



Monoclonal antibodies in chronic lymphocytic leukemia

Alessandra Ferrajoli[†], Stefan Faderl and Michael J Keating

Multiple options are now available for the treatment of chronic lymphocytic leukemia. Over the last 10 years, monoclonal antibodies have become an integral part of the management of this disease. Alemtuzumab has received approval for use in patients with fludarabine-refractory chronic lymphocytic leukemia. Rituximab has been investigated extensively in chronic lymphocytic leukemia both as a single agent and in combination with chemotherapy and other monoclonal antibodies. Epratuzumab and lumiliximab are newer monoclonal antibodies in the early phase of clinical development. This article will review the monoclonal antibodies more commonly used to treat chronic lymphocytic leukemia, the results obtained with monoclonal antibodies as single agents and in combination with chemotherapy, and other biological agents and newer compounds undergoing clinical trials.

Expert Rev. Anticancer Ther. 6(8), 1231–1238 (2006)

Chronic lymphocytic leukemia (CLL) is the most prevalent form of adult leukemia in Western countries. CLL is characterized by a heterogeneous clinical course, with a certain subset of patients not requiring treatment for a prolonged period of time [1]. However, since CLL patients tend to be older and have trouble tolerating myelosuppressive chemotherapeutic regimens, the relationship between treatment efficacy and toxicity becomes particularly important when therapeutic interventions are needed [2].

In an effort to understand CLL and thereby improve the balance between treatment efficacy and toxicity, significant work has been conducted into defining the biology of CLL. Over the past 20 years, ample progress has been made. For example, the immunophenotype of CLL has been characterized in detail, prognostic markers have been identified and more effective treatment options have emerged, with a move from simple oral alkylating agent-based monotherapies to complex multiagent modalities [3].

The concept of monoclonal antibodies has been studied for many years: monoclonal antibodies represent an ideal form of treatment as the agent is targeted to the malignant cell and

is able to selectively eliminate the neoplastic component but spare normal tissue. The development of human and chimeric monoclonal antibodies safe for use in humans has markedly advanced this form of treatment. The fact that these agents can now be produced in amounts sufficient for evaluation in clinical trials has been a further advance. Together, these two factors have contributed to a recent upsurge in the development and testing of monoclonal antibody-based therapies.

The exact mechanism of action of monoclonal antibodies in CLL has not been completely elucidated. Most monoclonal antibodies appear to have some common pathways of action, such as the ability to induce apoptosis and thereby move the cell into a proapoptotic state, and the ability to eliminate the targeted cells by activating physiological antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [4]. The antibodies that appear to be effective and safe in CLL are, for the most part, naked antibodies, with a few exceptions of antibodies linked to radioimmunoisotopes or toxins.

At present, several monoclonal antibodies are undergoing clinical trials in CLL: alemtuzumab and rituximab have been used more

CONTENTS

Alemtuzumab

Rituximab

Epratuzumab

Lumiliximab

Anti-CD40

Human anti-CD20

Bevacizumab

Radiolabeled antibodies

Expert commentary

Five-year view

Key issues

References

Affiliations

[†]Author for correspondence
University of Texas MD Anderson
Cancer Center, Department of
Leukemia, Unit 428, PO Box
301402, Houston,
TX 77230–1402, USA
Tel.: +1 713 792 2063
Fax: +1 713 794 4297
aferrajo@mdanderson.org

KEYWORDS:
chemoimmunotherapy, chronic
lymphocytic leukemia,
monoclonal antibodies, treatment

extensively in CLL. Alemtuzumab is the only monoclonal antibody that has received approval by the various regulatory agencies for use as second-line treatment of CLL in patients who are refractory to fludarabine. Rituximab, even if not approved for use in CLL, has been investigated extensively, both as a single agent and in combination with chemotherapy and other monoclonal antibodies.

This article reviews the current state of monoclonal antibody therapies for CLL, highlighting those agents that appear to be most promising (please refer to TABLE 1 for a list of monoclonal antibodies that are currently used or under investigation).

Alemtuzumab

Alemtuzumab is a humanized immunoglobulin (Ig)G₁ monoclonal antibody targeted against the CD52 antigen [5]. CD52 is an antigen of unknown function that is expressed in more than 95% of T and B lymphocytes. It is also present on monocytes, macrophages and eosinophils, but is not expressed on plasma cells, erythrocytes, platelets or bone marrow stem cells, and is only weakly expressed by a small subpopulation of granulocytes. The CD52 antigen is a large, complex structure and is the most abundant antigen on the surface of human lymphocytes, comprising approximately 5% of the cell membrane. It is an attractive target for monoclonal antibody therapies owing to its small size, high level of expression, stability and resistance to modulation [6].

In alemtuzumab, the human IgG₁ framework has been reshaped by incorporating six variable regions from the heavy- and light-chain domain of the rodent antibody, creating a humanized antibody with the ability to activate complement, as well as cause cell-mediated lysis. As with other agents of this category, the exact mechanism of action of alemtuzumab is unknown, although it has been shown that its ability to kill lymphoid cells is dependent on both ADCC and CDC [7]. Furthermore, there is also *in vitro* evidence indicating that binding of alemtuzumab to the CD52 antigen on the CLL cell surface triggers apoptosis [8–11]. Alemtuzumab was explored initially as prophylaxis against graft-versus-host disease in patients undergoing bone marrow transplantation. Only later was it evaluated as a treatment for CLL.

