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The role of alemtuzumab in the treatment of chronic lymphocytic leukemia (CLL) and the achievement of minimal residual disease negativity in relapsed/refractory CLL

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Chronic lymphocytic leukemia (CLL) is the most common adult hematologic malignancy in the developed world, predominantly affecting the elderly. It is a disease with a very heterogeneous outcome, with some patients surviving for many years without any evidence of progression and others rapidly developing aggressive disease, associated with early bone marrow failure and repeated infections. In recent years, the recognition of novel prognostic markers (e.g. mutational status of the variable region of the immunoglobulin heavy-chain gene [VH],[1-4] zeta-associated protein 70 [ZAP70],[5-8] and p53 deletion or mutation resistance)[9,10] has greatly improved the stratification of patients on the basis of their clinical risk profile, allowing treatment to be tailored accordingly, particularly in patients likely to develop progressive disease.

Treatment options have also expanded, initially with the introduction of purine analogs, such as fludarabine.[11] These chemotherapeutic agents produced higher complete remission (CR) rates and longer progression-free survivals than conventional alkylating agents, but no overall survival advantage has been demonstrated, with the majority of patients ultimately relapsing after achieving remission. The prognosis is poor for patients in whom fludarabine treatment fails, with only approximately 40% surviving longer than 12 months (median survival, 10 months)[12] (Figure 1). This prognosis is comparable with that for acute leukemias, such as relapsed acute lymphocytic leukemia. Alternative therapies and novel therapeutic strategies are therefore urgently required. For example, chemotherapy resistance in CLL is frequently due to p53 pathway dysfunction because the conventional chemotherapies used in CLL, namely the alkylating agents and purine analogs, appear to be effective, either directly or indirectly, by damaging DNA. DNA damage leads to cell-cycle arrest and cell death due to the activation of p53. In cases with p53 dysfunction, these chemotherapeutic agents damage DNA but do not lead to cell lysis. This is an inherently dangerous situation for a cell as further mutations would be acquired, which could theoretically lead to more rapid cell growth. Thus, the use of therapies which have different mechanisms of action to those of conventional chemotherapy, such as monoclonal antibodies and high-dose steroids, which both function in a p53-independent manner, are appropriate for patients with p53 dysfunction. In addition, the newer therapies produce better remissions, particularly alemtuzumab monotherapy.

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody that targets CD52 antigen, a non-modulating glycoprotein of unknown function. CD52 is present in large numbers (approximately $5x10^5$ binding sites per cell) on the surface of at least 95% of all normal peripheral blood lymphocytes of both T- and B-cell lineage, as well as on monocytes/macrophages and epithelial cells in the epidermis. CD52 is not, however, found on stem cells, so allowing hematopoietic recovery following treatment with alemtuzumab.[14] Monoclonal antibodies, such as alemtuzumab, act by binding to specific surface antigens on tumor cells and causing cell death through various effector mechanisms, including antibody-dependent cellular cytotoxicity,[15,16] complement-mediated cell lysis[17] and the induction of apoptosis.[18]

Clinical experience with alemtuzumab in CLL

Following a series of phase I and phase II clinical trials, alemtuzumab was approved in 2001 by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for the treatment of patients with refractory CLL who have been treated with alkylating agents and in whom fludarabine therapy failed.

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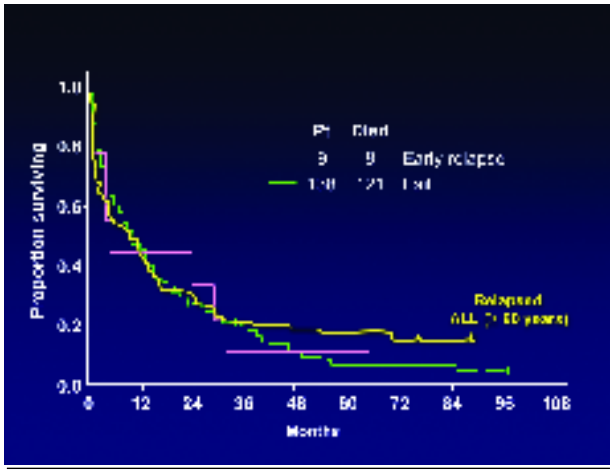

Figure 1. Survival of patients with fludarabine-refractory CLL relative to patients with relapsed acute lymphocytic leukemia.[12,13]

