Minimal residual disease following chemoimmunotherapy for patients with relapsed chronic lymphocytic leukemia

The goal of therapy in patients with chronic lymphocytic leukemia (CLL) is, whenever feasible, to induce sustained long-term complete remissions (CR). However, in most instances, patients in CR relapse after varying periods of progression-free survival (PFS). A number of studies have shown that patients, in whom detectable residual disease has been eradicated, have a longer duration of PFS than those in whom residual disease remains after treatment, especially when minimal residual disease (MRD) is measured using sensitive methods such as gated four-color flow cytometry (MRD Flow) or allele-specific oligonucleotide polymerase chain reaction (ASO-PCR).

This article first reviews fludarabine-based chemotherapeutic regimens and immunotherapy with alemtuzumab. The rationale for the treatment of CLL with fludarabine plus alemtuzumab combination therapy is then discussed, followed by a review of the results obtained to date, particularly the capacity of this therapeutic approach to induce molecular remissions and long-lasting clinical responses.

Fludarabine-based treatment protocols

Fludarabine and fludarabine-based combination regimens are becoming widely used in the treatment of CLL because of their higher efficacy compared with conventional regimens such as chlorambucil-based protocols.

In a study by Rai et al., fludarabine monotherapy was compared with chlorambucil alone, and with fludarabine plus chlorambucil combination therapy. The overall response rate (ORR) with fludarabine monotherapy was higher than that achieved with chlorambucil alone (Table 1). Chlorambucil plus fludarabine yielded similar response rates compared with fludarabine alone, but this arm of the protocol was stopped due to a high level of toxicity. The durations of response and of PFS were also significantly longer in patients treated with fludarabine, the median PFS being 20 months and 14 months for fludarabine and chlorambucil, respectively. Despite the improvements in ORR and PFS, there was no difference in overall survival (OS) between the two monotherapies, even though fludarabine resulted in longer remissions than chlorambucil.

Rai et al. reported an ORR of 63%, which included a 20% CR rate with fludarabine monotherapy. Conversely, Eichhorst et al. reported an ORR of 86%, which included a 9% CR rate with fludarabine monotherapy. Recently, Rossi et al. have reported similar efficacies for monotherapy with an oral fludarabine preparation.

Combining fludarabine in combination with appropriate agents increases efficacy further. Fludarabine plus cyclophosphamide (FC) increased the ORR to 94% and the CR to 20% in first-line treatment of CLL. These results are consistent with those previously reported by O’Brien et al. for first-line FC therapy: 88% ORR with a 35% CR rate, and a median PFS in excess of 41 months. In addition, this latter study also yielded an ORR >80% in previously treated patients who were not fludarabine-refractory at the start of treatment. A similar PFS was recently reported by Flinn et al., with a modified FC protocol.

Fludarabine plus rituximab protocols

Fludarabine is a purine analog that is phosphorylated to the triphosphate, leading to inhibition of DNA synthesis, and cyclophosphamide is a DNA alkylating agent. It is reasonable to suppose that adding an antibody, specific for a CLL surface marker, might yield further improvements in response, as the mechanisms of action of the antibody would differ from those of the antimitabolites. Several studies have examined the effect of combining fludarabine or FC with rituximab, a monoclonal antibody directed against the CD20 B-cell marker. The results, consistent with the above hypothesis, were encouraging and are included in the summaries shown in Table 1. Furthermore, a recent retrospective...
analysis indicated that fludarabine combined with rituximab results in greater PFS and OS than fludarabine alone, although the combination was associated with markedly higher levels of neutropenia.\textsuperscript{15} Response rates for these protocols are shown in Table 1, together with the responses obtained with single-agent rituximab and alemtuzumab therapies. At the doses used, a greater proportion of the responses to alemtuzumab were CRs than in the case of rituximab therapy. Interestingly, rituximab therapy appears to induce a transient downmodulation of CD20 expression.\textsuperscript{17} Furthermore, the level of circulating CD20 protein may have an inverse prognostic significance.\textsuperscript{18}

### Minimal residual disease

A CR should not be construed as the elimination of MRD. The majority of patients achieving a CR will eventually relapse due to the presence of MRD,\textsuperscript{1} which may or may not be detectable, depending on the methodology used.\textsuperscript{1} The assessment of MRD levels is discussed in more detail by Ritgen on page 5 of this supplement.

