were partial), with a median duration of response of 12.5 weeks.

Adverse events reported with the use of standard-dose rituximab monotherapy were generally mild, with the most frequently reported events being infusion-related reactions during the first infusion of rituximab. Additional references on rituximab monotherapy are provided in Appendix 1.

Rituximab Monotherapy Schedule and Dose Modifications

Based on the variable results seen with standard-dose rituximab monotherapy, investigators explored alternative dosing schedules and higher doses of rituximab. These efforts demonstrated higher response rates, although responses were primarily partial remissions as summarized in Table 10.[63,64]

Table 10. Rituximab Monotherapy in Relapsed/Refractory CLL

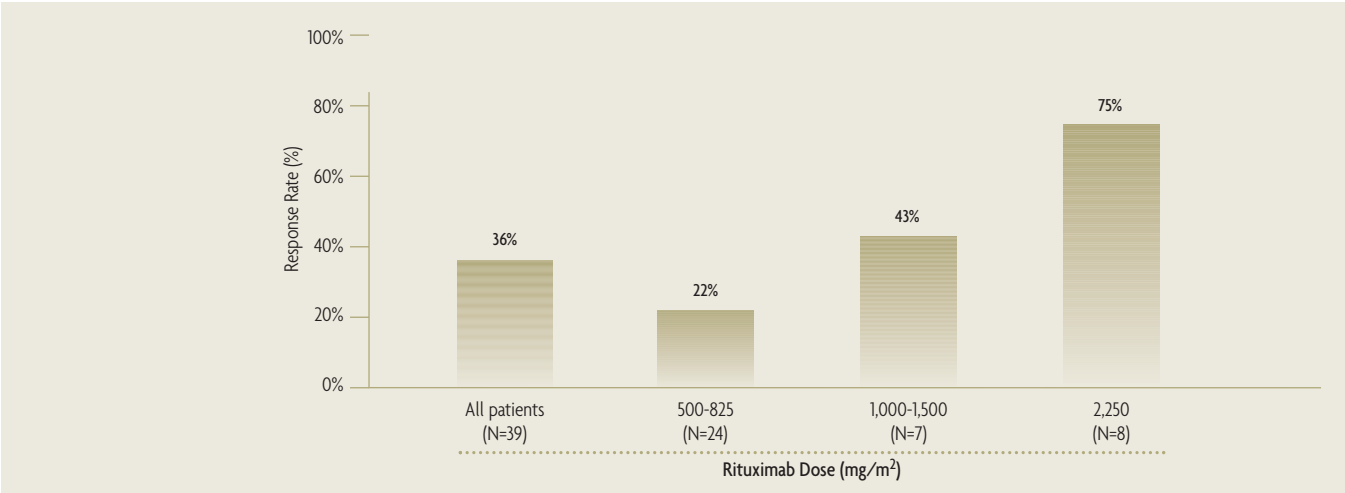
Author	N (evaluable)	Rituximab Dose	Response	Response Duration & Disease Control
O'Brien[64]	40 (CLL Subset)	Dose escalation 375 – 2250 mg/m^2	36% PR (all doses) 75% PR (highest doses)	Not reported
Byrd[63]	33 (CLL or SLL)	250 or 375 mg/m^2 day 3, thereafter 3 times weekly x 4	OR 45% 3% CR 42% PR	Median duration of response 10 months

Rituximab Dose Escalation Study

O'Brien and colleagues conducted a phase I/II dose-escalation study of rituximab in patients with CLL with the following objectives: to define the maximum tolerated dose; to evaluate first-dose reactions in patients with high circulating lymphocyte counts; and to assess the efficacy at higher versus lower doses.[64] Fifty patients with either previously treated CLL (n=40) or other B-cell lymphoid leukemias (n=10) were treated with four weekly infusions of rituximab. The first dose for all patients was 375 mg/m^2, which was followed by three weekly doses at an escalated level that ranged from 500 to 2250 mg/m^2.

The overall response rate was 40%, with 36% of patients achieving a partial response (PR). Analysis of response by dose is shown in Figure 4. The highest response rate was observed with the 2250 mg/m^2 dose.

Figure 4. Responses to higher doses of rituximab monotherapy in previously treated CLL



Adapted from O'Brien et al., 2001

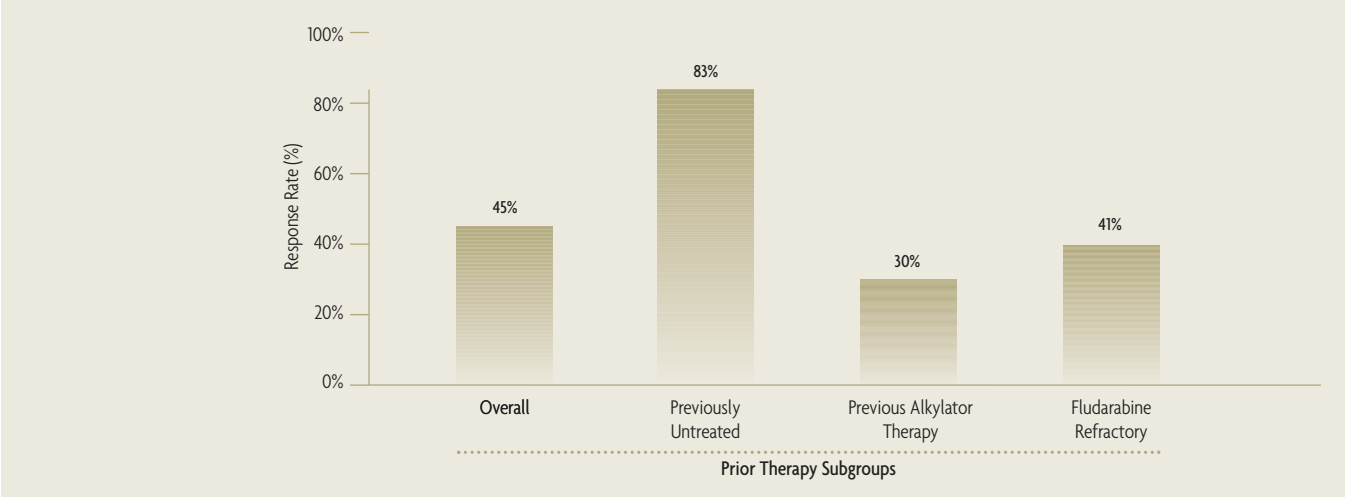
With the first dose (375 mg/m^2), the most common side effects were fever and chills observed in 94% of patients. Six patients experienced grade 3-4 toxicity including fever, chills, dyspnea and hypoxia; five patients had significant hypotension; one patient had severe hypertension. Toxicity was generally mild with subsequent doses which were escalated until 2250 mg/m^2 was reached. No grade 3-4 toxicity was noted, but 67% of patients experienced grade 2 toxicity: fever, chills, nausea and malaise.