One of the first studies to investigate alemtuzumab as a treatment for CLL was Keating and colleagues' multicenter Phase II study of 93 patients with fludarabine-refractory disease [12]. Most patients who received alemtuzumab in this trial were heavily pretreated and had advanced disease. Alemtuzumab was administered intravenously, with dose escalation to 30 mg during the first week; the dose was continued at 30 mg intravenously three times a week for a total of 12 weeks. The overall response rate in this study was 33%, with 2% of the patients achieving complete remission; 59% of the patients had evidence of stable disease. Other studies with smaller numbers of patients were conducted by Osterborg and collaborators [13] and Kennedy and collaborators [14]. In these studies, the overall response rates were 42 and 59%, respectively. This initial experience was able to highlight a pattern of antitumor response

Table 1. Monoclonal antibodies with confirmed or potential activity in CLL.

Target antigen	Monoclonal antibody	Development stage
CD52	Alemtuzumab	Approved in CLL
CD20	Rituximab	Approved in NHL
	Hu-max	Clinical trials
	¹³⁵ I-tositumomab	Approved in NHL
	⁹⁰ Y-ibritumomab tiuxetan	Approved in NHL
CD22	Epratuzumab	Clinical trials
CD23	Lumiluximab	Clinical trials
CD40	Anti-CD40 (CHIR12.12)	Clinical trials
VEGF	Avastin	Approved in solid tumors (colorectal cancer and nonsmall cell lung cancer)

CLL: Chronic lymphocytic leukemia; NHL: Non-Hodgkin's leukemia; VEGF: Vascular endothelial growth factor.

elicited by alemtuzumab: exquisite efficacy was noted in eliminating the disease in the peripheral blood and bone marrow, with a lesser effect on lymphadenopathy. This observation has been confirmed in several subsequent studies [15] and is helpful in selecting salvage strategies. Patients with bone marrow and peripheral blood involvement have a good chance of responding to alemtuzumab treatment, whereas patients with residual lymphadenopathies, especially if larger than 5 cm in diameter, may fare better with other treatments.

From the standpoint of toxicity, treatment with alemtuzumab is associated with reproducible infusion-related reactions, including fever, rigors, rash and sometimes hypotension. These symptoms mainly occur after the first administration and can usually be managed by premedication with antihist-amines and steroids [16]. The experience with alemtuzumab in previously treated patients also showed the immunosuppressive properties of this agent and increased risk for patients to develop cytomegalovirus (CMV) reactivation. Owing to the risk of CMV reactivation, prophylactic regimens are administered concomitant with alemtuzumab; new anti-CMV agents, such as valganciclovir, are being evaluated in this setting and are effective in preventing CMV reactivation [17].

Owing to the encouraging results in previously treated patients, alemtuzumab has also been evaluated as a frontline CLL therapy. In the largest such trial, 51 patients were treated at a dose of 30 mg three times a week using a different route of administration – subcutaneous rather than intra-venous [18]. The overall response rate was 87%. A complete remission was observed in 19% of patients and 68% had a partial response. The subcutaneous administration was well tolerated, with almost complete elimination of the classic first-dose reaction; most patients developed erythema and induration at the injection site, but these reactions usually disappeared with continuation of treatment. This study was also associated with lower

rates of infectious complications, CMV reactivation and neutropenic fevers, most probably due to the more preserved immune systems of these treatment-naïve patients.

Alemtuzumab for minimal residual disease

In recent years, the goal of CLL treatment has been to achieve a remission not only at the morphological level, but also at the molecular level. Various strategies have been tried to achieve this goal. O'Brien and colleagues administered alemtuzumab to 41 patients after treatment with chemotherapy; the drug was administered intravenously at an initial dose of 10 mg, with an increase to 30 mg after the first 24 patients in order to improve the response rate [19]. Alemtuzumab improved response in 46% of the patients and, in a small number of patients, even minimal residual disease was not evident. In the multicenter study coordinated by Moreton and colleagues, eradication of minimal residual disease with alemtuzumab was obtained in 20% of patients and resulted in prolonged survival [20], further confirming the use of this antibody in the treatment of CLL.

In another study, Montillo and collaborators used alemtuzumab as a consolidation strategy after treatment with fludarabine, with the aim of reducing the tumor burden, and allowed autologous bone marrow harvest and subsequent transplantation [21]. Alemtuzumab was administered subcutaneously at a dose of 10 mg three times a week for 6 weeks. Treatment with alemtuzumab was initiated a median of 6 months after treatment with fludarabine-based regimens. The complete response rate in this study improved from 35% after fludarabine treatment to 79% after alemtuzumab treatment.

Two cooperative groups have tested alemtuzumab as consolidation therapy. A two-part study conducted by the Cancer and Leukemia Cooperative Group B (CALGB) examined the use of alemtuzumab to consolidate response after fludarabine treatment in patients undergoing first-line therapy. In the first part of the study, alemtuzumab was given intravenously; in the second part the route of administration was changed and alemtuzumab was given subcutaneously. In both series, treatment with the alemtuzumab improved the complete response rate. Unfortunately, the response rates to fludarabine were quite different between the two parts of the study and it was therefore impossible to compare the activities of intravenous and subcutaneous alemtuzumab [22,23]. In a study carried out by the German CLL Cooperative Study Group, following treatment with fludarabine or a fludarabine-cytarabine combination, patients were randomized to receive consolidation treatment with alemtuzumab or placebo. This study was terminated early owing to severe infectious in the alemtuzumab arms, but showed that treatment with alemtuzumab was able to induce molecular remissions and was associated with a longer disease-free survival [24]. Both trials also taught us that alemtuzumab elicits better responses in patients without lymphadenopathy and that the risk for infectious complications is dependent on the patient's immunological status, with fewer infectious complications in less heavily treated patients and a higher rate of infectious complications when the interval between the administration of chemotherapy and alemtuzumab is short.

