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SYMPOSIUM IN WRITING

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Monoclonal antibody therapy of chronic lymphocytic leukemia

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Abstract Cure of patients with chronic lymphocytic leukemia (CLL) has been an elusive goal. The recent availability of active monoclonal antibodies has rekindled enthusiasm for new and innovative therapeutic approaches. Alemtuzumab, induces responses in about a third of patients with relapsed or refractory CLL following therapy with fludarabine and an alkylating agent. Whereas, rituximab has limited activity in previously treated patients, response rates of 50–70% have been reported in those without prior therapy. Recent data on combinations with rituximab and chemotherapy have shown promise for improving patient outcome. Newer antibodies in development include the primatized monoclonal antibody lumiliximab (IDEC-152), directed against CD23. Other biological approaches include the use of antisense oligonucleotides, proapoptic small molecules, and vaccines directed against the malignant B cells. The rational development of combinations of these promising approaches may eliminate the need for chemotherapy, leading to safer and more effective approaches for patients with CLL.

Introduction

Despite decades of clinical trials, chronic lymphocytic leukemia (CLL) remains an incurable disorder. Fludarabine induces higher response rates than alkylating agents, which are also more durable; however, evidence for a prolongation of survival is lacking [1, 2]. Thus, more effective therapies are clearly needed. Moreover, chemotherapy is associated with a number of untoward effects including myelosuppression, immunosuppression resulting in opportunistic infections and secondary malignancies.

The concept of an immune approach to therapy of infectious diseases and, potentially malignancies, dates back more than a century ago to the work of Paul Ehrlich, the founder of modern immunology. He theorized a ''magic bullet'', predicting the possibility of a monoclonal antibody that was capable of killing tumor cells with limited the damage to normal body tissues [3]. The first trials of monoclonal antibodies in B-cell malignancies dates back over 20 years following the identification of B-cell surface antigens. Early studies identified a signal of activity in non-Hodgkin's lymphomas (NHL) [4–6]. However, it took almost 2 decades until the availability of humanized or human antibody preparations were available, and for hybridoma technology to permit the generation of sufficient quantities of antibodies for large-scale clinical trials [7]. As a result, there is an ever increasing list of monoclonal antibodies being evaluated in CLL (Table 1).

Anti-CD5 monoclonal antibodies

Chronic lymphocytic leukemia cells are characterized by the expression not only of the B-cell antigens CD19 and CD20, but also CD5, CD23, and CD52. This characteristic phenotype makes these cells excellent targets for monoclonal antibody therapy. One of the earliest antibodies in CLL clinical trials was T101, which was directed against the CD5 antigen. Unfortunately, this antibody, when used either unconjugated or conjugated to an immunotoxin or radioisotope, was abandoned for further clinical study because of its limited activity with excessive toxicity [8–11]. Early trials with antiidiotype antibodies were also disappointing [12, 13].

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Table 1 Unconjugated monoclonal antibodies for CLL

Antibody	Target	Status in US
Alemtuzumab (campath)	CD52	Commercial
Rituximab (rituxan)	CD20	Commercial
Lumiliximab (IDEC-152)	CD23	Phase I
hA20s	CD20	Phase I
Bevacizumab (avastin)	VEG-F	Phase II

Alemtuzumab (CAMPATH-1H)

Alemtuzumab was the first monoclonal antibody approved by regulatory agencies for the treatment of CLL. Alemtuzumab is a humanized antibody that targets the CD52 antigen. CD52 is expressed on virtually all lymphocytes at various stages of differentiation, as well as monocytes, macrophages, and eosinophils [14]. The only other site of expression is the male reproductive tract. The highest levels of expression of CD52 appear to on T-prolymphocytic leukemia (PLL) cells, followed by B-CLL, with the lowest levels expressed on normal B cells [15]. Hematopoietic stem cells, erythrocytes, and platelets do not express this antigen and, therefore, they should be spared from direct antibody effects.

The first anti-CD52 antibodies were isolated in the 1980s in an attempt at identifying antibodies that could kill T cells by activating human complement. An IgM antibody was initially selected (CAMPATH-1M), followed by CAMPATH-1G, a murine derivative [16] demonstrated clinical activity in refractory CLL even in patients who had failed CAMPATH-1M. Alemtuzumab (CAMPATH-1H, the humanized antibody) was subsequently found to be effective and was brought to widespread clinical trials. The mechanisms of action of alemtuzumab include complement mediated cytotoxicity, antibody dependent cellular cytotoxicity, and induction of apoptosis [17].

Dose and schedule of administration

The optimal dose and schedule of alemtuzumab administration remains to be defined. The currently recommended dose and schedule was determined by several I/II studies in which alemtuzumab was administered from once to 10 days using doses that ranged from 0.5 mg to 80 mg [18]. Activity was observed in patients with rheumatoid arthritis; however, the associated lymphopenia was of concern to rheumatologists, limiting enthusiasm for further development. This same observation stimulated the interest of hematologists/oncologists in exploring this agent in lymphoid malignancies. In these initial studies, there were 175 NHL patients entered including 36 with CLL. While 25–80 mg could be safely delivered in the absence of premedication, subsequent clinical trials have included premedication with diphenhydramine, and acetaminophen, along with prophylaxis for *pneumocystis carinii* and herpes.

The currently approved schedule of alemtuzumab involves a dose of 3 mg delivered on day 1, escalating to 10 mg on day 2 and then to 30 mg three times weekly as soon as the infusion related reactions are tolerable. The duration of therapy generally lasts 12 weeks, although some studies suggested that additional benefit may be achieved with longer treatment duration [19].