Analysis of response by prior fludarabine exposure revealed that the response rate in fludarabine-sensitive patients was 56% versus 20% in fludarabine-refractory patients. The authors concluded that rituximab has significant activity in CLL at the higher dose levels.[64]

Thrice-Weekly Schedule

In an attempt to improve the efficacy of single agent rituximab, Byrd and colleagues explored a thrice-weekly schedule.[63] Thirty-three patients with CLL or SLL were enrolled, the majority of whom had been treated previously. The median age was 66 years (range 50 – 80 years) and the median number of prior treatments was 2. Six previously untreated patients were included.

Figure 5. Response to rituximab thrice weekly schedule by previous therapy



Adapted from Byrd et al, 2001[63]

The overall response rate in this trial was 45%. Median response duration for the 15 responding patients was 10 months (range 3-17+) and the median time to progression was 11 months (range 0-18+). The investigators observed that, although not significant, responses appeared to correlate with prior treatment status, but did not correlate with age, stage, presence of bulky disease, or CD20 density. Specifically, as shown in Figure 5, patients who were previously untreated had a higher response rate (83%) than those who were treated with alkylator therapy (30%) or who were refractory to fludarabine (41%).

Overall, the thrice-weekly schedule was well tolerated. Thirteen patients were observed to have transient hypoxemia, hypotension, or dyspnea requiring temporary cessation of therapy and supportive intervention.

The authors concluded that rituximab administered in a dose-dense approach had acceptable toxicity and demonstrated clinical efficacy in patients with CLL.

Rituximab Monotherapy in Initial Therapy for CLL

Based on the activity observed in previously treated CLL, investigators evaluated single agent rituximab in the first-line setting, as summarized in Table 11.

Table 11. Rituximab in First-Line CLL Therapy

Author	N	Regimen	Response	Response Duration & Disease Control
Thomas[65]	21	R	OR 90% 19% CR 19% NPR 48% PR	At a median follow up of 8 months, one patient had progressed
Hainsworth[66]	44	R (+ R maintenance)	OR 58% 9% CR	At a median follow up of 20 months, median progression-free time was 18.6 months

R= rituximab; OR= overall response; CR= complete response; NPR= nodular partial response; PR= partial response

SINGLE AGENT RITUXIMAB AS INITIAL THERAPY

Thomas and colleagues explored single agent rituximab as a potentially active, low-toxicity alternative to observation for asymptomatic patients with high risk CLL.[65] Patients were eligible if they had untreated Rai stage 0–II CLL with an elevated beta-2 microglobulin and no indication for therapy according to the NCI Working Group criteria. Rituximab was administered at 375 mg/m^2 weekly for 8 weeks. Thirty one patients were enrolled and twenty-one were evaluable for response. The overall response rate observed was 90%, with 19% complete responses, 19% nodular partial responses, and 48% partial responses.

No unexpected toxicities were observed. The majority of adverse events consisted of grade I-II fever, chills, and/or hypotension related to the first infusion. The authors concluded that rituximab has significant activity in early stage CLL; however, its impact on survival and time to progression will require further evaluation.

RITUXIMAB MAINTENANCE THERAPY

Hainsworth and colleagues also evaluated the use of rituximab monotherapy in previously untreated patients with CLL. A total of 44 patients with either SLL (N=5) or CLL (N=39) received weekly rituximab at a dose of 375 mg/m^2 for four weeks. Patients who achieved an objective response were given a further maintenance course of rituximab using the standard 4-week schedule every six months for a maximum of four courses. Following initial treatment, 22 of 43 evaluable patients (51%) achieved an objective response. Following scheduled maintenance treatment, further disease reduction was noted in five patients for an overall response rate of 58%. After a median follow-up of 20 months, 24 patients remained progression-free and the median progression-free interval was 18.6 months.

Rituximab treatment was well tolerated with only two episodes of grade 3 to 4 infusion-related toxicity reported. No cumulative toxicity or opportunistic infections occurred.

The authors concluded that rituximab was active as first-line therapy for CLL/SLL, producing substantially higher responses than previously reported in relapsed or refractory patients. However, additional follow-up is required to fully assess the impact of this treatment strategy.

Clinical Efficacy of Rituximab Combinations

Recent studies have focused on the use of rituximab in combination with other agents to evaluate for possible synergy and the potential for greater efficacy. Pre-clinical studies have suggested that fludarabine may downregulate complement resistance proteins, such as CD46, CD55, and CD59, thereby enhancing the activity of rituximab when used in combination with fludarabine (FR) or fludarabine and cyclophosphamide (FCR).[51] Hence, rituximab has been evaluated as part of combination therapy for both relapsed/refractory and previously untreated patients with CLL.

Combination Therapy for Relapsed/Refractory CLL

Numerous trials, some of which are shown in Table 12, have reported promising activity of rituximab combinations in the treatment of relapsed/refractory CLL.