According to the Dohner genomic classification, CLL patients with abnormalities of 17p or 11q23 have a relatively poor response to treatment and poor survival [25]. This subgroup of patients also often has evidence of fludarabine-refractory disease, either primary or acquired. Several investigators have reported that alemtuzumab was able to elicit responses in such cases [26,27].

Alemtuzumab in combination treatments

Several recent studies have explored the use of alemtuzumab in combination with other monoclonal antibodies or chemotherapeutic agents. Study of the latter combination has been prompted by the different toxicity profiles of monoclonal antibodies and chemotherapies and by *in vitro* evidence that the combination of monoclonal antibodies and chemotherapies can offer potential advantages.

Kennedy and collaborators demonstrated that patients with refractory CLL benefited from treatment with a combination of fludarabine and alemtuzumab [28]. The two agents were administered at the same time and three patients in this small study had a better response to the combination than to the single agents used alone. The combination of fludarabine and alemtuzumab was also evaluated in a larger cohort of previously treated patients. A high response rate (85%) was observed [29].

Faderl and collaborators pioneered the use of a combination of monoclonal antibodies in refractory and relapsed lymphoid malignancies [30]. They combined alemtuzumab and rituximab, owing to these agents' different toxicity profiles and patterns of clinical response (with rituximab active in lymph nodes, as demonstrated in the lymphoma literature, and alemtuzumab preferentially active in the bone marrow and peripheral blood). The study showed that responses obtained with the combination of rituximab and alemtuzumab in previously treated patients were superior to those obtained with each antibody alone; the overall response rate was 63% and the complete response rate 28%. Another clinical trial investigating the combination of rituximab and alemtuzumab was reported by Nabhan and colleagues [31], with rituximab administered once a week for 5 weeks and alemtuzumab given three times a week in weeks 2 and 5. In this small trial of 12 patients with extensively previously treated disease, only one partial response was obtained.

Rituximab

Rituximab is a chimeric IgG1-κ monoclonal antibody containing mouse variable and human constant regions. Rituximab recognizes the CD20 antigen, which is expressed in both malignant and normal B cells. Rituximab has been used extensively in patients with non-Hodgkin's lymphoma and has marked activity in patients with follicular lymphoma [32]. Owing to its activity in malignant lymphoma and favorable toxicity profile, rituximab has also been studied alone and in combination for the treatment of CLL.

Single-agent rituximab has typically demonstrated only modest activity in CLL. Several small Phase II trials evaluating the efficacy of standard doses of rituximab in previously treated

patients have indicated that responses can be obtained in a maximum of 30–35% of patients; most of the responses are partial responses, with only isolated complete responses [33,34]. The exact reason for the disappointing activity of single-agent rituximab in CLL is not known; it has been hypothesized that contributing factors are the low CD20 surface expression by CLL cells and lower circulating levels of rituximab when compared with the one detected in follicular lymphoma. Thus, many strategies have been tested to improve the efficacy of rituximab in CLL. In one such effort, O'Brien and collaborators conducted a dose-escalation study in 50 patients, using rituximab doses of up to 2250 mg/m² [35]. The investigators observed that increasing the dose increased the number of responses, with up to 80% of the patients responding to the highest doses. A similar strategy was investigated by Byrd and collaborators, who administered rituximab at a standard dose but on an intensive schedule of three times a week for 4 weeks [36]. As with dose escalation, this form of dose intensification was associated with an improved response rate: the overall response rate was 45% and occasional complete responses were seen.

Currently, our group is exploring the use of rituximab in combination with granulocyte macrophage colony-stimulating factor (GM-CSF), based on several possible synergistic interactions between these agents. For instance, rituximab-induced cell clearance is dependent on ADCC and CDC. The use of GM-CSF could thus potentiate both types of cytotoxicity, resulting in improved tumor cell clearance, as shown *in vitro* [37]. It has also been postulated that one reason for rituximab's limited activity in CLL is weak surface expression of CD20 by CLL cells; GM-CSF may improve the antibody's performance by increasing CD20 expression on the surface of CLL cells, as indicated by *in vitro* data from Venugopal and collaborators [38]. The combination of rituximab and GM-CSF has so far shown an overall response rate of 47% and a complete response rate of 16% in previously treated patients [39].

Rituximab has also been investigated as a frontline therapy in CLL. Thomas and collaborators investigated the effects of 8 weeks of treatment with standard-dose rituximab in patients with early stage disease and high risk for progression indicated by elevation of β -2 microglobulin [40]. In this study, an overall response rate of 83% was observed, with 17% of the patients achieving a complete remission. In a multicenter study, Hainsworth and collaborators assessed the response of CLL/small lymphocytic lymphoma (SLL) to four weekly standard doses of rituximab in 51 patients, with the indications for treatment defined according to National Cancer Institute (NCI) working group criteria [41]. The rate of response to an initial 4 weeks of treatment was 51%, with occasional complete responses. This study also included a maintenance program, with rituximab administered weekly four times every 6 months for 2 years. The median time to progression for patients on this regimen was 18.6 months. The combination of rituximab and GM-CSF was also investigated in previously untreated patients with an indication for treatment based on a high level of disease-associated symptoms or elevation of β -2 microglobulin. In a preliminary

report covering 35 patients, the overall response rate was 80% and the complete response rate 13%. This combination has also been offered to patients at least 70 years old who met NCI criteria for treatment. So far, 14 patients have been treated; they have shown an overall response rate of 86% and a complete response rate of 7% [39].