Alemtuzumab is associated with important clinical activity in patients with CLL [19–27] (Table 2). In one of the initial studies, Österborg et al. [19] reported a phase II study in previously treated patients with CLL only three of whom had received prior fludarabine. The response rate was 42%, with 4% complete remission (CR). The likelihood of disease eradication varied with the site of involvement; CLL cells were rapidly cleared from the blood of 97% of patients, while the bone marrow became normal in 36%, with a reduction in splenomegaly in 32%. The median duration of response was 12 months [6–25+].

The data that led to the approval of alemtuzumab by regulatory agencies were primarily derived from a pivotal trial that included 93 patients, who had failed prior therapy with fludarabine and who had also received an alkylating agent [22]. Patient characteristics included a median age of 66 years, 76% with Rai stage II or IV disease, and a median of 4.1 months from the previous therapy. Almost half of the patients had never responded to nucleoside analog therapy. The response rate was 33%, including 2% CR. In addition, six patients had clearing of CLL from all sites, but with persistent anemia or thrombocytopenia that improved on long-term follow-up. The median time to response was 1.5 months. The likelihood of response was inversely related to lymph node size: while 74% of those with adenopathy responded including 27% complete responders, 64% of lymph nodes 0–<2 cm completely resolved, but only 15% of those 2–<5, and none >5 cm. The median duration of response was 8.7 months with a median survival of 16 months, 32 months in responders. Infections occurred in 55% of patients during the study, which were mild to moderate in 26, and severe or life-threatening in 25. Of concern was the reactivation of cytomegalovirus (CMV) in seven

Table 2 Alemtuzumab in CLL

Investigator [ref.]	Pts	Prior theraphy	CR (%)	RR (%)
Österborg [19]	29	+	4	42
Bowen [20]	6	+	50	50
Rawstron [21]	17	+	50	70
McCune [24]	13	+	31	46
Stilgenbauer [27]	11	+	18	55
Rai [26]	136	+	2	33
Keating [22]	92	+	7.4	39.8
Österborg [23]	11	–	33	89
Lundin [25]	41	–	19	87

patients. Causes of death included progressive disease in 37 patients, two due to autoimmune hemolytic anemia or immune thrombocytopenia, and infection in 17 patients.

Rai et al. [26] reviewed the data from a compassionate use protocol including 136 heavily pretreated, fludarabine refractory patients, which confirmed the safety and efficacy of this agent in a broad community setting. Overall 39.8% of patients responded, with 7.4% CR and a median time to progression of 7.3 months for the responders.

Toxicities of alemtuzumab

The major toxicities of alemtuzumab can be distinguished into those that are immediate and others that are more delayed. Despite premedication with acetominophen and diphenhydramine in the pivotal trial, infusion of this antibody was associated with rigors in 90% of patients, that were grade III in 14%; fever was noted in 85% (17% grade III, 3% grade IV), grade I or II nausea in 53%, vomiting in 38% (1% grade III), while a third of patients experience a mild to moderate rash [22]. These adverse events tend to decrease in frequency and severity over the subsequent weeks of treatment. Because of the risk of opportunistic infections, patients also received prophylactic antimicrobial therapy with trimethoprim sulfamethoxazole and acyclovir or famciclovir. Despite administration of these agents, at least one infection occurred in 55% of these patients, which were severe or life-threatening in 27% of patients. CMV reactivation was detected in seven patients and Herpes simplex in six. Opportunistic infections occurred in 11 patients during treatment and an additional seven events during follow-up. Nevertheless, patients who responded to therapy tended to experience fewer Infections. Results from the compassionate use experience were similar in that 32% of patients experienced at least one infection that were fatal in two of them [26]. The frequency of infections in relapsed and refractory patients appears to be lower than with fludarabine in a similar patient group [28].

In an attempt at reducing the acute adverse events, Lundin et al. [25] administered the antibody by the subcutaneous route to 41 patients with previously untreated, advanced CLL requiring treatment. The response rate was 87% including 19% complete remissions; however, most responses occurred after 18 weeks of therapy as a result of the more delayed attainment of serum levels by this route [29]. Although 90% of patients experienced local injection site reactions, most were only grades I–II in severity, with a single grade III toxicity, and these reactions generally disappeared within 2 weeks despite continued therapy. Three patients withdrew from study within the first week because of the local reactions. Of note was that the infusional reactions were relatively mild, with only 17% of patients experiencing rigors. More serious reactions were not reported. Recently, the German CLL Study Group presented an interim analysis of their trial of subcutaneous alemtuzumab in previously treated patients [30]. The response rate of 36% with 2% complete remissions is comparable to the pivotal trial using the intravenous preparation [22]. The Cancer and Leukemia Group B (CALGB) conducted a phase II study of fludarabine for 4 months followed by subcutaneous alemtuzumab for 6 weeks [31]. The investigators concluded that the activity of alemtuzumab by the subcutaneous route was lower than their previous experience with a similar study design in which the alemtuzumab was given intravenously [32]. The explanation for this observation is not clear, but may reflect an effect of the differences in pharmacokinetics of the two routes given the short course of antibody therapy [29].

Alemtuzumab combinations and sequences

Alemtuzumab has been combined with other active agents including fludarabine. Kennedy et al. [33] combined alemtuzumab with fludarabine in six patients with refractory diseases, who had failed a median of eight courses and 12 weeks of either agent, respectively. Five patients responded to the combination, including one CR. Long-term follow-up of those cases is not available. More recently, Elter et al. [34] reported a combination of these two drugs given on days 1–3 monthly for up to six cycles. The overall response rate in the first 34 patients was 85% with 29% complete remissions. Using four-color flow cytometry, the absence of minimal residual disease in the peripheral blood was noted in 44%. CMV reactivation was detected in two patients, one of whom died of bacterial sepsis; two other patients developed fungal pneumonia.