Table 12. Rituximab Combinations in Relapsed/Refractory CLL

Author	N	Regimen	Response	Response Duration & Disease Control
Weirda[67]	177	FCR	OR 73% 25% CR: 12/37 (32%) achieved molecular remission 16% NPR 32% PR	Median time to progression 28 mo. (range 3 – 49 mo)
Savage[68]	10 (CLL, 4 pre-treated)	FR	4/10 CR 6/10 PR	Not reported
Gupta[69]	22	RCD	OR 77% 36% (8/22) CR 41% (9/22) PR	Median duration of response 7 mo (range 2 – 13+ mo)
Tsimberidou[70]	19 (CLL)	Hyper-CVXD + R + GM-CSF	OR 37% (7/19) 5% CR (1/19)	Not reported for CLL group Median survival of entire group (N=49) 8.5 mo
Faderl[71]	32	R+A	OR 52% 8% CR 4% NPR 40% PR	Not reported for CLL group Median survival of entire group (N=48) 6 mo
Nabhan[72]	12	R+A (dose escalation)	1/12 PR	Not reported

F=fludarabine, C=cyclophosphamide, R=rituximab, A=alemtuzumab, D=dexamethasone

MD ANDERSON FCR REGIMEN

Since fludarabine is considered the most active single agent in CLL, investigators at the MD Anderson Cancer Center developed a combination of fludarabine, cyclophosphamide, and rituximab (FCR as detailed in Figure 6) based on in vitro and in vivo data indicating complementary activities of these agents.[67] In a report by Weirda and colleagues, 177 previously treated patients with CLL received up to 6 cycles of FCR. Complete responses were achieved in 25% of patients (45/177), and nodular partial remissions and partial remissions were achieved in 16% (28/177) and 32% (57/177) of patients respectively, for an overall response rate of 73%. Of note, 12 (32%) of the 37 of the complete responders achieved a molecular remission in the bone marrow, demonstrating good quality remissions.

The authors reported that FCR was reasonably well tolerated. Adverse events associated with rituximab were primarily infusion-related and occurred in 63% of patients, similar to the toxicity seen in other studies. Myelosuppression was the most frequent toxicity, with grade 3 and 4 neutropenia noted in 21% and 41% of 529 assessable cycles.

Figure 6. MD Anderson FCR Regimen

Total of 6 treatment cycles, each with a duration of 28 days:

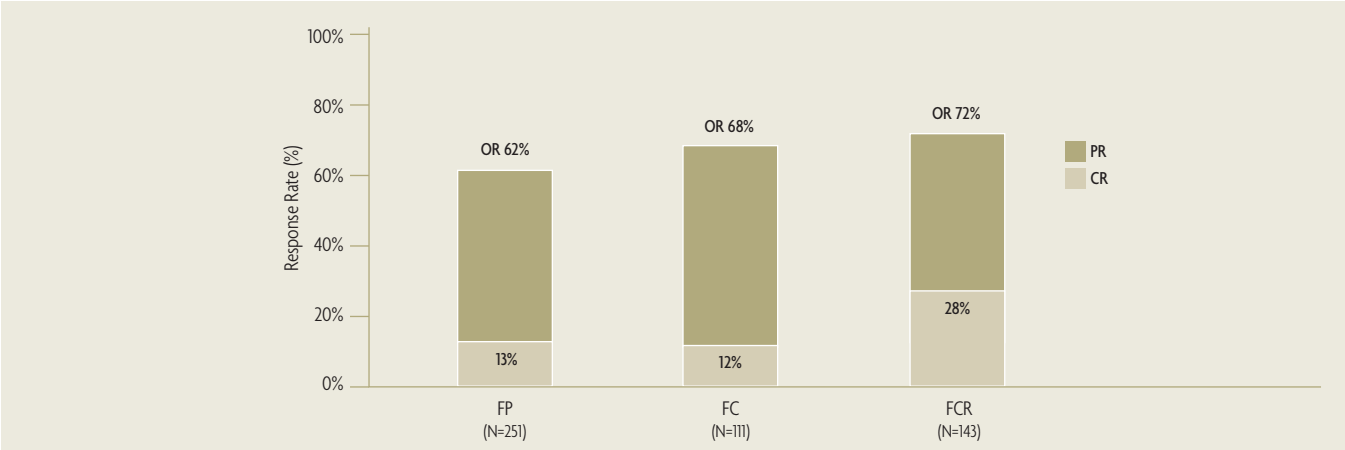
Cycle 1:
Rituximab 375 mg/m^2 i.v. day 1
Fludarabine: 25 mg/m^2/day i.v. days 2-4
Cyclophosphamide: 250 mg/m^2/day i.v. days 2-4

Cycles 2-6:
Rituximab 500 mg/m^2 i.v. day 1
Fludarabine: 25 mg/m^2/day i.v. days 1-3
Cyclophosphamide: 250 mg/m^2/day i.v. days 1-3

MD ANDERSON FCR VS HISTORICAL CONTROL

To explore whether the FCR regimen represented true progress in the treatment of relapsed/refractory CLL, the same group of investigators compared preliminary results of the FCR trial with results from two previous studies performed at MD Anderson Cancer Center.[73] Specifically, they compared 251 patients treated with fludarabine plus prednisone (FPN) between October 1984 and July 1993; 111 patients treated with fludarabine plus cyclophosphamide (FCN) between April 1995 and September 1999; and 143 patients treated with FCR between November 1999 and December 2001. The results from the comparison are illustrated in Figure 7.

Figure 7. Comparison of FCR vs Historical Populations



Adapted from Wierda et al., 2003 Abbreviations: F, fludarabine; P, prednisone; C, cyclophosphamide; OR, Overall Response; PR, Partial Response; CR, Complete Remission

In the analysis of all 505 patients, treatment with FCR was associated with a significant improvement in overall survival ($p<0.0001$) adjusting for significant pretreatment prognostic variables. For patients achieving a CR, the median survival for the FP and FC groups was 49 and 67+ months, respectively, and was not yet reached for the FCR group. FCR was significantly ($p<0.0001$) associated with an increased CR rate compared to other regimens when adjusted for pretreatment prognostic variables. Additionally, for patients achieving a partial response (nodular PR and PR), median survivals for the FR and FC groups were 36 months and 38 months, respectively; but median survival was not reached for the FCR group (Table 13). Median survival for the non-responders was similar for all three groups, suggesting that improved supportive care over time did not have a major impact on the improvement seen in overall survival.