Rituximab in combination treatments

Combinations of chemoimmunotherapy are able to achieve a high response rate in both untreated and previously treated patients with CLL than either the antibody or the chemotherapy alone. These combinations are based on the ability of purine analogs to inhibit the alkylating-induced DNA repair mechanisms when the two agents are given concomitantly and also on the reported ability of rituximab to potentiate the activity of chemotherapy by inducing apoptosis [42,43]. The CALGB carried out a Phase III randomized trial of fludarabine alone and in combination with rituximab [44]; once patients had received six cycles of treatment with fludarabine or fludarabine–rituximab, the response was consolidated by treatment with rituximab for 4 more weeks. In this trial, the combination of fludarabine and rituximab was superior to fludarabine alone in the induction phase and, even after consolidation with the rituximab, patients who had received both agents concomitantly had superior response rates. In terms of toxicity, the concomitant administration of purine analogs and rituximab was associated with a significantly increased incidence of neutropenia but no significant change in the incidence of severe infection.

Keating and collaborators used the combination of fludarabine, cyclophosphamide and rituximab (FCR) in 224 previously untreated patients meeting the NCI criteria for indication for treatment [45]. The overall response rate was 95%; 70% of the patients achieved a complete remission, 10% a nodular partial remission and 15% a partial response. In a subgroup of patients in this trial, minimal residual disease was measured by flow cytometry as well as by polymerase chain reaction (PCR). FCR was able to reduce the number of CD5–CD19-positive cells in the bone marrow to less than 1% in two-thirds of the patients, and molecular remission was attained in 50% of patients. The investigators projected that 69% of patients will be free from relapse at 4 years. Toxic effects, such as myelosuppression and infectious complication, were manageable. A similar approach was used by Kay and collaborators, who treated 33 patients with the combination of pentostatin, cyclophosphamide and rituximab [46]. The overall response rate was 93%, with 33% of patients showing complete response, 21% showing nodular partial responses and 39% showing partial responses. In this study, flow cytometry was used to identify minimal residual disease, with the number of detectable leukemia cells significantly reduced in the majority of patients. Toxicities were similar to those observed with other chemoimmunotherapies. In both these studies of treatment-naïve patients, it is too early to discern a survival benefit from the combination therapies, but the high rate of complete responses and the quality of these responses are encouraging.

Combination chemoimmunotherapy has also been investigated in patients with previously treated disease. For example, our group at the University of Texas MD Anderson Cancer Center (TX, USA) treated 177 CLL patients with FCR; 21% of the patients had fludarabine-refractory disease. The overall response rate was 73%, with 25% of the patients achieving a complete response and 38% obtaining a partial response. Toxicity was similar to that seen in patients receiving it as frontline therapy, except for higher incidences of myelosuppression and infection. Factors predicting good response to this treatment were fewer prior treatments, low pretreatment β -2 microglobulin and normal pretreatment platelet count [47]. Lamanna and collaborators evaluated the combination of pentostatin, cyclophosphamide and rituximab in 46 previously treated patients who had CLL [48]. Rituximab was added in the second cycle, after debulking was done with pentostatin and cyclophosphamide. The overall response rate was 79%, with 29% of patients showing complete response and 50% showing a partial response.

The activity of FCR and alemtuzumab in combination was evaluated by Wierda and colleagues in patients with either fludarabine-refractory CLL or CLL that had undergone Richter's transformation [49]. The rationale for this combination was based on the high response rate elicited by the rituximab-containing chemoimmunotherapy and alemtuzumab's activity against CLL with poor genomic features. Preliminary results of this combination are encouraging.

Although alemtuzumab and rituximab have undergone the most extensive use and testing so far, other monoclonal antibodies are also being evaluated in clinical trials of agents for CLL or other lymphoid malignancies. The following sections look at some of the most promising of these potential agents.

Epratuzumab

Epratuzumab is a humanized monoclonal antibody against CD22, a glycoprotein normally expressed on the surface of B cells in the mature stages of differentiation. CD22 is not expressed by embryonic stem cells, T cells or plasma cells. The function of CD22 is uncertain, but it has been implicated in the B-cell activation complex and has been shown to function as an adhesion molecule. *In vitro* data indicate that when epratuzumab binds to CD22, the antigen is internalized and serves as a proapoptotic signal for neoplastic cells [50]. Epratuzumab has shown clinical activity in patients with relapsed non-Hodgkin's lymphoma. No experience is available at the present time in CLL, but its activity in non-Hodgkin's lymphoma and the fact that CD22 is expressed in 30% of cases of CLL make it a potentially clinically useful antibody in the treatment of chronic lymphoproliferative disorders [51–53].