Magnac\textsuperscript{19} et al.\textsuperscript{19} reported on a series of 12 patients in CR, nine following chemotherapy and three after autologous bone marrow transplantation (ABMT). Using consensus PCR, MRD was detectable in all but one of the chemotherapy patients and none of the ABMT patients. However, when the more sensitive ASO-PCR method was used, which is based on individual complementarity determining region-3 sequences, MRD was detectable in all patients with the sole exception of one of the ABMT patients. Of note, the one chemotherapy patient who was MRD-negative by consensus PCR, but MRD-positive by ASO-PCR, relapsed at 1 year. The clear inference to be drawn is that assessment of MRD status must include consideration of the method(s) used\textsuperscript{4} and, furthermore, that when highly sensitive detection methods are employed, elimination of MRD by chemotherapy alone is rare. This is consistent with the conclusion of Brüggemann et al.\textsuperscript{1} that patients in CR are nevertheless likely to have significant levels of MRD.

### Alemtuzumab therapy

The above results notwithstanding, alemtuzumab appears to offer even greater potential than previous protocols for the eradication of MRD. Alemtuzumab is a humanized recombinant IgG1κ monoclonal antibody with human Fc and V region framework sequences. The complementarity determining regions (CDR) are derived from rodent (rat) gene sequences.\textsuperscript{20} The antibody is specific for the CD52 cell-surface glycoprotein, which is found at densities of up to $5 \times 10^5$ binding sites per cell on the surface of normal and malignant B and T cells, as well as on natural killer (NK) cells, monocytes and macrophages. However, CD52 does not appear to be expressed by granulocytes, or myeloid or erythroid bone marrow cells. Furthermore,
treatment of bone marrow with the non-humanized parent campath-1 antibody did not delay engraftment following autologous transplantation in rhesus monkeys.\textsuperscript{20} In addition, incubation with campath-1H did not affect the numbers of CD34\(^+\) hematopoietic precursor cells or their growth in long-term bone marrow cultures.\textsuperscript{22} Collectively, these results indicate that treatment with alemtuzumab should not affect hematopoietic recovery after transplantation, an important consideration for its therapeutic application.

In 1996, Osterborg et al.\textsuperscript{21} reported the results of a pilot study using alemtuzumab as first-line treatment in nine patients with CLL. Three patients achieved a CR, and five a partial response (PR). Furthermore, malignant cells were cleared from the peripheral blood in all patients and from the marrow in seven patients. Response durations ranged from 8 to 24 months and, with the exception of one case of cytomegalovirus (CMV) pneumonitis, adverse events were mild. These results suggested that alemtuzumab might be a highly effective and well-tolerated treatment option for first-line use. In addition, the same group also reported that alemtuzumab had significant activity in patients who had relapsed disease following chemotherapy\textsuperscript{23} or refractory disease. Subsequently, they reported the results of a first-line phase II trial with subcutaneous (s.c.) alemtuzumab in 41 patients with CLL.\textsuperscript{14} An ORR of 87% and a CR rate of 19% were obtained in 38 evaluable patients. Infections were rare, although one patient suffered CMV reactivation. Twenty-one percent of the patients developed a transient grade 4 neutropenia. The median time to treatment failure (TTF) had not been reached after a median follow-up of 18 months.

Rai et al.\textsuperscript{24} reported the results of a phase II pilot study in 24 patients with CLL whose disease had not responded to prior fludarabine chemotherapy regimens, or who had relapsed after a response duration of less than 6 months. Eight of the patients (33%) achieved a PR, although no CRs were attained. Nonetheless, prolonged response durations were obtained in responders. Ten patients developed major infections and the authors recommended that future alemtuzumab protocols should include prophylaxis against infections. It is, however, important to note that the incidence of infections in this trial could have been a consequence of the patients' characteristics rather than of alemtuzumab therapy per se. Fludarabine-refractory patients have a markedly higher rate of infection than those with fludarabine-sensitive disease (48% vs. 18%).\textsuperscript{25} Furthermore, response status is the factor primarily responsible for reducing infectious morbidity and mortality. Thus, patients with heavily pretreated fludarabine-refractory CLL are considerably more likely than fludarabine-sensitive patients to experience rapid disease progression, bone marrow failure, immunosuppression, high infection rates and significant short-term mortality.\textsuperscript{24} Set in this context, the ability of alemtuzumab to produce prolonged responses acquires correspondingly greater importance.