Several groups have delivered alemtuzumab after other chemotherapy to try to eradicate minimal residual disease (MRD). CALGB investigators treated patients with four cycles of fludarabine; alemtuzumab was delivered after a 2-month hiatus to patients with stable disease or better [32]. Disappointing response rates following fludarabine of 4% CR and 52% PR, were probably related to the reduced number of courses. However, following alemtuzumab, the response rate increased to 42% CR and 50% PR. Unfortunately, infections with CMV occurred in eight patients during or within 4 months of alemtuzumab, with one fatality. In their subsequent trial substituting subcutaneous alemtuzumab [31], three of 18 evaluable patients experienced reactivation of CMV, with no deaths from that infection. Four of eight partial responders after fludarabine became complete responders after alemtuzumab, while four of ten patients with stable disease improved to a partial response. The overall complete response rate was 18%, which was lower than the 42% noted above using intravenous alemtuzumab. Montillo et al. [35] demonstrated that stem cell transplantation could successfully be performed in patients in whom

alemtuzumab following chemotherapy had eradicated MRD (51%). They used an escalating schedule of alemtuzumab up to 10 mg three times a week, starting at least 8 weeks after fludarabine. This schedule was associated with CMV reactivation in 57%. O'Brien et al. [36] demonstrated an improved response rate with fewer patients with MRD following the alemtuzumab consolidation; but, three patients developed a large cell NHL as a result of this therapy. In a randomized trial, patients treated with either fludarabine or fludarabine plus cyclophosphamide were randomized to alemtuzumab or observation. There were only 212 evaluable patients because the study was stopped early when 7/11 randomized to antibody experienced a severe infection [37].

Moreton et al. [38] reported on 91 previously treated patients, 44 of whom were refractory to purine analogs. Following treatment with a median of 9 weeks of alemtuzumab, there were 36% complete responses and 19% partial responses. Treatment free survival was significantly prolonged in patients with a molecular CR than in those with a clinical and morphological CR, but with persistent MRD, whose survival was comparable to those with a partial response. Moreover, overall survival was not yet reached for the MRD− complete responders, but was 84% at 60 months compared with a median of 60 months for the MRD+ complete responders, 70 months for the partial responders and 15 months for the nonresponders.

A combination of cyclophosphamide, fludarabine, alemtuzumab and rituximab has been studied; however, the data on the 21 evaluable patients are preliminary [39]. However, five of these patients experienced reactivation of CMV.

Predicting response to alemtuzumab

In contrast to therapy with alkylating agents and nucleoside analogs, responses to alemtuzumab can be achieved in patients with p53 gene mutations [27, 40], which confers resistance to nucleoside analog therapy [41], as well as in those with unmutated immunoglobulin VH genes, 11q- or 17p-cytogenetic abnormalities [30]. FcgammaRIIIA and RIIA polymorphisms do not appear to predict response [42]. Early disappearance of CLL cells from the peripheral blood also predicts bone marrow clearance [43].

Rituximab

Rituximab has revolutionized the approach to patients with a wide variety of B-cell malignancies because of its activity, noncross resistance with chemotherapy, and favorable safety profile [44]. Following the demonstration of its activity in NHL, it soon entered clinical trials for patients with CLL/small lymphocytic lymphoma (SLL), two entities similar enough to be considered as one diagnosis in the World Health Organization (WHO) classification [45]. Nevertheless, response rates were lower in patients with CLL/SLL (10–15%) than in the those with relapsed or refractory follicular NHL (46–58%) [44, 46–55] (Table 3). There is no clear explanation for this observation; however, it has been attributed to the low density of expression of CD20 on these cells. However, this finding does not entirely explain the higher response rates in previously untreated patients and, therefore, may also relate to the reduction by prior treatment of the number of residual effector cells needed for antibody dependent cellular cytotoxicity (ADCC).

Response rates are significantly higher when rituximab is used in patients with CLL/SLL who have not been previously treated (Table 3). Hainsworth et al. [54, 55] noted that the response rate of 70% in 24 patients with SLL was comparable to that in 38 patients with a follicular histology (76%); however, the time to disease progression following maintenance rituximab was longer in the NHL patients. In a subsequent study by the same investigators, 44 previously untreated patients with stage III or IV CLL were treated with single agent rituximab, and those achieving a response by the sixth week received an additional 4 weeks of the antibody every 6 months. The response rate was 58% including 9% CR. Thus, it appears that single agent rituximab is less effective in inducing complete and overall response rates, with less durable remissions than standard fludarabine in previously untreated CLL [2].