Lumiliximab

Lumiliximab is a primatized monoclonal antibody that binds to the CD23 antigen, which is highly expressed on CLL cells. The function of CD23 is not completely known, but this antigen is considered important in Ig production and the maturation of normal lymphocytes. The CLL cells express CD23 in more

than 90% of patients. Preclinical models of CLL have shown increased apoptosis in CLL cells exposed to lumiliximab, and this antibody reportedly has antilymphoproliferative activity in mouse models of severe combined immunodeficiency [54]. In a Phase I study of 25 patients with extensively previously treated CLL, weekly infusions of lumiliximab (125 mg/m²) were given for 4 consecutive weeks. The white cell count was notably reduced in 24 patients and a reduction in node size was also documented. A randomized trial of the combination of FCR with or without lumiliximab has been completed recently [55]. The major toxicity of lumiliximab in that study appeared to be myelosuppression, with mild systemic symptoms (such as fatigue and nausea) noted during the infusion. This antibody thus appears to be safe. Confirmatory data can be derived from studies of patients with refractory asthma [56].

Anti-CD40

Anti-CD40 (CHIR-12.12) is a fully humanized IgG₁ antibody that binds to the CD40 receptor, which is expressed on the surface of B cells from the early stage of development. CD40 has important roles in the development and function of B cells [57]. The ligation of CD40 by the CD40 ligand results in cell proliferation and protects the cell from apoptosis. CD40 also plays a large role in T-cell-mediated antitumor response. Anti-CD40 binds to the CD40 antigen with high affinity; it is not internalized and blocks signaling by CD40 ligands. Anti-CD40 has been found to inhibit the proliferation and survival of CLL cells *in vitro* but only when CD40 ligand is present; anti-CD40 antibody has shown no activity in the absence of CD40 ligand [58,59]. Studies of anti-CD40 in monkeys have shown the antibody to be safe; it is now undergoing a Phase I study in humans.

Human anti-CD20

Several second-generation anti-CD20 monoclonal antibodies have been created in an effort to optimize antitumor activity. The human anti-CD20 (HuMax) is one example: it is a fully human monoclonal antibody (IgG₁) that has been produced by means of transgenic technology, and *in vitro* studies have shown that it binds to a site on the human CD20 molecule different from that bound by rituximab. Once anti-CD20 has become bound to tumor B cells, CD20 aggregates into clusters on the cell surface, similar to the effect elicited by rituximab. The two monoclonal antibodies differ in that the human anti-CD20 dissociates from the cell surface more slowly than rituximab. Owing to its different kinetics, human anti-CD20 may be superior to rituximab in terms of ADCC and equivalent in terms of ADCC [60]. The human anti-CD20 monoclonal antibody has been evaluated in murine and other animal models and has been investigated in clinical trials. In a Phase I/II study conducted in patients with CLL, human anti-CD20 was administered weekly at doses varying from 100 to 2000 mg. The agent showed a favorable toxicity profile and, in previously treated patients, it elicited a response rate of 46%, indicating its strong potential utility in the treatment of CLL [61].

Bevacizumab

Bevacizumab is a recombinant humanized monoclonal antibody (IgG₁) that binds to the biologically active human vascular endothelial growth factor (VEGF) and prevents the interaction of VEGF with its receptors (Flt-1 and kinase domain region receptor). Interaction between VEGF and its receptors leads to endothelial cell proliferation and neovascularization [62]. This monoclonal antibody has been granted FDA approval for use with 5-fluorouracil-based chemotherapy in the treatment of metastatic colorectal cancer. The use of anti-VEGF strategies may be particularly appropriate for CLL since CLL cells produce VEGF and high VEGF levels are associated with aggressive disease [63,64]. VEGF receptors are also expressed on the surface of CLL cells and high levels of expression of VEGF receptor-2 are associated with aggressive disease [65], providing another strong rationale for use of this agent. Studies of bevacizumab's effects on lymphoproliferative disease are ongoing.

Radiolabeled antibodies

Two radiolabeled anti-CD20 monoclonal antibodies have been approved for use in patients with low-grade lymphomas: yttrium⁹⁰-labeled ibritumomab tiuxetan and iodine¹³¹-labeled tositumomab. Both of these antibodies have been studied in CLL, but their role in the disease appears to be limited by the prolonged myelosuppression associated with their use. It is believed that myelosuppression is probably a consequence of the extensive bone marrow involvement, as well as the different bone marrow reservoir of patients with CLL [66]. Radioimmunoconjugates approved for the treatment of non-Hodgkin's lymphoma also appear to have limited use in CLL.

Expert commentary

Thanks to the technology that allows us to produce monoclonal antibodies in quantities sufficient to be used in the clinical practice and to the discoveries regarding the pathophysiology of neoplasms such as CLL, our knowledge on the potential therapeutic application of monoclonal antibodies has increased

significantly. This field is undergoing an explosive growth and, in the years to come, we will see an increasing use of monoclonal antibodies. Novel combinations of monoclonal antibodies and chemotherapy and combination of different monoclonal antibodies will be developed keeping pace with the increasing knowledge of the pathophysiology of CLL.

Five-year view

Over the past 20 years, major achievements have been made in the treatment of CLL. A large number of patients can obtain complete responses with combinations of chemotherapy and monoclonal antibodies. The quality of response has been progressively improving and achievement of a molecular response (defined as no evidence of disease using sensitive techniques such as flow cytometry and/or PCR) is nowadays a realistic goal for many patients. In the next 5 years, we anticipate that the therapy of CLL will focus on achievement of molecular remission by precisely tailoring treatment to the individual characteristics of each patient. We have already opened this era by selectively offering treatment with alemtuzumab to patients who carry poor prognostic genomic abnormalities. In the years to come, the characterization of clinically relevant prognostic factors will be paralleled by the development of agents specifically targeted toward their biological function.