Table 13. Survival Comparison – FCR vs Historical Populations

Median Survival	FP	FC	FCR
Patients achieving a CR	49 mo	67+ mo	not reached
Patients achieving a NPR or PR	36 mo	38 mo	Not reached

RITUXIMAB, CYCLOPHOSPHAMIDE, AND DEXAMETHASONE (RCD)

Gupta and colleagues reported results with the RCD combination in 22 previously treated patients with advanced CLL.[69] The RCD regimen consisted of rituximab 375 mg/m^2 IV on day 1, cyclophosphamide 750 mg/m^2 IV on day 2, and dexamethasone 12 mg/day on days 1-7. Cycles were repeated every 4 weeks until a maximum response was observed.

After a median of four cycles, eight patients (36%) achieved a complete response and nine patients (41%) a partial response. The median duration of response was seven months (range 2+ - 13+).

Toxicities in this series were minimal, with grade 1 or 2 rituximab infusion-related reactions experienced by 10 of 20 patients; grade 4 neutropenia occurred in three patients, two patients developed pulmonary infections, and one developed sepsis. No patients were taken off treatment due to toxicity.

The authors concluded that this combination was effective in achieving durable responses in patients with previously treated advanced CLL.

RITUXIMAB + ALEMTUZUMAB

As both rituximab and alemtuzumab are active in patients with CLL, and both differ in their basic targets, it has been hypothesized that combining them may be of potential benefit in refractory patients.[74] Preliminary studies investigating their use in combination, with or without other cytotoxics, have been reported.[71,72,75] Nabhan and colleagues published a pilot study of 12 patients with refractory CLL who were given escalating doses of alemtuzumab in combination with rituximab administered weekly at a dose of 375 mg/m^2. They demonstrated that the combination was feasible and associated with acceptable toxicity. Activity was demonstrated, with one patient achieving a partial response.[72]

Similarly, Faderl and colleagues reported results of the combination of rituximab and alemtuzumab in 48 patients with relapsed/refractory lymphoid malignancies, of whom 32 had CLL and 9 had CLL/prolymphocytic leukemia.[71] The overall response rate was 52%, including 8% CR, 4% nodular PR, and 40% PR. At a median follow up of 6.5 months, median time to progression was 6 months (range 1-20 months) and median survival was 11 months (11+ months for responders vs 6 months for non-responders). Most toxicities were infusion-related, primarily grade 2 or lower. The authors concluded that the combination was feasible and clinically active with an acceptable safety profile in a group of patients with poor prognosis.

CFAR REGIMEN

In an effort to improve upon the results of the FCR regimen in previously treated patients, Wierda and colleagues added alemtuzumab to the combination. They reported results from 31 patients, 21 of whom were evaluable for response.[75] The CFAR regimen consisted of cyclophosphamide 250 mg/m^2 on days 3-5; fludarabine 25 mg/m^2 on days 3 to 5; alemtuzumab 30 mg on days 1, 3, and 5; and rituximab 375-500 mg/m^2 on day 2. Cycles were repeated every 28 days for a total of 6 planned cycles. Overall response rate was 52%, including complete responses in 14% and partial responses in 38%. All patients who achieved a CR were negative for minimal residual disease in the bone marrow as assessed by 2-colour flow cytometry.

The most common non-hematologic toxicities reported were grades 1-2 and included fatigue, fever, rash/hives, nausea, upper respiratory tract infection, and sinusitis. Grade 3-4 toxicities were much less common, consisting of nausea/vomiting and shortness of breath. Grade 3 and 4 neutropenia occurred in 23% and 16% of 70 evaluable treatment cycles, respectively.

The authors concluded that this early analysis indicated that CFAR was an active regimen in patients with relapsed/refractory CLL and that toxicities were in keeping with the known toxicities of these agents.

Rituximab Combinations in Initial Therapy for CLL

Rituximab combinations have also been evaluated as part of initial therapy for patients with CLL, and recent reports on these combinations are summarized in Table 14.

Table 14. Rituximab Combinations in First-Line CLL Therapy

Author	N	Regimen	Response	Response Duration & Disease Control
Schultz[76]	31 (20 previously untreated)	FR	OR 87% (27/31) 32% (10/31) CR OR in first-line 85%	Median duration of response 75 weeks (19 mo) at a median follow-up of 54 weeks.
Byrd[49]	104 Total (51 concurrent FR) (53 sequential FR)	FR	Concurrent FR OR 90% 47% CR, 43% PR Sequential FR OR 77% 28% CR, 49% PR	Median duration of response not reached at a median follow-up of 23 months.
Keating[77]	224	FCR	OR 95% 70% CR 10% NPR 15% PR	As of 2003, 154 (99%) of the 156 CRs were alive; 9 had relapsed but remained alive.

Abbreviations: R, rituximab; FR, fludarabine + rituximab; FCR, fludarabine, cyclophosphamide, rituximab; Overall Response, OR; Partial Response, PR; Nodular PR, NPR; Complete Remission, CR

FLUDARABINE PLUS RITUXIMAB (FR))

An early phase II study of the combination of fludarabine and rituximab was reported by Schultz and colleagues.[76] This trial evaluated the FR combination in 34 patients (31 evaluable for response), of whom 20 were previously untreated. Patients received fludarabine 25 mg/m^2/day on days 1-5 every 28 days for four cycles and rituximab 375 mg/m^2 on days 57, 85, 113, and 151. The overall response rate was 87% (27 of 31 evaluable patients) with ten patients achieving a complete response. Of the 20 previously untreated patients, 17 responded (85%) and 5 achieved a complete response. The median duration of response was 75 weeks.

