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Re-evaluation of response criteria in chronic lymphocytic leukemia and the significance of minimal residual disease

Chronic lymphocytic leukemia (CLL) is one of the most common lymphoid malignancies in the developed world, affecting approximately 120,000 people annually in the USA and Europe.¹ The disease has a very variable clinical course; many patients survive for decades without requiring treatment and die of unrelated causes, whereas other patients develop more aggressive forms of the disease that lead to an early death. Traditionally, the approach to the management of CLL has been *watchful waiting*, with clinical staging (Rai or Binet) providing limited prognostic information.^{2,3}

Improvement in prognostic factors for CLL

Over the past few years, however, the establishment of additional prognostic factors has enabled patients to be better differentiated into risk categories. Elevated levels of several new serologic parameters are predictive of an unfavorable outcome; these parameters include β_2 microglobulin (β_2 M), thymidine kinase (TK), and soluble CD23.⁴⁻⁶

In addition, genetic parameters, especially the mutational status of immunoglobulin variable heavy-chain genes (VH), and cytogenetic abnormalities such as 17p (p53 gene) and 11q mutations, have been shown to correlate with clinical course. Thus, somatic mutations of the VH gene have been associated with stable disease and long survival, whereas an unmutated germ line VH configuration has been associated with a relatively poor prognosis.⁷⁻¹⁰ Low expression of cell-surface CD38 on CLL cells has also been associated with a mutated phenotype and a favorable prognostic outcome.⁷

One of the strongest prognostic factors for survival appears to be p53 deletion or mutation, increasing the risk of death by 13-fold compared with that in patients without p53 deletion; p53 deletion or mutation also appears to predict drug resistance.¹¹ More recently, gene expression pro-

file analysis has demonstrated that zeta-associated protein 70 (ZAP70), a tyrosine kinase enzyme, is also associated with an unmutated phenotype, and is itself an independent prognostic factor for CLL.^{12,13} It is therefore increasingly likely that it will become possible to differentiate those patients with minimal risk of dying from CLL from those with more aggressive forms of the disease, and to be able to tailor treatment accordingly.

The potential for cure rather than palliation of CLL

As well as the development of better prognostic factors, treatment options have also expanded in recent years and now include monoclonal antibodies, such as rituximab and alemtuzumab, combination chemotherapy and allogeneic stem cell transplantation (SCT). These newer therapeutic strategies have dramatically improved response rates and are likely to lead to an improvement in overall survival.¹⁴

It is hoped that the development of more effective therapies will transform CLL into a curable disease in selected subsets of patients, as has occurred with other previously incurable lymphoid malignancies, such as Hodgkin's disease, acute lymphocytic leukemia (ALL) and diffuse large B-cell lymphoma. The therapeutic goal of CLL is, therefore, shifting away from that of palliative therapy towards one of achieving a long duration of response, and ultimately a cure.

The history of response criteria in CLL

Following the use of Binet and Rai prognostic criteria for several decades, the response criteria for CLL were updated by the National Cancer Institute (NCI) Working Group and the International Working Group on CLL (IWCLL).^{15,16} The criteria include features that allowed for comparability between studies. The updated definition of complete remission (CR) includes the disappearance of clinical evidence of disease on examination and the normalization of bone marrow and hematopoiesis. Initially,

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Table 1. National Cancer Institute Working Group Criteria for complete remission in CLL.¹⁶

Physical examination	Normal
Symptoms	None
Lymphocytes	4x10 ⁹ /L
Neutrophils	≤1.5x10 ⁹ /L
Platelets	> 100x10 ⁹ /L
Hemoglobin	> 11 g/dL (untransfused)
Bone marrow lymphocytes	< 30%; no nodules

however, patients were still classified as attaining CR even in the presence of persistent nodules in the bone marrow biopsy.^{15,17} It soon became obvious that the persistence of these nodules was associated with persistence of CLL, with a consequently higher likelihood of relapse.¹⁸

The NCI criteria for CR in CLL did not require radiological imaging of disease or assessment of minimal residual disease (MRD)¹⁶ (Table 1). However, the advent of flow cytometry, detecting monoclonal CD5/CD19 co-expressing cells, was able to show residual disease in patients who had otherwise achieved a CR, according to NCI criteria, including bone marrow biopsy remission (<30% lymphocytes).¹⁸

The role of MRD status in CLL

MRD is usually detectable in patients treated with conventional chemotherapy, including purine analogs.^{19,20} Furthermore, a number of studies have suggested that the persistence of MRD after autologous transplant can predict lower overall survival.²¹⁻²⁴ Improved understanding of MRD may therefore help to identify those patients in remission who are at risk of relapse. Early therapeutic intervention based on the presence of MRD may improve outcome and prolong survival, and monitoring MRD could therefore help to direct treatment strategy. Thus, eradication of MRD may become a new therapeutic target, especially for patients with a poor prognosis.