Key issues

- Monoclonal antibodies are active in chronic lymphocytic leukemia.
- Combinations of monoclonal antibodies and chemotherapy are associated with a high response rate.
- Alemtuzumab is effective in patients with an unfavorable genomic profile.
- Novel and improved monoclonal antibodies are undergoing clinical evaluation.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

- Byrd JC, Stilgenbauer S, Flinn IW. Chronic lymphocytic leukemia. *Hematology* 1, 163–183 (2004).
- Ferrajoli A, O'Brien S, Faderl S *et al.* Rituximab in combination with sargramostin (GM-CSF) in patients with chronic lymphocytic leukemia. *Ann. Oncol.* 16(Suppl. 5), 204 (2005).
- Keating MJ, Rai KR, Wierda WG. Evolution of treatment strategies in chronic lymphocytic leukemia. Part 2 of a 3-part series: advances in the treatment of hematologic malignancies. *Clin. Adv. Hematol. Oncol.* 8, 1–10 (2005).
- Review of chemoimmunotherapy for chronic lymphocytic leukemia (CLL) and its background.
- Tabrizi MA, Tseng CM, Roskos LK. Elimination mechanisms of therapeutic monoclonal antibodies. *Drug Discov. Today* 11, 1–2 (2006).
- Waldmann H, Hale G. CAMPATH: from concept to clinic. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 1707–1711 (2005).
- Historical paper that highlights the milestone of alemtuzumab development.
- Hale G. The CD52 antigen and development of the CAMPATH antibodies. *Cytotherapy* 3, 137–143 (2001).
- Xia MQ, Hale G, Waldmann H. Efficient complement-mediated lysis of cells containing the CAMPATH-1 (CDw52) antigen. *Mol. Immunol.* 30, 1089–1096 (1993).
- Rowan WC, Hale G, Tite JP *et al.* Cross-linking of the CAMPATH-1 antigen (CD52) triggers activation of normal human T lymphocytes. *Int. Immunol.* 7, 69–77 (1995).
- Mone AP, Cheney C, Banks AL *et al.* Alemtuzumab induces caspase-independent cell death in human chronic lymphocytic leukemia cells through a lipid raft-dependent mechanism. *Leukemia* 20, 272–279 (2006).
- Stanglmaier M, Reis S, Hallek M. Rituximab and alemtuzumab induce a nonclassic, caspase-independent apoptotic pathway in B-lymphoid cell lines and in chronic lymphocytic leukemia cells. *Ann. Hematol.* 83, 634–645 (2004).