Adverse events consisting of fever, chills, and rash were generally mild and were mainly associated with the first rituximab infusion. Hematologic toxicity consisted of neutropenia grade I-II in 26% and grade III-IV in 42%; and thrombocytopenia grade I-II in 19% and grade III-IV in 9%. One patient died of cerebral bleeding during prolonged thrombocytopenia after the second cycle of fludarabine. There were a total of 32 infections in 16 patients, none of which were fatal.

The authors concluded that the combination of rituximab and fludarabine was a feasible and effective treatment in this group of patients.

In a larger study of the FR regimen in initial therapy of CLL, the Cancer and Leukemia Group B randomized patients with previously untreated CLL to sequential or concurrent FR. Patients in the sequential arm received fludarabine at a dose of 25 mg/m^2 on days 1-5 every 28 days for six cycles, followed by rituximab two months later at a dose of 375 mg/m^2 weekly for four weeks in patients with stable disease or better.[49] Patients in the concurrent arm received therapy identical to that in the sequential arm except that rituximab 375 mg/m^2 was also given with the fludarabine on day 1 and day 4 of cycle 1 and then on day 1 of cycles 2–6. A total of 104 patients were randomized, with 53 patients given the sequential regimen and 51 patients the concurrent regimen.

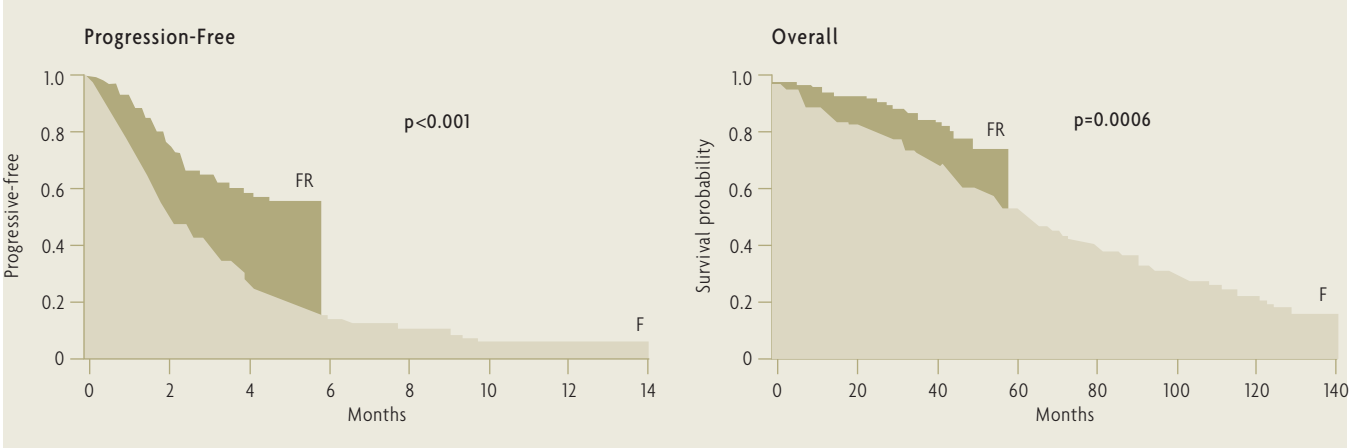
The overall response rate with the sequential regimen was 77% (28% CR, 49% PR) compared with 90% (47% CR, 43% PR) with the concurrent regimen. At a median follow up of 23 months, the median response duration had not been reached with either regimen. The authors concluded that rituximab administered concurrently with fludarabine in previously untreated CLL patients demonstrated marked clinical efficacy and acceptable toxicity. They suggested that a phase III trial of the combination be done; it is currently underway.

CALGB 9712 FR vs Historical Control

To further assess the value of the addition of rituximab to fludarabine therapy, Byrd and colleagues recently carried out a retrospective comparison of patients treated with FR in the CALGB 9712 trial with patients treated with fludarabine alone in a previous CALGB trial (CALGB 9011).[78] Patient entry criteria were similar in both trials. In a multivariate analysis controlling for pretreatment characteristics, patients receiving fludarabine and rituximab had a significantly better progression-free survival (p<0.0001) and overall survival (p<0.0006) compared with patients receiving fludarabine alone (Figure 8).

Further analysis of these two trials by Morrison and colleagues demonstrated that the addition of rituximab to fludarabine therapy did not increase the risk or severity of infections in first-line therapy of CLL and that there were no major differences in infections between concurrent and sequential administration.[79]

Figure 8. Survival Comparison: CALGB 9712 FR vs CALGB 9011



Adapted from Byrd et al, 2005

MD Anderson FCR Regimen in Initial Therapy of CLL

The FCR regimen has also been investigated in first-line therapy for CLL. In a recent report by Keating and colleagues, 224 patients were treated with FCR (as detailed previously in Figure 6) as initial therapy for CLL.[77] The use of FCR in this setting resulted in an impressive 95% overall response rate, including 70% complete responses, 10% nodular partial responses, and 15% partial responses. Notably, two thirds of patients evaluated with flow cytometry had less than 1% CD5/CD19 co-expressing cells in their bone marrow after therapy. There was a strong correlation between the lack of detectable disease on flow cytometry and the risk of relapse.

Overall, a moderate level of toxicity was seen. Grade 3 to 4 neutropenia occurred in 52% of treatment cycles and major and minor infections were seen in 2.6% and 10% of courses, respectively. One-third of the 224 patients experienced at least one episode of infection and 10% had fever of unknown origin.

The authors concluded that FCR produced a high rate of complete responses in previously untreated CLL. Furthermore, most patients had no detectable disease on flow cytometry at the end of therapy. At the time of reporting, 15 responders had clinically relapsed and thirteen patients had died. The time-to-treatment failure analysis demonstrated that 69% of patients were projected to be failure-free at 4 years (95% CI, 57% to 81%).