Neither increasing the dose or dose density of administration of rituximab has improved the benefit of rituximab in CLL. Byrd et al. [52] treated 33 previously treated or untreated patients with a schedule of thrice weekly rituximab. Thirteen patients experienced transient hypoxemia, hypotension or dyspnea, and one patient discontinued therapy because of infusion related toxicity, despite a schedule of stepped-up dosing. The overall response rate was 45% including 42% partial responses. Four patients were considered inevaluable for response; one died on day 3 of a pulmonary hemorrhage, one of a septic arthritis during week 2, and another a month later from sepsis and a gastrointestinal

Table 3 Rituximab in CLL/SLL

Investigator [ref.]	Patients	Prior Tx	CR (%)	RR (%)
McLaughlin [45]	33	+	0	13
Maloney [48]	3	+	0	0
Nguyen [47]	15	+	0	7
Piro [49][a]	7	+	0	14
Winkler [50]	9	+	0	11
Foran [51]	29	+	0	14
Huhn [54]	28	+	0	25
Byrd [53][b]	33	±	3	45
O'Brien [52][c]	40	+	0	36
Hainsworth [55][d]	15	–	NA	57
Hainsworth [56]	21	–	90	19

[a]Eight infusions; [b]three times weekly; [c]phase I dose escalation; [d]includes SLL patients; *NA* not available

bleed, the last developed immune thrombocytopenia and required alternative therapy. The overall median duration of response was 10 months, but it was only 6 months patients who had failed prior fludarabine. Moreover, the median time to progression was only 6 months. Higher response rates were obtained in previously untreated patients and in disease that was relapsed after fludarabine compared with patients whose CLL was refractory to that therapy.

In a phase I trial, O'Brien et al. [51] escalated the dose of rituximab dose from 375 mg/m^2 to 2,250 mg/m^2 . The dose limiting toxicity had not been reached. There was no clear correlation between dose and response rate in patients with CLL. Thus, this expensive regimen is not sufficiently promising or cost-effective to warrant further study.

More encouraging results have been produced with the combination of rituximab and chemotherapy. Investigators from the CALGB [56] conducted a randomized phase II trial of concurrent and sequential fludarabine and rituximab with 51 and 53 patients per arm, respectively. The overall response rate in the concurrent arm was 90% including 47% CR, and, in the sequential arm, 77% with 28% CR. However, if anything, the disease-free and overall survival suggest a trend in favor of the sequential approach. Myelosuppression was greater in the concurrent than the sequential arm with grade III and IV neutropenia of 37% versus 18%, respectively. However, this observation did not translate into a clinically meaningful difference as there were opportunistic infections in eight patients in the concurrent arm and 14 on the sequential arm. This combination appeared to prolong survival compared with historical controls treated with fludarabine alone [57]. The CALGB investigators completed a subsequent trial of fludarabine and rituximab followed by alemtuzumab that is undergoing analysis.

Building on their experience with the combination of fludarabine and cyclophosphamide, Keating et al. [58] used the combination of fludarabine, rituximab, and cyclophosphamide in 224 previously untreated patients. The regimen included six cycles of fludarabine at 25 mg/m^2, cyclophosphamide 250 mg/m^2, days 2–4 of cycle 1 and days 1–3 of cycles 2–6, with rituximab at 375 mg/m^2 on day 1 of cycle 1 and 500 mg/m^2, day 1 of cycles 2–6. The median age of the patients was 58 years, and only a third hade Rai stage III–IV disease. The complete remission rate was 70%, with 10% nodular PR, and 15% PR. At the time of the report, there were nine relapses, all of whom were alive. Grade 3 neutropenia occurred in 24% and grade 4 in 28% of patients, although only 2.6% of courses were associated with major infections. This regimen has also been reported in 127 previously treated patients, who had received a median of two prior therapies; only 21% were refractory to prior fludarabine [59]. The CR rate was 25% with 16% nodular PR and 32% PR. The CR rate was 33% in fludarabine-sensitive compared with 6% in fludarabine-refractory patients, with 19% and 9% nodular PRs, respectively. Whether FCR is superior to FR is a subject of considerable controversy. The patients in the CALGB study [56] were older and had more advanced disease than in the experience of Keating et al. [58] (Table 4). Remaining questions that require prospective comparative trials include whether rituximab clearly adds to either F or FC alone, and, if so, how FR and FCR compare with one another. However, the former would be difficult to conduct in the US because of the increasing bias toward using rituximab as part of the initial therapy of CLL.

Rituximab has also been used successfully to treat the complications of CLL or its therapy, including pure red cell aplasia [60], and fludarabine induced immune thrombocytopenia [61].

The combination of the two antibodies Rituximab + alemtuzumab has been studied by several groups [62, 63]. Faderl et al. [63] treated 48 patients; 32 with CLL, 9 with CLL/PLL, 1 with PLL, 4 with mantle cell lymphoma, and 2 with Richter's transformation. The overall response rate was 52%, with only 8% CR. The median time to progression was 6 months. More than half the patients experienced an infection, with reactivation of CMV in 27%. Nabhan et al. [62] treated 12 patients with a single PR. Clearly, this regimen is not ready for general clinical use.

Rituximab toxicities

Although rituximab is considered to be relatively well tolerate, more than 90% of patients with NHL experience some infusion related reaction, that is generally mild to moderate in severity [44, 64]. However, patients with CLL and others with a markedly increased number of circulating malignant lymphocytes may be at an increased risk of more serious complications including respiratory insufficiency and a rapid tumor clearance syndrome [49, 65]. The latter differs from typical "Tumor Lysis Syndrome" in that, in the former, the abnormalities of potassium, calcium, and phosphorus tend to be milder, and renal insufficiency is generally not as severe [49, 65, 66]. This syndrome generally begins within 30–60 min of the initial infusion, and is characterized by a rapid disappearance of lymphocytes from the peripheral blood with marked increases in uric acid and LDH, along with fevers, rigors, dyspnea, hypoxia, and hypotension, and may progress despite interruption of the infusion. Thrombocytopenia has been common,

Table 4 Fludarabine–rituximab (FR) versus fludarabine–cyclophosphamide–rituximab (FCR) in previously untreated CLL

Regimen	CR/ORR[a] (%)	Setting	Median (age)	Stage 0–II (%)
FR (57;58)	47/90	Coop group	63	61
FCR (59)	69/95	Single center	58	67

[a]Complete remission/overall response rate

with abnormal coagulation studies in several cases. In the event that such complications occur, infusion of the antibody should be immediately interrupted and the patient managed with hydration, allopurinol, oxygen, and bronchodilators. None of the patients who have received subsequent infusions of the antibody experienced a recurrence of this adverse event. Patients with high white blood cell counts should be considered for prophylactic hydration, allopurinol and, in some cases, alkalinization of the urine.