- 11 Smolewski P, Szmigielska-Kaplon A, Cebula B *et al.* Proapoptotic activity of alemtuzumab alone and in combination with rituximab or purine nucleoside analogues in chronic lymphocytic leukemia cells. *Leuk. Lymphoma* 46, 87–100 (2005).
- 12 Keating MJ, Flinn I, Jain V *et al.* Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. *Blood* 99, 3554–3561 (2002).
- Clinical trial of alemtuzumab in previously treated CLL.
- 13 Osterborg A, Dyer MJ, Bunjes D *et al.* Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H treatment in chronic lymphocytic leukemia. *J. Clin. Oncol.* 15, 1567–1574 (1997).
- 14 Kennedy B, Hillmen P. Immunological effects and safe administration of alemtuzumab (MabCampath) in advanced B-CLL. *Med. Oncol.* 19, 49–55 (2002).
- 15 Ferrajoli A, O'Brien SM, Cortes J *et al.* Phase II study of alemtuzumab in chronic lymphoproliferative disorders. *Cancer* 98, 773–778 (2003).
- 16 Ferrajoli A, O'Brien S, Keating MJ. Alemtuzumab: a novel monoclonal antibody. *Expert Opin. Biol. Ther.* 1, 1059–1065 (2001).
- 17 O'Brien S, Ravandi-Kashani F, Wierda W *et al.* A randomized trial of calacyclovir versus valganciclovir to prevent CMV reactivation in patients with CLL receiving alemtuzumab. *Blood* 106, 830A (2005).
- 18 Lundin J, Kimby E, Bjorkholm M *et al.* Phase II trial of subcutaneous anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) as first-line treatment for patients with B-cell chronic lymphocytic leukemia (B-CLL). *Blood* 100, 768–773 (2002).
- Experience with subcutaneous alemtuzumab administration and experience in frontline treatment of patients with CLL.
- 19 O'Brien SM, Kantarjian HM, Thomas DA *et al.* Alemtuzumab as treatment for residual disease after chemotherapy in patients with chronic lymphocytic leukemia. *Cancer* 98, 2657–2663 (2003).
- 20 Moreton P, Kennedy B, Lucas G *et al.* Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. *J. Clin. Oncol.* 23, 2971–2979 (2005).
- 21 Montillo M, Tedeschi A, Miqueleiz S *et al.* Alemtuzumab as consolidation after a response to fludarabine is effective in purging residual disease in patients with chronic lymphocytic leukemia. *J. Clin. Oncol.* (2006) (In Press).
- Original study of alemtuzumab as an agent prior to autologous bone marrow transplantation.
- 22 Rai KR, Byrd JC, Peterson B *et al.* Subcutaneous alemtuzumab following fludarabine for previously untreated patients with chronic lymphocytic leukemia (CLL): CALGB study 19901. *Blood* 102, 676–677 (2003).
- 23 Rai KR, Byrd JC, Peterson B *et al.* A Phase II trial of fludarabine followed by alemtuzumab (CAMPATH-1H) in previously untreated chronic lymphocytic leukemia (CLL) patients with active disease: Cancer and Leukemia Group B (CALGB) Study 19901. *Blood* 100, 205A (2002).
- 24 Wendtner CM, Ritgen M, Schweighofer CD *et al.* Consolidation with alemtuzumab in patients with chronic lymphocytic leukemia (CLL) in first remission – experience on safety and efficacy within a randomized multicenter Phase III trial of the German CLL Study Group (GCLLSG). *Leukemia* 18, 1093–1101 (2004).
- 25 Dohner H, Stilgenbauer S, Benner A *et al.* Genomic aberrations and survival in chronic lymphocytic leukemia. *N. Engl. J. Med.* 343, 1910–1960 (2000).
- 26 Stilgenbauer S, Dohner H. Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. *N. Engl. J. Med.* 347, 452–453 (2002).
- 27 Lozanski G, Heerema NA, Flinn IW *et al.* Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. *Blood* 103, 3278–3281 (2004).
- 28 Kennedy B, Rawstron A, Carter C *et al.* Campath-1H and fludarabine in combination are highly active in refractory chronic lymphocytic leukemia. *Blood* 99, 2245–2247 (2002).
- 29 Elter T, Bochmann P, Schulz H *et al.* Fludarabine in combination with alemtuzumab is effective and feasible in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: results of a Phase II trial. *J. Clin. Oncol.* 23, 7024–7031 (2005).
- 30 Faderl S, Thomas DA, O'Brien S *et al.* Experience with alemtuzumab plus rituximab in patients with relapsed and refractory lymphoid malignancies. *Blood* 101, 3413–3415 (2003).
- Experience of combination of monoclonal antibodies in CLL.
- 31 Nabhan C, Patton D, Gordon LI *et al.* A pilot trial of rituximab and alemtuzumab combination therapy in patients with relapsed and/or refractory chronic lymphocytic leukemia (CLL). *Leuk. Lymphoma* 45, 2269–2273 (2004).
- 32 McLaughlin P, Grillo-Lopez AJ, Link BK *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J. Clin. Oncol.* 16, 2825–2833 (1998).
- 33 Huhn D, von Schilling C, Wilhelm M *et al.* Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. *Blood* 98, 1326–1331 (2001).
- 34 Itala M, Geisler CH, Kimby E *et al.* Standard-dose anti-CD20 antibody rituximab has efficacy in chronic lymphocytic leukaemia: results from a Nordic multicenter study. *Eur. J. Haematol.* 69, 129–134 (2002).
- 35 O'Brien SM, Kantarjian H, Thomas DA *et al.* Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J. Clin. Oncol.* 19, 2165–2170 (2001).
- 36 Byrd JC, Murphy T, Howard RS *et al.* Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. *J. Clin. Oncol.* 19, 2153–2164 (2001).
- 37 Voso MT, Pantel G, Rutella S *et al.* Rituximab reduces the number of peripheral blood B-cells *in vitro* mainly by effector cell-mediated mechanisms. *Haematologica* 87, 918–925 (2002).
- 38 Venugopal P, Sivaraman S, Huang XK *et al.* Effects of cytokines on CD20 antigen expression on tumor cells from patients with chronic lymphocytic leukemia. *Leuk. Res.* 24, 411–415 (2000).
- 39 Ferrajoli A, O'Brien S, Faderl S *et al.* Rituximab plus GM-CSF for patients with chronic lymphocytic leukemia. *Blood* 106, 214A (2005).
- 40 Thomas DA, O'Brien S, Giles F *et al.* Single agent Rituxan in early stage chronic lymphocytic leukemia (CLL). *Blood* 98, 364A (2001).
- 41 Hainsworth JD, Litchy S, Barton JH *et al.* Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a Phase II trial of the Minnie Pearl Cancer Research Network. *J. Clin. Oncol.* 21, 1746–1751 (2003).