Current Research Directions: Rituximab in CLL

Data to date have demonstrated that rituximab in combination with chemotherapy has considerable activity in CLL, producing a high rate of clinical and molecular responses. Although curative therapy may not yet exist for CLL, these trials demonstrate that immuno-chemotherapy can achieve a high rate of durable remissions and may improve overall survival. Moreover, the trials establish a rationale for further clinical research into rituximab combination therapy for CLL.[45]

Moving forward, it will be important to explore how different prognostic subsets of patients respond to rituximab therapy, as it is likely that risk-adaptive strategies will be increasingly applied in the management of CLL.[45]

Prediction of Response

Several newer prognostic factors, including unmutated IgV_H gene mutational status and cytogenetic abnormalities such as p53 mutations, have been associated with a shortened progression-free survival and overall survival in patients with CLL. The impact of these factors on outcome following rituximab combination therapy has not been fully explored. Hence, Byrd and colleagues assessed a number of these factors within the data set from the CALGB 9712 trial.[80] Of the 104 patients enrolled, pre-treatment samples were available for 88 patients. The impact of IgV_H gene mutational status on outcome was examined. A total of 46 out of 75 patients (61%) had IgV_H unmutated CLL and 29 patients (39%) had IgV_H mutated CLL. The complete response rate was not significantly different between patients with mutated CLL and those with unmutated CLL (52% versus 43%, p=0.49). However, the median progression-free survival was significantly longer in patients with mutated CLL than in those with unmutated CLL (46 versus 32 months, p=0.03).

In addition, these investigators sought to define a high-risk group of patients with CLL based on these newer molecular markers. They proposed that patients with any of the following characteristics should be considered high-risk: IgV_H unmutated, del (17p), del (11q), or non-silent p53 mutations. Using this classification, 35 patients in the trial were assigned to the low-risk and 53 to the high-risk groups. The authors concluded that high-risk patients did in fact have a shorter progression-free survival with the fludarabine and rituximab combination and thus defined a subset of patients for whom additional novel treatment approaches should be considered.

Loss of CD20 Expression

Loss of CD20 expression following rituximab therapy has been reported infrequently in various B-cell non-Hodgkin's lymphomas and this remains an area of ongoing research. Savage and colleagues reported thirteen CLL cases with loss of expression of CD20 on malignant lymphocytes following treatment with rituximab.[81] Two cases demonstrated Richter's transformation concurrent with loss of CD20 expression in the large cell component. In six cases, the loss of CD20 expression was transient with subsequent biopsies demonstrating CD20 positivity. In one additional case, partial reversion was suggested by the emergence of a population of cells with dim CD20 expression. In some cases, the emergence of a CD20 negative clone was delayed by several years following rituximab therapy. The underlying mechanism and prevalence of conversion to a CD20 negative state is unclear. However, re-evaluation of CD20 expression may be warranted if retreatment with rituximab is considered.

Special Considerations: Autoimmune Complications

Autoimmune-related cytopenias can be observed in patients with CLL, manifesting as autoimmune hemolytic anemia (AIHA), immune-mediated thrombocytopenia, immune-mediated granulocytopenia, and pure red cell aplasia.[10] Of these events, AIHA is the most frequent and its severity does not necessarily correlate with the severity of CLL. Prednisone is the most commonly used treatment for autoimmune complications, with high initial response rates, although relapses are not uncommon. Cyclosporin A may be effective in steroid-refractory patients. The monoclonal antibodies, rituximab and alemtuzumab, have been reported to produce satisfactory responses in patients failing standard therapies.[5] In an open-label nonrandomized trial, 8 patients with CLL and steroid-refractory AIHA were treated with rituximab at a dose of 375 mg/m^2 administered on day 1 in combination with cyclophosphamide and dexamethasone (cyclophosphamide 750 mg/m^2 on day 2; and dexamethasone 12 mg given intravenously on days 1 and 2 and orally on days 3 through 7), with cycles repeated every 4 weeks until best response. An improvement in hemoglobin levels was seen in all 8 patients, 5 of whom converted to a negative Coombs test following therapy.[82] Similarly, rituximab achieved an improvement in hemoglobin level in all of 5 patients with a CD20-positive lymphoproliferative disorder complicated by AIHA refractory to steroids and chemotherapy.[83] In both of these reports, infusion-related reactions such as chills and fever were noted but were minor in nature.

Conclusions: Rituximab in CLL Therapy

Fludarabine remains the most effective agent in the management of CLL. However, trials investigating different chemotherapy regimens have failed to yield a curative therapy for CLL and have not demonstrated a survival advantage of one regimen over another. There is a need for newer and more effective treatment strategies. Recently, rituximab has been investigated in patients with relapsed/refractory and previously untreated CLL. Although rituximab monotherapy appears to have modest activity, the combination of rituximab and chemotherapy significantly improves response rates and the quality of the responses achieved. In fact, newer combinations such as FCR have demonstrated an ability to achieve a high rate of molecular complete remissions, which has been associated with an improvement in outcome.