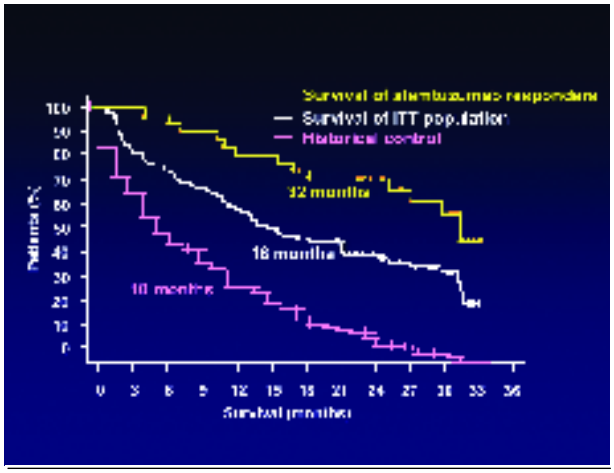

Figure 2. Overall survival following treatment with alemtuzumab.[19]

CAM 211 pivotal trial of alemtuzumab in CLL

The CAM 211 pivotal trial[19] was a prospective, multicenter, international, phase II trial designed to investigate the safety and efficacy of 12 weeks of intravenous (i.v.) alemtuzumab therapy in 93 patients with B-CLL who had previously received alkylating agents and failed treatment with fludarabine. The findings of the CAM 211 trial confirmed the response data produced by a number of smaller phase II clinical trials (Table 1).

Dosage was increased gradually (target 30 mg, three times weekly, for a maximum of 12 weeks) and responses were assessed at weeks 4, 8 and 12, with follow-up continuing for up to 34 months. The overall response rate (ORR) in the intent-to-treat (ITT) population (n=93) was 33% (CR rate 2%, partial response [PR] rate 31%), with 86% of responders showing a response in the blood and 45% of responders showing a bone marrow response to treatment. An additional 54% of patients achieved stable disease that did not progress during the treatment phase. Although only two patients (2%) achieved a CR using National Cancer Institute sponsored Working Group (NCIWG) criteria, six additional patients (7%) had clearing of B-CLL from all sites, but had persistent anemia and/or thrombocytopenia, most probably due to prior therapy. The median time to progression was 4.7 months overall, but 9 months for responders. The median overall survival (OS) was 16 months (95% CI 11.8–21.9) and 32 months for responders (Figure 2). These data indicate that monotherapy with alemtuzumab was an improvement over standard regimens for this patient population. The most common adverse events were infusion-related, generally grade 1 or 2, including fever and rigors. These reactions occur mainly with the first few i.v. doses of alemtuzumab, although in some patients they can persist throughout therapy. Given the high incidence of infections reported in the study by Rai *et al.*,[21] in which infection prophylaxis had not been mandatory, the CAM 211 trial included prophylaxis against infection beginning on day 8 and continuing for a minimum of 2 months after treatment. As a result, the infection rate was reduced compared with earlier studies without anti-infective prophylaxis, with the grade 3/4 infection rate found to be lower in responders (9.7%) than in the ITT population (26.9%).

Table 1. Summary of response rates from phase II trials of alemtuzumab in patients with relapsed/refractory CLL.

Author (reference)	n	Complete response	Overall response rate
Osterborg *et al.*[20]	29	4%	42%
Keating *et al.*[9]	93	2%*	33%*
Rai *et al.*[21]	24	–	33%
Rai *et al.*[22]	152	5%	43%
Moreton *et al.*[23]	91	35%**	54%**

**9% MRD negative; ** 20% MRD negative,*

Alemtuzumab as first-line therapy in CLL

Alemtuzumab has also been used as first-line treatment for CLL. A phase II trial of subcutaneous (s.c.) alemtuzumab was conducted for 18 weeks in 41 patients with symptomatic, previously untreated B-CLL.[24] An ORR of 87% (PR 68%, CR 19%) was achieved in 38 evaluable patients, with clearance of CLL cells from the blood in 95% of patients occurring in a median time of 21 days. In addition, CR or nodular PR was achieved in the bone marrow in 66% of patients, usually within 18 weeks of treatment. Furthermore, an ORR of 87% was achieved in the lymph nodes, with