G3139 plus rituximab

G3139 (oblimersen sodium; Genta Incorporated, Berkeley Heights, NJ, USA) is the first antisense molecule to be widely tested in the clinic for the treatment of human tumors. Bcl-2 is overexpressed in cells from most patients with CLL, which may contribute to chemotherapy resistance. The phase I/II experience with oblimerson as a single agent demonstrated that this agent was well tolerated, although at doses lower than those used to treat patients with solid tumors, and showed evidence of activity [67]. In a recently reported phase III trial, the addition of oblimerson to fludarabine and cyclophosphamide in relapsed patients resulted in a significant improvement in complete remission/nodular PR rate [68]. In vitro data suggest a positive interaction with rituximab, and clinical trials of the combination are being conducted. A multicenter study conducted at the Lombardi Comprehensive Cancer Center, Roswell Park Cancer Institute and Long Island Jewish Hospital is currently testing the feasibility and activity of the combination of fludarabine, rituximab, and oblimerson in both previously untreated and previously treated patients with CLL [69].

Other antibodies under investigation

Anti-CD23

Since expression of CD23 is a characteristic feature of CLL cells, an antibody directed at this antigen is of interest. An anti-CD23 (IDEC-152; Lumiliximab) is a primatized monoclonal antibody, with strong similarity to the human antibody and no mouse component. The antibody inhibits IgE secretion in vitro and induces apoptosis of lymphoma cell lines. It binds complement and mediates ADCC by binding FcgammaRI and RII. In vitro data suggest a favorable interaction with rituximab [70]. The initial phase I trial with this agent included patients with progressive CLL, who had received a median of three prior regimens [71]. Toxicities included fatigue, nausea, and cough, with the dose limiting toxicities being neutropenia and headache. A reduction in the number of circulating lymphocytes occurred in 24 of 25 patients, while other experienced a reduction in lymph node size. A combination trial with rituximab, fludarabine and cyclophosphamide has been conducted and is being analyzed.

Bevacizumab

Data suggest a potential role for angiogenesis in CLL. Angiogenesis factors such as basic fibroblast growth factor (bFGF) upregulate bcl-2, delaying programmed cell death [72]. Bevacizumab is an anti-VEGF monoclonal antibody currently being evaluated in solid tumors and lymphomas [73–75]. This agent may also be of interest in CLL.

Radioimmunotherapy

The use of radiolabeled antibodies has expanded the therapeutic options for patients with NHL [76, 77]. However, radioimmunotherapy for the treatment of CLL has been limited by the frequent extensive bone marrow involvement. Lym-1 is a radioimmunoconjugate consisting of an I^{131} labeled mouse IgG kappa. The antibody recognizes a 31–35 kDa antigen presumed to be a polymorphic variant of the HLA-DR antigen, and thought to be specific for B cells, with a particular avidity for malignant B-cells [78–82]. Although responses were reported in patients with NHL and CLL, the failure to use standardized criteria for response made the data difficult to interpret. The future if this antibody is unclear. In a phase Ia, trial including ten patients, the naked antibody was well tolerated, but not active [83].

Y-90 ibritumomab tiuxetan and I-131 tositumomab are currently restricted to patients with less than 25% bone marrow involvement because of safety concerns. Nevertheless, Kaminski et al. [84] reported their experience in 14 patients with SLL and a median of four prior regimens. Responses occurred in 64% of patients including 21% complete remissions. The median duration of response was 24.7+ months. Strategies are under development to reduce bone marrow involvement prior to RIT therapy.

The use of radioimmunotherapy in CLL is limited by the extent of bone marrow involvement. Thus, reducing the number of CLL cells in the bone marrow prior to radiotherapy by the administration of an agent that is effective in clearing the bone marrow, such as alemtuzumab, is a potentially interesting strategy.

Future directions

The increasing number of monoclonal antibodies active in the treatment of CLL provides promise that treatment for this disease may be moving from nonspecific cytotoxic agents to more specific biological therapies.

Recent advances in molecular technology may permit a more meaningful treatment selection based on specific targets. Other approaches being studied for the immu-

nologic management of CLL include new, active proapoptotic small molecules [85], and vaccine therapy. Under experimental conditions, T cells isolated from patients with CLL can recognize and lyse allogeneic CLL cells demonstrating that these cells are functionally intact [86–89].

The availability of an expanding menu of monoclonal antibodies provides great promise for therapeutic advances in patients with CLL. Individually, these agents are unlikely to lead to significant outcome benefit. However, their ability to eliminate minimal residual disease may prolong survival of treated patients, thus providing an important goal for treatment strategies. The rational development of combinations of multiple antibodies directed at different targets, along with antisense oligonucleotides, proapoptotic small molecules, or other cytokines may provide a strategy that minimizes the dependency on chemotherapy while extending the lifespan of patients with CLL.