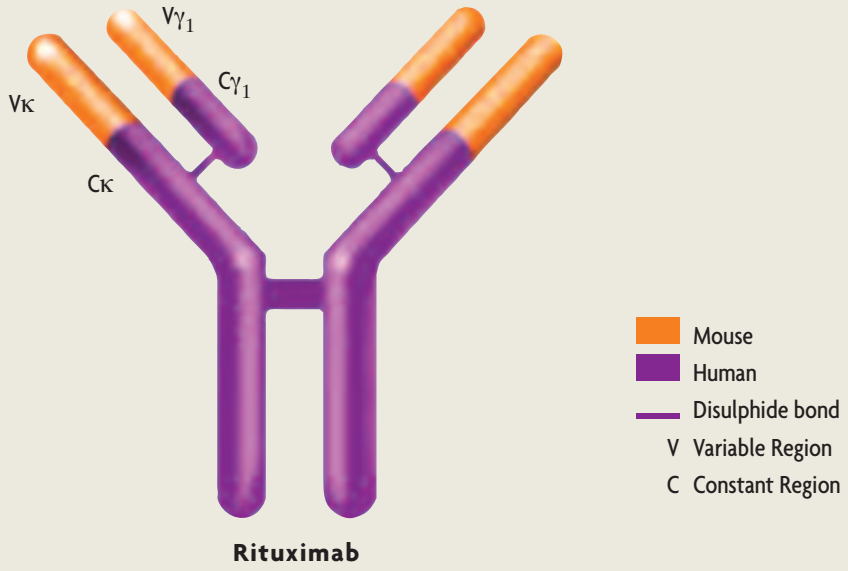
The body of evidence to date supporting the use of rituximab in the management of patients with CLL includes numerous phase II studies and two historical cohort comparisons. In addition to improving response rates, the addition of rituximab to chemotherapy appears to improve progression-free survival and possibly overall survival compared with chemotherapy alone. Notably, these benefits have been observed in relapsed/refractory disease as well as in patients receiving initial therapy. These encouraging results support the use of rituximab in combination with chemotherapy as an effective therapeutic option in these patients. Ongoing phase III trials will further clarify the role of rituximab in the management of CLL.

Advances in molecular biology have greatly improved our understanding of the biology of CLL. Numerous molecular prognostic markers have been elucidated, and may allow us to identify subsets of patients who may benefit from earlier intervention or alternative treatment approaches. However, these markers remain investigational at this time and should be evaluated further in the context of well designed clinical trials assessing risk-adapted strategies for CLL.

Rituximab Clinical Profile

Rituximab (RITUXAN) is a chimeric mouse/human monoclonal antibody directed against CD20. It is used extensively in the treatment of B-cell lymphomas.[84,85,86,87] The antibody is an IgG1 kappa immunoglobulin containing murine light-and heavy-chain variable region sequences and human constant region sequences (Figure 9).[86,88]

Figure 9. Rituximab Monoclonal Antibody



Adapted from Onrust et al., 1999.

Pharmacodynamics

MECHANISM OF ACTION

Rituximab uniquely targets and binds only to the CD20 antigen (human B-lymphocyte-restricted differentiation antigen, Bp35), a hydrophobic transmembrane protein, which is located on pre-B and mature B lymphocytes. The antigen is also expressed on >90% of B-lymphocyte-derived cell non-Hodgkin's lymphomas,[88,89] but it is not found on hematopoietic stem cells, pro-B cells, normal plasma cells, or other normal tissues. The Fab domain of rituximab binds to the CD20 antigen on B-lymphocytes and Fc domain recruits immune effector functions to mediate B cell lysis in vitro.

The antigen regulates the early step(s) in the activation process for cell cycle initiation and differentiation. CD20 is not shed from the cell surface and does not internalize upon antibody binding. Free CD20 antigen is not found in the circulation. Rituximab is thought to deplete CD20-positive cells via antibody-dependent cell- and complement-mediated cytotoxicity.[88] It has been shown to induce apoptosis (programmed cell death) in B lymphoma cells in vitro.[89]

EFFECTS ON B LYMPHOCYTES

After a single infusion of rituximab 250 or 500 mg/m^2, peripheral B lymphocyte counts were reduced by approximately 90% in less than or equal to 3 days in patients with relapsed indolent lymphoma. Peripheral B lymphocyte counts began to recover within 90 days.[84] After 4 weekly infusions of rituximab 375 mg/m^2 for the treatment of indolent lymphomas, peripheral B lymphocyte counts were reduced for 6 months but recovered after 9 to 12 months.[57]

Pharmacokinetics

The pharmacokinetics of rituximab has been studied in low-grade or follicular, B-cell non-Hodgkin's lymphoma patients. The findings are summarized in Table 15.

Table 15. Rituximab Pharmacokinetics[89,90]

Absorption	No oral absorption
Plasma concentration after intravenous administration (after 4 infusions of rituximab 375 mg/m^2 once weekly)	Peak plasma concentration: 465 mg/L. Area under the plasma concentration-time curve: 86, 125 mg/L•h.
Distribution	In 9 patients given single doses of 10, 50, 100, 250, or 500 mg/m^2 as an IV infusion, serum levels and the half-life of rituximab were proportional to dose. Rituximab binding was found on lymphoid cells in the thymus, the white pulp of the spleen, and a majority of B-lymphocytes in the peripheral blood and the lymph nodes. Little or no binding was observed in non-lymphoid tissues examined.
Metabolism	The metabolism of rituximab is not fully understood. There are no active or inactive metabolites.
Excretion	Clearance of rituximab decreases remarkably with accumulation of the drug (occurs after multiple infusions). Rituximab is detectable in serum for 3 to 6 months after completion of treatment. No information on urinary excretion. In 9 patients given 375 mg/m^2 as an IV infusion for 4 doses, the mean serum half-life ($t^1/_2$) was 59.8 hours (range 11.1 to 104.6 hours) after the first infusion and 174 hours (range 26 to 442 hours) after the fourth infusion.

Health Canada approved indications

Rituximab is approved for treatment of patients:[90]

- with relapsed or refractory low grade or follicular, CD20 positive, B-cell non-Hodgkin's lymphoma;
- with CD20 positive diffuse large B-cell non-Hogkin's lymphoma in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy; and
- with previously untreated Stage III/IV follicular, CD20 positive, B-cell non-Hodgkin's lymphoma in combination with CVP (cyclophosphamide, vincristine and prednisone) chemotherapy.