Based on the significant activity observed by Rai et al.\textsuperscript{24} in pretreated poor-prognosis patients, a larger trial (CAM 211) was initiated.\textsuperscript{25} Ninety-three patients were enrolled for this study; their median age was 66 years and 76% of them had advanced disease (Rai stage III/IV). Eligibility criteria included B-CLL with up to seven previous therapies, which must have included at least one alkylating agent regimen and failure with fludarabine (defined as failure to respond to at least one fludarabine regimen, disease progression while receiving fludarabine, or response duration of \(< 6\) months following the last fludarabine treatment). The primary endpoint was ORR with safety as a secondary endpoint. Following an initial dose escalation period, patients were treated with intravenous (i.v.) alemtuzumab for 4–12 weeks, with response evaluations every 4 weeks. In the event of disease progression or a CR, treatment was discontinued, otherwise alemtuzumab therapy continued for up to 12 weeks.\textsuperscript{24,25}

The ORR obtained in this trial was 33% with a 2% CR rate by National Cancer Institute sponsored Working Group (NCIWG) assessment.\textsuperscript{25} However, an additional 7% of patients had clearance of B-CLL cells from all sites, but with persistent anemia or thrombocytopenia. The median response duration was 8.7 months (2.5–22.6). The median time to progression (TTP) in the intent-to-treat (ITT) population was 4.7 months and 9.5 months in responders. Furthermore, there was a substantial reduction in disease in both the peripheral blood and the bone marrow, although MRD was not assessed. Overall, median survival was 16 months (32 months in responders), results that compare favorably with the 10-month median survival observed with other salvage regimens in fludarabine-refractory CLL.\textsuperscript{26}

Anti-infective prophylaxis was employed in this trial and continued for at least 2 months after treatment cessation. Overall, 55% of patients developed an infection during the study. The most frequent opportunistic infection was CMV reactivation, which occurred in seven patients. Five of these cases resolved and the other two patients discontinued alemtuzumab treatment. The authors concluded that alemtuzumab was effective, and had an acceptable safety profile in treating high-risk patients with advanced disease.\textsuperscript{25} The optimal management of alemtuzumab therapy in the treatment of CLL has been discussed in recent reviews.\textsuperscript{24,27} Alemtuzumab has also been used as consolidation therapy following prior tumor reduction by chemotherapy.\textsuperscript{24,25} Alemtuzumab consolidation thera-
Rationale for fludarabine/alemtuzumab combination therapy

The above discussion underscores the availability of two different therapeutic approaches, each of which has significant efficacy as a first-line monotherapy. These observations provided compelling arguments for assessing the potential efficacy of fludarabine and alemtuzumab combination treatment: (i) these two agents have different mechanisms of action; (ii) there is, as yet, no standard therapy for patients with relapsed CLL; and (iii) the additive or synergistic effects of combining chemotherapeutic and immuno-therapeutic modalities have been demonstrated in malignant lymphomas, in addition to the rituximab studies cited above.

In a small but seminal pilot study, Kennedy et al. selected 6 patients with fludarabine-refractory CLL, who had also failed to benefit from alemtuzumab monotherapy, and treated them with fludarabine and alemtuzumab in combination. Five of the 6 patients responded (1 CR, 4 PR) and 5 were alive at 12 months of follow-up, which compares favorably with an expected median survival of 10 months. Furthermore, 2 of the patients became MRD-negative in the bone marrow, assessed using MRD flow. Hence, alemtuzumab, when combined with fludarabine, achieves significant responses, including immunophenotypic remissions, in patients in whom both fludarabine and alemtuzumab had failed as single agents. In light of these highly favorable results, and the apparent synergy between these agents, a phase II trial was conducted.