References

1. Leporrier M, Chevret S, Cazin B et al (2001) Randomized comparison of fludarabine, CAP, and ChOP, in 938 previously treated stage B and C-chronic lymphocytic leukemia. Blood 98:2319–2325
2. Rai KR, Peterson BL, Kolitz J et al (2000) Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. New Engl J Med 343:1750–1757
3. Ehrlich P (1900) The Croonian lecture: ''on immunity with special reference to cell life''. Proc Royal Soc 66:424–448
4. Nadler LM, Ritz J, Hardy R et al (1981) A unique cell surface antigen identifying lymphoid malignancies of B cell origin. J Clin Invest 67:134–140
5. Nadler LM, Stashenko P, Hardy R et al (1980) Serotherapy of a patient with a monoclonal antibody directed against a human lymphoma-associated antigen. Cancer Res 40:3147–3154
6. Miller RA, Maloney DG, Warnke R, Levy R (1982) Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. New Engl J Med 306:517–522
7. Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497
8. Dillman RO, Shawler DL, Dillman JB, Royston I (1984) Therapy of chronic lymphocytic leukemia and cutaneous T-cell lymphoma with T101 monoclonal antibody. J Clin Oncol 2:881–891
9. Hertler AA, Schlossman DM, Borowitz MJ et al (1988) A phase I study of T101-ricin A chain immunotoxin in refractory chronic lymphocytic leukemia. J Biol Resp Mod 7:97–113
10. Zimmer AM, Kaplan EH, Kazikiewicz JM et al (1988) Pharmacokinetics of I-131 T101 monoclonal antibody in patients with chronic lymphocytic leukemia. Antibody Immunoconjugates Radiopharm 1:291–303
11. Laurent G, Pris J, Farcet J-P et al (1986) Effects of therapy with T101 ricin A-chain immunotoxin in two leukemia patients. Blood 67:1680–1687
12. Capel PJA, Preijers FWMB, Allebes WA, Haanen C (1985) Treatment of chronic lymphocytic leukaemia with monoclonal anti-idiotype antibody. Neth J Med 28:112–118
13. Allebes WA, Preijers FWMB, Haanen C, Capel PJA (1988) The development of non-responsiveness to immunotherapy with monoclonal anti-idiotypic antibodies in a patient with B-CLL. Br J Haematol 70:295–300
14. Hale G, Xia M-Q, Tighe HP et al (1990) The CAMPATH-1 antigen (CDw52). Tissue Antigens 35:118–127
15. Ginaldi L, De martinis M, Matutes E et al (1998) Levels of expression of CD52 in normal and leukemic B and T cells: correlation with in vivo therapeutic responses to CAMPATH-1H. Leuk Res 22:185–191
16. Dyer MJS, Hale G, Hayhoe FGJ, Waldmann H (1989) Effects of CAMPATH-1 antibodies in vivo in patients with lymphoid malignancies: Influence of antibody isotype. Blood 73:1431–1439
17. Dyer MJ (1999) The role of CAMPATH-1 antibodies in the treatment of lymphoid malignancies. Semin Oncol 26:52–57
18. Dyer MJ, Hale G, Marcus RE, Waldmann H (1990) Remission induction in patients with lymphoid malignancies using unconjugated CAMPATH-1 monoclonal antibodies. Leuk Lymph 2:179–190
19. Österborg A, Dyer MJS, Bunjes D et al (1997) Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. J Clin Oncol 15:1567–1574
20. Bowen AL, Zomas A, Emmett E et al (1997) Subcutaneous CAMPATH-1H in fludarabine-resistant/relapsed chronic lymphocytic and B-prolymphocytic leukaemia. Br J Haematol 96:617–619
21. Rawstron AC, Davies FE, Evans P et al (1997) CAMPATH1H therapy for patients with refractory chronic lymphocytic leukemia (CLL). Blood 90:529a (abstr 2356)
22. Keating MJ, Flinn I, Jain V et al (2002) Therapeutic role of alemtuzumab (CAMPATH-1H) in patients who have failed fludarabine: results of a large international study. Blood 99:3554–3561
23. Österborg A, Fassas AS, Anagnostopoulos A et al (1996) Humanized CD52 monoclonal antibody Campath-1H as first-line treatment in chronic lymphocytic leukaemia. Br J Haematol 93:151–153
24. McCune SL, Gockerman JP, Moore JO et al (2002) Alemtuzumab in relapsed or refractory chronic lymphocytic leukemia and prolymphocytic leukemia. Leuk Lymph 43:1007–1011
25. Lundin J, Kimby E, Björkholm M et al (2002) 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
26. Rai KR, Coutré S, Rizzieri D et al (2001) Efficacy and safety of alemtuzumab (CAMPATH-1H) in refractory B-CLL patients treated on a compassionate basis. Blood 98:365a (abstr 1538)
27. Stilgenbauer S, Döhner H (2002) Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 mutation and resistance to chemotherapy. New Engl J Med 347:452–452
28. Perkins JG, Flynn JM, Howard RS, Byrd JC (2002) Frequency and type of serious infections in fludarabine-refractory B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma: implications for clinical trials in this patient population. Cancer 94:2033–2039
29. Hale G, Rebello P, Breitman LR et al (2004) Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration. Blood 104:948–955
30. Stilgenbauer S, Winkler D, Kröber A et al (2004) Subcutaneous Campath-1H (alemtuzumab) in fludarabine-refractory CLL: Interim analysis of the CLL2h study of the German CLL Study Group (GCLLSG). Blood 104:140a (abstr#478)
31. Rai KR, Byrd JC, Peterson B et al (2003) Subcutaneous alemtuzumab following fludarabine for previously untreated patients with chronic lymphocytic leukemia (CLL): CALGB study 19901. Blood 102:676–677a (abstr#2506)
32. Rai KR, Byrd JC, Peterson B, Larson RA (2002) 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 (abstr 772)
33. Kennedy B, Rawstron A, Carter C et al (2002) CAMPATH-1H and fludarabine in combination are highly active in refractory chronic lymphocytic leukemia. Blood 99:2245–2247