At the present time, rituximab is not approved for use in the treatment of chronic lymphocytic leukemia and any such use must be considered investigational.[90]

Dosage form and route of administration

Rituximab is available as a sterile, clear, colorless liquid concentrate for intravenous administration. The suggested rituximab dose and schedule for the treatment of autoimmune hematological disorders has been borrowed from earlier trials in which the antibody was used to treat relapsed or refractory low-grade or follicular B-cell non-Hodgkin's lymphoma (Table 16).[84,85,90,91]

Table 16. Rituximab Dosing Recommendations[90]

	Rituximab Dosing in Adults*
Recommended dose	375 mg/m^2
	First infusion: initial rate of 50 mg/h, then escalate in 50 mg/h increments every 30 minutes, to a maximum of 400 mg/h.
	Subsequent infusions: initial rate of 100 mg/h, increments every 30 minutes, to a maximum of 400 mg/h as tolerated.
	Observe closely for infusion-related reactions. After an interruption for a severe reaction, the infusion can be restarted at a 50% reduction in rate once the symptoms have resolved.
Route of administration	Intravenous. Do not administer as an intravenous push or bolus.
Frequency of administration	Once weekly for 4 weeks.
Concomitant therapy	Acetaminophen and diphenhydramine before each dose of rituximab. Premedication with a corticosteroid should be considered.
Special considerations	Administration requires no equipment outside of that normally associated with infusion of drugs in a chemotherapy clinic.
	Rituximab infusions should be administered in a setting where full resuscitation facilities are immediately available, and under the close supervision of someone experienced and capable of dealing with severe infusion-related reactions.
	The infusion may have to be stopped temporarily and the infusion-related effects treated.

* Safety and effectiveness in children have not been established. Rituximab should not be administered to pregnant women unless the possible benefit outweighs the potential risk.

Contraindications

Rituximab is contraindicated in patients with known hypersensitivity to any of its components or to murine proteins.[90]

Safety

The safety data are based on 356 patients treated in five single-agent studies of rituximab in the treatment of low-grade or follicular B-cell non-Hodgkin's lymphoma. It includes patients with bulky disease, those who have received more than one course of rituximab, and patients receiving 375 mg/m^2 for eight doses.[90]

ADVERSE REACTIONS

The most common adverse reactions of rituximab are infusion-related, including fever and chills/rigors, urticaria, pruritus, angioedema, and flushing. In about 10% of people, these reactions are accompanied by hypotension and bronchospasm. Infusion-related events generally occur within 30 minutes to 2 hours after initiating the first infusion and resolve with slowing or interruption of the rituximab infusion and with supportive care (i.e., intravenous saline, diphenhydramine and acetaminophen). The incidence of infusion-related reactions decreases with subsequent infusions (77%, falling to 14% at the eighth infusion).

There have been post-marketing reports of more serious infusion-related reactions in a very small proportion of people. Rituximab infusion should be interrupted for severe hypersensitivity reactions, including hypotension, bronchospasm, angioedema, and rapid tumor lysis syndrome. The infusions may be resumed at 50% reduction in rate when symptoms have completely resolved. Aggressive treatment of these symptoms with diphenhydramine, steroids, and acetaminophen is recommended; additional treatments with bronchodilators or intravenous saline may be indicated. Fatal outcomes have been reported for people who developed features of cytokine-release syndrome and/or signs and symptoms of tumor-lysis syndrome.

Severe mucocutaneous reactions (Stevens-Johnson syndrome, toxic epidermal necrolysis, paraneoplastic pemphigus, lichenoid dermatitis or vesiculobullous dermatitis) have been described and may be fatal. The onset of these reactions can vary from days to several months following exposure to rituximab.

Severe pulmonary reactions with dyspnea, bronchospasm, hypoxia and pulmonary infiltrates or edema have been reported, including a case of fatal bronchiolitis obliterans. Acute symptoms appear within 1-2 hours of the initiation of the first infusion, while pneumonitis may appear 1-4 weeks after the infusion. Patients with pre-existing lung disease and those cancer patients with pulmonary involvement may be at increased risk. Rituximab infusion should be interrupted; may be restarted at a slower rate if all symptoms resolve.

Reactivation of HBV infection may occur with fulminant hepatitis, hepatic failure, and death. High risk patients should be tested for HBV and monitored closely.

It is beyond the scope of this monograph to list the incidence of adverse events observed in rituximab monotherapy clinical studies. A summary of adverse events reported in ≥5% of 356 patients receiving rituximab monotherapy is found in the RITUXAN® Product Monograph.[90]

ELDERLY PATIENTS

Elderly patients (≥65 years): The incidence of adverse events in rituximab monotherapy clinical studies was similar in elderly and younger patients.

PREGNANCY

Rituximab should be avoided during pregnancy unless the potential benefit to the mother outweighs the risk of B-lymphocyte depletion in the fetus. It is also contraindicated in women who are breast-feeding. Effective contraception is required during treatment and for 12 months after treatment.

DRUG INTERACTIONS

Table 17 lists drug interactions that have been deduced from the action and clinical pharmacology of rituximab.

Table 17. Drug Interactions[90]

Agent	Effect	Mechanism	Management
Antihypertensive medications	Potentiation of hypotension with infusion of rituximab	Additive hypotensive effects	Consider instructing patients taking antihypertensive medications to hold their medications 12 hours prior to an infusion.
Cisplatin	Renal failure	Unknown	Use with extreme caution.
Live vaccines	Systemic viral infection	Rituximab-induced immunosuppression	Avoid/Caution

Stability and Storage

RITUXAN (rituximab) vials are stable at 2 to 8°C. The vial should be protected from light. The drug should not be used beyond the expiration date.

The preparation of RITUXAN solutions for infusion should be done under aseptic conditions. RITUXAN solutions for infusion are stable at 2 to 8°C for 24 hours and at room temperature for an additional 12 hours. However, administration should take place as per standard practices after the aseptic preparation of intravenous admixtures. No incompatibilities between RITUXAN and polyvinylchloride or polyethylene bags have been observed.

Availability

RITUXAN (rituximab) is supplied as 100 mg and 500 mg single-use vials containing a sterile, preservative-free solution.

100 mg: each carton contains two 100 mg/10 mL vials (10 mg/mL).

500 mg: each carton contains one 500 mg/50 mL vial (10 mg/mL).

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