FluCam phase II study

Inclusion criteria for this study included relapsed or refractory CLL, at least one prior treatment regimen (no upper limit was set) and a World Health Organization (WHO)/Eastern Cooperative Oncology Group (ECOG) performance status 0–2. The primary endpoint was ORR, with response duration, toxicity and MRD as secondary endpoints. The protocol was initiated with a dose escalation of alemtuzumab up to 30 mg over 3 days, followed by the FluCam schedule. Fludarabine was administered at 30 mg/m² on days 1–3, followed immediately on each day by a 2-hour infusion of 30 mg alemtuzumab. The cycle was repeated four times at 4-weekly intervals. Compared with the standard monotherapy regimens (fludarabine 6 cycles of 5 x 25 mg/m²; alemtuzumab 12 weeks of 3 x 30 mg), the FluCam schedule has a markedly lower overall administered dose (Table 2). In addition, antimicrobial prophylaxis was administered throughout treatment duration and for at least 2 months afterwards.

Thirty-four of 37 patients are evaluable to date. Their median age was 61 years (range 38–80 years), and the median number of prior treatment regimens was 2 (range 1–8). Seven of the 34 evaluable patients had autoimmune hemolytic anemia or thrombocytopenic purpura before the initiation of treatment. The ORR was 85%, which included 10 CR and 19 PR. Transient grade 3/4 hematologic toxicity was observed in patients with considerable bone marrow involvement, and one heavily pretreated patient who was refractory to both fludarabine and alemtuzumab died of fungal infection. Two patients experienced CMV reactivation, one of whom died from Escherichia coli sepsis. However, in general, side effects were mild and related only to the first 2 cycles of treatment.

There was impressive clearance of tumor cells from both the marrow and peripheral blood (Figure 1). Significantly, 15 of the 34 evaluable patients became MRD-negative in the peripheral blood, based on MRD Flow. Four of 6 patients evaluated also became MRD-negative in the bone marrow. Median TTF, to date, is 15.3 months.

These data show that the FluCam regimen is feasible, highly effective, and well tolerated in patients with...
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ure 2, alemtuzumab is administered subcutaneously in this study rather than intravenously. Other studies have indicated that the s.c. route of administration has equivalent efficacy to i.v. administration but with fewer side effects.¹⁴ Unfortunately, no data are available from this study yet.

Further studies

CLL2L study

Fludarabine/cyclophosphamide combination therapy has been shown to have a higher efficacy than fludarabine alone.¹ Hence, building on the above phase II study, cyclophosphamide has been added to the above FluCam regimen (FC-Cam), as shown in Figure 2.

Eligibility criteria for the CLL2L study are B-CLL in Binet stage C or A/B, relapsed or refractory disease after 2 prior regimens, adequate organ function, and any prior treatment with fludarabine or alemtuzumab at least 6 months earlier. In addition, as shown in Figure 2, alemtuzumab is administered subcutaneously in this study rather than intravenously. Other studies have indicated that the s.c. route of administration has equivalent efficacy to i.v. administration but with fewer side effects.¹⁴ Unfortunately, no data are available from this study yet.

CAM 314 phase III trial

Following on from the encouraging results of the phase II FluCam trial described above, a phase III trial (CAM 314) has been initiated to compare the FluCam protocol with standard fludarabine monotherapy (four cycles of 25 mg/m² for 5 days). Inclusion criteria are relapsed or refractory B-CLL after only one prior regimen. Patients who previously responded to fludara-

bine or alemtuzumab are eligible provided the response lasted at least 12 months. Patients will be treated for 4 weeks and then restaged. At the time of writing, the study is still recruiting patients and no results are yet available.

Conclusions

These studies show that FluCam and FC-Cam regimens are highly effective and well tolerated in patients with previously treated CLL. Significantly, MRD-negative status can be obtained in both bone marrow and peripheral blood. Longer follow-ups are now needed to determine whether or not these MRD-negative remissions actually confer longer PFS and OS than those in patients who remain MRD positive. Lastly, a phase III trial is underway comparing FluCam with fludarabine monotherapy.

Collectively, these results indicate that the combination of these highly efficacious drugs, in either the FluCam or FC-Cam regimens, has the potential to yield significant response rates and highly durable remis-

References