34. Elter T, Bochmann P, Schulz H et al (2004) FluCam - a new, 4-weekly combination of fludarabine and alemtuzumab for patients with relapsed chronic lymphocytic leukemia. Blood 104:690a (abstr 2517)
35. Montillo M, Tedeschi A, Rossi V et al (2004) Alemtuzumab as consolidation after a response to fludarabine is effective to purge residual disease in patients with chronic lymphocytic leukemia. Blood 104:140a (abstr#479)
36. O'Brien SM, Kantarjian HM, Thomas DA et al (2003) Alemtuzumab as treatment for residual disease after chemotherapy on patients with chronic lymphocytic leukemia. Cancer 98:2657–2663
37. Wendtner C-M, Ritgen M, Schweighofer CD et al (2004) 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. Leukemia 18:1093–1101
38. Moreton P, Kennedy B, Lucas G et al (2005) Eradication of minimal residual disease in B-cell chronic lymphocytic leukemia after alemtuzumab therapy is associated with prolonged survival. J Clin Oncol epub, Feb 28
39. Wierda W, Faderl S, O'Brien S et al (2004) Combined cyclophosphamide, fludarabine, alemtuzumab, and rituximab (CFAR) is active for relapsed and refractory patients with CLL. Blood 104:101a (abstr#340)
40. Lozanski G, Heerema NA, Flinn IW et al (2004) Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 103:3278–3281
41. Döhner H, Fischer K, Bentz M et al (1995) p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. Blood 85:1580–1589
42. Lin TS, Flinn IW, Modali R et al (2004) FCGR3A and FCGR2A polymorphisms may not correlate with response to alemtuzumab (CAMPATH-1H) in chronic lymphocytic leukemia. Blood (in press)
43. Rawstron AC, Kennedy B, Moreton P et al (2004) Early prediction of outcome and response to alemtuzumab therapy in chronic lymphocytic leukemia. Blood 103:2027–2031
44. McLaughlin P, Grillo-López AJ, Link BK et al (1998) Rituximab chimeric anti-CD20 monoclonal antibody therapy of relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 16:2825–2833
45. Harris NL, Jaffe ES, Diebold J et al (1999) World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the clinical advisory committee meeting—Airlie House, Virginia. J Clin Oncol 17:3835–3849
46. Nguyen DT, Amess JA, Doughty H et al (1999) IDEC-C2B8 anti-CD20 (rituximab) immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoproliferative disorders: evaluation of response on 48 patients. Eur J Haematol 62:76–82
47. Maloney DG, Grillo-López AJ, White CA et al (1997) IDEC-C2B8 (Rituximab) anti-CD20- monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 90:2188–2195
48. Piro LD, White CA, Grillo-Lopez AJ et al (1999) Extended rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. Ann Oncol 10:655–661
49. Winkler U, Jensen M, Manzke O et al (1999) Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (Rituximab, IDEC-C2B8). Blood 94:2217–2224
50. Foran JM, Rohatiner AZ, Cunningham D et al (2000) European phase II study of rituximab (Chimeric anti-CD20 monoclonal antibody) for patients with newly diagnosed mantle-cell lymphoma and previously treated mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma. J Clin Oncol 18:317–324
51. O'Brien SM, Kantarjian H, Thomas DA et al (2001) Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 19:2165–2170
52. Byrd JC, Murphy T, Howard RS et al (2001) 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
53. Huhn D, von Schilling C, Wilhelm M et al (2001) Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. Blood 98:1326–1331
54. Hainsworth JD, Lichty S, Barton JH et al (2003) 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
55. Hainsworth JD, Lichty S, Burris HA III et al (2002) Rituximab as first-line and maintenance therapy for patients with indolent non-Hodgkin's lymphoma. J Clin Oncol 20:4261–4267
56. Byrd JC, Peterson B, Morrison VA et al (2003) 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 B 9712 (CALGB 9712). Blood 101:6–14
57. Byrd JC, Rai K, Peterson BL et al (2005) The addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: An updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. Blood 105:49–53
58. Keating MJ, O'Brien S, Albitar M et al (2005) Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab (FCR) as initial therapy for chronic lymphocytic leukemia. J Clin Oncol epub ahead of print, March 14
59. Wierda W, O'Brien S, Wen S et al (2005) Chemoimmunotherapy with fludarabine, cyclophosphamide and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol epub ahead of print, March 14
60. Ghazal HH (2001) Successful treatment of pure red cell aplasia (PRA) with rituxan in patients with CLL. Blood 98:219a (abstr 914)
61. Hegde UP, Wilson WH, White T, Cheson BD (2002) Rituximab treatment of refractory fludarabine -associated immune thrombocytopenia in chronic lymphocytic leukemia. Blood 100:2260–2262
62. Nabhan C, Patton D, Gordon L et al (2004) A pilot trial of rituximab and alemtuzumab combination therapy in patients with relapsed and/or refractory chronic lymphocytic leukemia. Leuk Lymph 45:2269–2273
63. Faderl S, Thomas DA, O'Brien S et al (2003) Experience with alemtuzumab plus rituximab in patients with relapsed and refractory lymphoid malignancies. Blood 101:3413–3415
64. Dillman RO (1999) Infusion reactions associated with the therapeutic use of monoclonal antibodies in the treatment of malignancy. Cancer Metastasis Rev 18:465–471
65. Byrd JC, Waselenko JK, Maneatis T et al (1999) Rituximab therapy in hematologic malignancy patients with circulating tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. J Clin Oncol 17:791–795
66. Cheson BD, Frame JN, Vena D et al (1998) Tumor lysis syndrome: an uncommon complication of fludarabine therapy of chronic lymphocytic leukemia. J Clin Oncol 16:2313–2320
67. Rai KR, O'Brien S, Cunningham C et al (2002) Genasense (Bcl-2 antisense) monotherapy in patients with relapsed or refractory chronic lymphocytic leukemia: phase I and II results. Blood 100:384a (abstr 1490)
68. Rai KR, Moore JO, Boyd TE et al (2004) Phase 3 randomized trial of fludarabine/cyclophosphamide chemotherapy with or without oblimerson sodium (Bcl-2 antisense; Genasense;