complete resolution of lymphadenopathy occurring in 29%, with the effect of alemtuzumab on lymph nodes being more pronounced in first-line use than in the relapsed/refractory setting. The median time to treatment failure had not been reached after more than 18 months (range 8–44+ months). Thus, alemtuzumab appears to achieve high rates of response as first-line therapy in CLL. Furthermore, s.c. administration of alemtuzumab resulted in fewer, first-dose, *flu-like* symptoms compared with i.v. administration. Although injection-site skin reactions occurred in 90% of patients, rigor, rash, nausea, dyspnea or hypotension were rare or absent, and transient grade 4 neutropenia developed in 21%. Infections were rare; cytomegalovirus (CMV) reactivation occurred in 10% of patients but responded rapidly to ganciclovir. In addition, a recent report of alemtuzumab use in patients with CLL who were refractory to fludarabine demonstrated that the monoclonal antibody is also effective when given subcutaneously.[25] Pharmacological studies suggest that alemtuzumab takes longer to achieve therapeutic levels when given via the s.c. route. Consequently, s.c. alemtuzumab given for 18 weeks is probably roughly equivalent to 12 weeks of i.v. therapy, but it is still unclear whether patients will have an excess of complications such as the development of human anti-human antibodies.

Duration of treatment with alemtuzumab

Prolonged treatment is important in achieving high-quality remissions in the bone marrow, as pivotal studies have demonstrated that although disease is cleared promptly from the blood (~ 4 weeks), the bone marrow takes longer to respond. Thus, it has been recommended that treatment should be continued for long enough to clear leukemic cells from the bone marrow, and unless contraindicated, treatment should be given for up to 12 weeks.

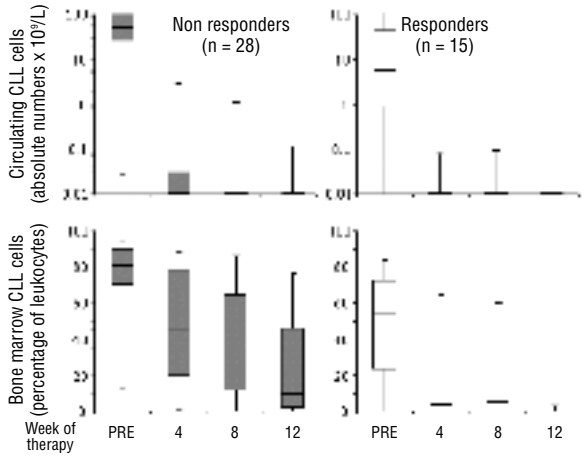

Figure 3. Monitoring of circulating CLL cells to identify disease progression. Patients with CLL who will probably not benefit from a full course of alemtuzumab therapy are likely to have a peripheral B-cell count > 0.001x10⁹/L at week 2 with less than 1 log depletion of circulating B cells between weeks 2 and 4.[26]

However, early identification of non-responders would help to minimize toxicity and/or facilitate more effective strategies. Recently, we have demonstrated that monitoring blood for clearance of circulating CLL cells is useful for early prediction of response and relapse in patients with CLL.[26] Four-color flow cytometry was performed in 887 blood samples and 201 marrow samples from 43 patients receiving i.v. alemtuzumab therapy. Although absolute lymphocytosis was resolved in all patients by week 4, a significant depletion of bone marrow tumor load occurred only if circulating B-lymphocyte counts were persistently less than 0.001×10^9/L (Figure 3). The majority of patients (16/28) who did not benefit from a full course of therapy were identified with 100% positive predictive value as having a peripheral B-cell count > 0.001×10^9/L at week 2 with a less than 1 log depletion of circulating B cells between weeks 2 and 4. Thus, monitoring CLL levels after treatment could help to identify patients at risk of disease progression and guide patient management. If these results are validated in prospective studies, blood monitoring at 2 and 4 weeks may be used to optimize therapy.

Response to alemtuzumab among CLL patients with p53 deletion or mutation

Alemtuzumab works in a p53-independent manner and has thus been hypothesized to be effective in patients with 17p (p53) mutation or deletion. An initial case study demonstrated that alemtuzumab was effective in a patient with a p53 deletion.[27] A 72-year old woman with refractory CLL and poor prognostic factors (unmutated VH and 17p [p53] deletion) had shown no response to previous treatment with chlorambucil, mitoxantrone, vincristine, prednisone or fludarabine. Complete remission was achieved in the patient following treatment with alemtuzumab, three times weekly for 12 weeks (Figure 4).