- 42 Gandhi V, Huang P, Chapman AJ *et al.* Incorporation of fludarabine and 1- β -D-arabinofuranosylcytosine 5'-triphosphates by DNA polymerase α : affinity, interaction, and consequences. *Clin. Cancer Res.* 3, 1347–1355 (1997).
- 43 Cerny T, Borisch B, Introna M *et al.* Mechanism of action of rituximab. *Anticancer Drugs* 13, 3–10 (2002).
- 44 Byrd JC, Peterson BL, Morrison VA *et al.* Randomized Phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B9712 (CALGB 9712). *Blood* 101, 6–14 (2003).
- 45 Keating MJ, O'Brien S, Albitar M *et al.* Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J. Clin. Oncol.* 23, 4079–4088 (2005).
- Combination of chemoimmunotherapy in treatment of CLL.
- 46 Kay NE, Geyer SM, Lin T *et al.* Combination chemotherapy with pentostatin, cyclophosphamide and rituximab induces high rate of remissions including complete responses and achievement of minimal residual disease in previously untreated B-cell chronic lymphocytic leukemia. *Blood* 104, 100A (2004).
- 47 Wierda W, O'Brien S, Wen S *et al.* Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. *J. Clin. Oncol.* 23, 4070–4078 (2005).
- 48 Lamanna N, Kalaycio M, Maslak P *et al.* Pentostatin, cyclophosphamide, and rituximab is an active, well-tolerated regimen for patients with previously treated chronic lymphocytic leukemia. *J. Clin. Oncol.* 24, 1575–1581 (2006).
- 49 Wierda W, O'Brien S, Ferrajoli A *et al.* Salvage therapy with combined cyclophosphamide (C), fludarabine (F), alemtuzumab (A), and rituximab (R) (CFAR) for heavily pre-treated patients with CLL. *Blood* 106, 213A (2005).
- 50 Decker T, Oelsner M, Kreitman RJ *et al.* Induction of caspase-dependent programmed cell death in B-cell chronic lymphocytic leukemia cells by anti-CD22 immunotoxins. *Blood* 102, 439A (2003).
- 51 Carnahan J, Wang P, Kendall R *et al.* Epratuzumab, a humanized monoclonal antibody targeting CD22: characterization of *in vitro* properties. *Clin. Cancer Res.* 9, 3982–3990 (2003).
- 52 Leonard JP, Coleman M, Ketas JC *et al.* Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: Phase I/II clinical trial results. *Clin. Cancer Res.* 10, 5327–5334 (2004).
- 53 Leonard JP, Coleman M, Ketas JC *et al.* Phase I/II trial of epratuzumab (humanized anti-CD22 antibody) in indolent non-Hodgkin's lymphoma. *J. Clin. Oncol.* 21, 3051–3059 (2003).
- 54 Pathan N, Hariharan K, Hopkins M *et al.* Induction of apoptosis by IDEC-152 (anti-CD23 antibody) therapy for relapsed or refractory CLL. *Blood* 98, 367A (2001).
- 55 Byrd J, O'Brien S, Flinn I *et al.* Interim results from a Phase I study of lumiliximab (IDEC-152, anti-CD23 antibody) therapy for relapsed or refractory CLL. *Blood* 102, 74A (2003).
- 56 Rosenwasser LJ, Meng J. Anti-CD23. *Clin. Rev. Allergy Immunol.* 29, 61–72 (2005).
- 57 Tong X, Georgakis FV, Long L *et al.* *In vitro* activity of a novel fully human anti-CD40 antibody CHIR-12.12 in chronic lymphocytic leukemia: blockade of CD40 activation on induction of ADCC. *Blood* 104, 606A (2004).
- 58 Younes A, Snell V, Consoli U *et al.* Elevated levels of biologically active soluble CD40 ligand in the serum of patients with chronic lymphocytic leukaemia. *Br. J. Haematol.* 100, 135–141 (1998).
- 59 Fiumara P, Younes A. CD40 ligand (CD154) and tumor necrosis factor-related apoptosis inducing ligand (Apo-2L) in haematological malignancies. *Br. J. Haematol.* 113, 265–274 (2001).
- 60 Leonard JP. Targeting CD20 in follicular NHL: novel anti-CD20 therapies, antibody engineering, and the use of radioimmunoconjugates. *Hematology* 335–339 (2005).
- 61 Coiffier B, Tilly H, Pedersen LM *et al.* HuMax CD20 fully human monoclonal antibody in chronic lymphocytic leukemia. Early results from an ongoing Phase I/II clinical trial. *Blood* 135A (2005).
- 62 Langer CJ, Natale RB. The emerging role of vascular endothelial growth factor receptor tyrosine kinase inhibitors. *Semin. Oncol.* 32, 23–29 (2005).
- 63 Molica S, Vitelli G, Levato D *et al.* Clinicoprognotic implications of increased serum levels of vascular endothelial growth factor and basic fibroblastic growth factor in early B-cell chronic lymphocytic leukaemia. *Br. J. Cancer* 86, 31–35 (2002).
- 64 Kay NE, Bone ND, Tschumper RC *et al.* B-CLL cells are capable of synthesis and secretion of both pro- and anti-angiogenic molecules. *Leukemia* 16, 911–919 (2002).
- 65 Ferrajoli A, Manshouri T, Estrov Z *et al.* High levels of vascular endothelial growth factor receptor-2 correlate with shortened survival in chronic lymphocytic leukemia. *Clin. Cancer Res.* 7, 795–799 (2001).
- 66 Pohlman B, Sweetenham J, Macklis RM. Review of clinical radioimmunotherapy. *Expert Rev. Anticancer Ther.* 6, 445–461 (2006).

Affiliations

- Alessandra Ferrajoli, MD
University of Texas MD Anderson Cancer Center, Department of Leukemia, Unit 428, PO Box 301402, Houston, TX 77230–1402, USA
Tel.: +1 713 792 2063
Fax: +1 713 794 4297
aferrajo@mdanderson.org
- Stefan Faderl, MD
University of Texas MD Anderson Cancer Center, Department of Leukemia, Unit 428, PO Box 301402, Houston, TX, 77230–1402, USA
Tel.: +1 713 745 4613
Fax: +1 713 794 4297
sfaderl@mdanderson.org
- Michael J Keating, MB, BS
University of Texas MD Anderson Cancer Center, Department of Leukemia, Unit 428, PO Box 301402, Houston, TX, 77230–1402, USA
Tel.: +1 713 745 2376
Fax: +1 713 794 1602
mkeating@mdanderson.org