G3139) for patients with relapsed or refractory chronic lymphocytic leukemia (CLL). Blood:abstr 338
69. Chanan-Khan AA, Mavromatis B, Rai KR et al (2004) A pilot study of Genasense (Oblimersen sodium, Bcl-2 antisense oligonucleotide), fludarabine and rituximab in previously treated and untreated subjects with chronic lymphocytic leukemia. Blood 104:abstr 4827
70. Pathan N, Hariharan K, Hopkins M et al (2001) Induction of apoptosis by IDEC-152 (anti-CD23) in lymphoma cells. Blood 98:367a (abstr 1545)
71. Byrd J, O'Brien S, Flinn I et al (2003) Interim results from a phase I study of lumiliximab (IDEC-152, anti-CD23 antibody) therapy for relapsed or refractory CLL. Blood 102:abstr 248
72. König A, Menzel T, Lynen S et al (1997) Basic fibroblast growth factor (bFGF) upregulates the expression of bcl-2 in B cell chronic lymphocytic leukemia cell lines resulting in delaying apoptosis. Leukemia 11:258265
73. Langmuir VK, Cobleigh MA, Herbst RS et al (2002) Successful long-term therapy with bevacizumab (Avastin) in solid tumors. Proc Amer Soc Clin Oncol 21:9a (abstr 32)
74. Figg D, Fruger EA, Price DK (2002) Inhibition of angiogenesis: treatment options for patients with metastatic prostate cancer. Inv New Drugs 20:183–194
75. Karp JE, Gojo I, Gocke CD et al (2002) Timed sequential therapy (TST) of relapsed and refractory adult acute myelogenous leukemia (AML) with the anti-vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab. Blood 100:198a (abstr 744)
76. Cheson BD (2003) Radioimmunotherapy of non-Hodgkin's lymphomas. Blood 101(in press) (Jan 15)
77. Dillman RO (2002) Radiolabeled anti-CD20 monoclonal antibodies for the treatment of B-cell lymphoma. J Clin Oncol 20:3545–3547
78. DeNardo GJ, Lewis JP, DeNardo SJ, O'Grady LF (1993) Effect of Lym-1 radioimmunoconjugate on refractory chronic lymphocytic leukemia. Cancer 73:1425–1432
79. Epstein AL, Marder RJ, Winter JN et al (1987) Two new monoclonal antibodies, Lym-1 and Lym-2, reactive with human B-lymphocytes and derived tumors, with immunodiagnostic and immunotherapeutic potential. Cancer Res 47:830–840
80. Lewis JP, DeNardo GL, DeNardo SJ, O'Grady LF (1990) Impact of Lym-1 radioimmunoconjugate on refractory chronic lymphocytic leukemia (CLL). Blood 76:295a (abstr 1171)
81. DeNardo SJ, DeNardo GL, O'Grady LF et al. (1988) Pilot studies of radioimmunotherapy of B cell lymphoma and leukemia using I-131 Lym-1 monoclonal antibody. Antibody Immunoconjugates Radiopharm 1:17–33
82. DeNardo GL, DeNardo SJ, O'Grady LF et al (1990) Fractionated radioimmunotherapy of B-cell malignancies with ^{131}I-Lym-1. Cancer Res 50:1014s–1016s
83. Hu E, Epstein AL, Naeve GS et al (1989) A phase Ia clinical trial of LYM-1 monoclonal antibody serotherapy in patients with refractory B cell malignancies. Hematol Oncol 7:155–166
84. Kaminski MS, Press OW, Lister TA et al (1999) Iodine I131 tositumomab for patients with small lymphocytic lymphoma (SLL): overall clinical trial experience. Blood 94:abstr 386
85. McGreivy JS, Marshall J, Cheson BD et al (2005) Initial results from ongoing Phase I trials of a novel pan bcl-2 family small molecule inhibitor. Proc ASCO (in press)
86. Krackhardt AM, Harig S, Witzens M et al (2002) T-cell responses against chronic lymphocytic leukemia cells: implications for immunotherapy. Blood 100:167–173
87. Kater AP, Remmerswaal EBM, Nolte MA et al (2004) Autologous cytomegalovirus-specific T cells as effector cells in immunotherapy of B cell chronic lymphocytic leukaemia. Br J Haematol 126:512–516
88. Gitelson E, Hammond C, Mena J et al (2003) Chronic lymphocytic leukemia-reactive T cells during disease progression and after autologous tumor cell vaccines. Clin Cancer Res 9:1656–1665
89. Müller MR, Tsakou G, Grünebach F et al (2004) Induction of chronic lymphocytic leukemia (CLL)-specific CD4- and CD8-mediated T-cell responses using RNA-transfected dendritic cells. Blood 103:1763–1769