A recent study of 36 patients with fludarabine-refractory CLL treated with alemtuzumab[28] has substantiated this finding: 10/36 (31%) patients responded according to NCI criteria (2 CR, 8 PR). p53 deletion or mutation was found in 15/36 (42%) patients; 40% of patients (6/15) with p53 deletion or mutation achieved a response (CR or PR) compared with 19% of patients with no p53 abnormality. The median duration of remission was 10 months (range 3–36 months). Furthermore, data from an interim analysis of patients with fludarabine-refractory CLL treated with s.c. alemtuzumab[29] demonstrated that 3/7 patients (43%) with

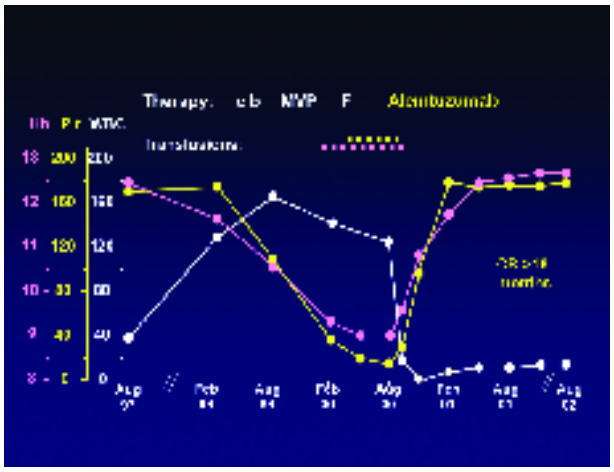

Figure 4. ***In vivo*** **response to alemtuzumab in a patient with 17p (p53) mutation in CLL.[27]**

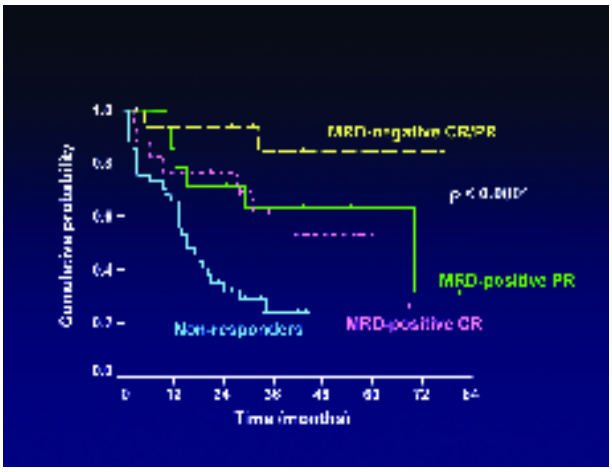

Figure 5. Overall response according to MRD status following alemtuzumab.[23]

p53 mutations responded to alemtuzumab. These data suggest that alemtuzumab may be an effective therapy for patients with CLL with p53 mutation or deletion.

The importance of minimal residual disease

Within the past decade, the introduction of therapies for CLL, such as monoclonal antibodies (e.g. alemtuzumab) and non-myeloablative allogeneic stem cell transplant (NST), has resulted in a significant proportion of patients achieving much more profound responses to treatment and is likely to lead to an improvement in OS.[30] The availability of more effective therapies has begun to shift the focus of treatment away from that of palliative care towards one of achieving a long duration of response, and ultimately a cure. As previously mentioned in this supplement, the detection of minimal residual disease (MRD) has become significant within the context of assessing therapeutic response and the need for further treatment.

The various methods of detecting MRD have different sensitivities and it is important to be aware of the sensitivity of the method used when considering the responses achieved with different therapeutic strategies. An important recent advance in detecting MRD has been the development of gating analysis, which is able to identify discrete cell populations with specific characteristics, including the sequential gating method known as 'MRD Flow'. This method utilizes CD5, CD19, CD20 and CD79b antibodies to discriminate CLL cells. The assay is more sensitive than conventional four-color flow cytometric analysis and can detect one tumor cell in 50–100,000 leukocytes or 2% of B cells.[31] The assessment of MRD in cells is currently being evaluated and standardized by an international group of expert laboratories.[32]

The ability of alemtuzumab to induce MRD negativity

The efficacy of alemtuzumab is reduced by the presence of bulky lymph nodes and extranodal masses, which tend to be associated with more severe, refractory disease. Some researchers have therefore suggested that the role of alemtuzumab, including the eradication of MRD, may be greater in less extensive disease.[33]

In a study by Moreton *et al.*,[23] single-agent alemtuzumab was demonstrated to be effective in eradicating MRD. In the study, 91 patients with relapsed/refractory CLL were treated with single-agent alemtuzumab between 1996 and 2003. Bone marrow assessment for disease using MRD Flow cytometry was performed regularly during therapy with the aim of achieving the best possible response to alemtuzumab, including MRD negativity.

A total of 74 men and 17 women with a median age of 58 years (range 32-75 months) received a median of 12 weeks' alemtuzumab, 30 mg three times weekly. The median number of previous treatment regimens was 3 (range 1-8). Lymphadenopathy was present in 58 (64%) patients. According to NCI criteria CR was achieved in 32/91 (35%) patients, PR in 17/91 (19%) patients, and no response in 42 (46%) patients. According to MRD Flow cytometry, which was conducted using bone marrow and peripheral blood, CLL was eradicated (MRD-negative) in 18 (20%) patients. There was no difference in response or survival between purine analog-refractory patients and purine analog-sensitive patients.

The median survival was found to be significantly longer in MRD-negative patients than in MRD-positive patients. Median survival had not been reached in patients with an MRD-negative CR, whereas median

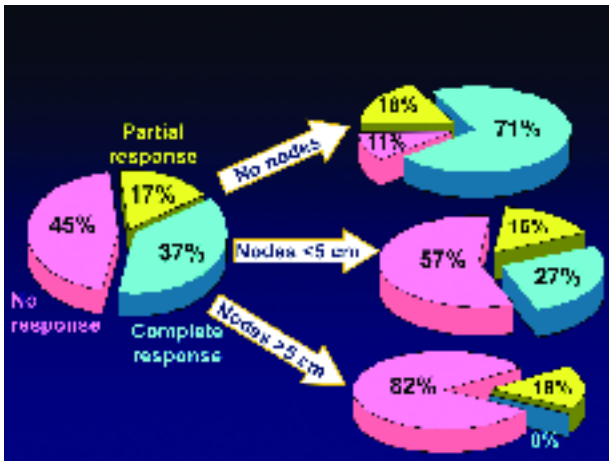

Figure 6. Lymphadenopathy is predictive of a poor response to alemtuzumab; patients with less bulky disease are more likely to show MRD-negative remissions.[23]

survival was 41 months in those with an MRD-positive CR, 30 months in those with a PR, and 15 months in non-responders (p=0.0004) (Figure 5). The mean treatment-free survival following alemtuzumab, compared with the immediate prior therapy, was 34+ months versus 10 months. Patients achieving an MRD-negative CR had a significantly longer treatment-free survival than patients with an MRD-positive CR, PR or non-responders (not reached for those with MRD-negative CR, 20 months in patients with MRD-positive CR, 13 months in patients with PR, and 6 months in non-responders) (p<0.0001). The overall survival for patients with MRD-negative remissions (n=18) was 84% at 5 years. Eight (47%) of the MRD-negative patients had converted to an MRD-positive status after a median of 28 months.

These data from Moreton *et al.*[23] have clearly demonstrated that MRD-negative remission is an achievable goal with alemtuzumab in patients of all ages who have refractory disease. The results also show that MRD status at treatment completion was a better predictor of response duration and OS than NCI response criteria. The use of alemtuzumab to eradicate MRD after tumor debulking with other agents is a major step in the progress towards durable remissions.

Moreton *et al.*[23] also showed that lymphadenopathy was predictive of a poor response to alemtuzumab, and that patients with less bulky disease are more likely to show MRD-negative remissions with alemtuzumab (Figure 6). The combination of fludarabine with alemtuzumab[34] has been suggested as a means of maximizing eradication of CLL at all disease sites since fludarabine is effective at reducing bulky disease in lymph nodes. Combination therapy with fludarabine is further discussed by A. Engert *et al.* on page 23 of this supplement.

The role of alemtuzumab in transplantation protocols

Alemtuzumab has been administered as part of a conditioning regimen in non-myeloablative allogeneic SCT for patients with CLL, and has been shown to minimize graft-versus-host disease (GVHD) without compromising the graft-versus-tumor effect of non-myeloablative conditioning. The roles of autologous and allogeneic SCT in the management of CLL are further discussed in the article by M. Ritgen *et al.* on page 9 of this supplement.

Conclusions

Monoclonal antibodies, such as alemtuzumab, have a different mechanism of action than cytotoxic chemotherapeutic agents. Alemtuzumab is licensed for the treatment of patients with fludarabine-refractory CLL, but its efficacy has been demonstrated in a variety of settings, including chemotherapy-refractory CLL with p53 dysfunction, which usually has a very poor prognosis.

MRD negativity is a better predictor of response duration and OS than are NCI response criteria. Alemtuzumab has a significant role in the attainment of MRD negativity and the eradication of MRD has been demonstrated with single-agent alemtuzumab in up to 20% of patients with refractory disease. MRD-negative remission is an achievable goal with alemtuzumab.

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