The aims of research on MRD in leukemia include improving the measurement of treatment response, providing independent prognostic information and optimizing therapeutic strategies. In CLL, MRD may be detected by immunophenotype (multiple-color flow cytometry) or quantitative real-time polymerase chain reaction (QR-PCR).

The monitoring of MRD has provided independent prognostic information in other leukemias, including childhood acute lymphoblastic leukemia, chronic myeloid leukemia and acute promyelocytic leukemia.²⁵⁻²⁸

In CLL, the traditional goal of conventional therapy has been symptom palliation, with most patients show-

ing a partial response (PR). As even patients with CR are likely to have a significant level of residual disease,^{19,20} the measurement of MRD is not relevant in patients with conventionally treated CLL. However, the introduction of therapeutic strategies, such as autologous or allogeneic SCT and monoclonal antibodies, has resulted in a significant proportion of patients being able to achieve much better responses. The evaluation of MRD has therefore gained greater importance as a prognostic factor.

Treatment options in CLL: potential to achieve MRD negativity

Attempts have been made to achieve MRD negativity by using combination chemotherapy, such as combinations of fludarabine and cyclophosphamide in advanced previously untreated CLL,²⁹ combined fludarabine, cyclophosphamide and mitoxantrone (FCM) in relapsed/refractory CLL,³⁰ and cladribine combined with cyclophosphamide.³¹

Some patients treated with these combinations have achieved PCR negativity. In a study by Bosch *et al.*,³⁰ 30 of 60 (50%) patients achieved CR, of whom 10 achieved MRD-negative CR, whereas in a study by Robak *et al.*,³¹ 24 of 82 (29.3%) patients achieved CR, of whom 18 patients achieved MRD negativity by immunophenotyping. Cazin *et al.*²⁹ reported that 40 of 75 (53%) patients achieved CR; however, although 20 of 30 (66%) CR patients tested by four-color flow cytometry were MRD-negative, only four of 15 (27%) CR patients tested with PCR were found to be MRD-negative.

Monoclonal antibodies

Moreton *et al.*³² demonstrated that MRD-negative response following treatment with alemtuzumab was a better predictor of response duration and overall survival than NCI response criteria in patients with relapsed/refractory CLL. According to the NCI response criteria, CR was achieved in 32 of 91 (35%) patients, whereas according to flow cytometry (bone marrow and blood), MRD-negative CR was obtained by 18 of 91 (20%) patients. The overall survival for the 18 patients with MRD-negative remission was 84% at 60 months, with 8 (47%) of the MRD-negative patients converting to MRD-positive status after a median of 38 months.

Chemoimmunotherapy

More recently, the development of monoclonal antibodies has led to the use of chemoimmunotherapy, using purine nucleoside analogs (fludarabine or pentostatin) combined with rituximab (anti-CD20 monoclonal antibody), with or without cyclophosphamide. Although fludarabine combined with cyclophosphamide and rituximab has resulted in an overall response rate (ORR) of 95% (69% CR, 10% NPR, 16% PR),³³ the

method used to measure MRD sensitivity was a semi-quantitative method. Using FAND (a fludarabine-containing combination regimen) combined with alemtuzumab,³⁴ only one in four evaluable patients with a poor prognosis for CLL achieved molecular remission, even though all four had shown a cytometric response (3 CR, 1 PR). However, in a phase II trial of fludarabine combined with alemtuzumab (FluCam), an ORR of 85% was achieved in 34 of 37 evaluable patients (10 CR, 19 PR). Fifteen of the 34 patients were found to have MRD-negative blood by four-color flow cytometry, with four of six patients found to have MRD-negative bone marrow.^{35,36} At the time of reporting, the median time to treatment failure was 15.3 months. The FluCam regimen appears to be feasible, highly effective and well tolerated in patients with relapsed or refractory CLL, and these findings have provided a rationale for further studies using alemtuzumab chemoimmunotherapy. However, in order to compare the efficacy of different treatment regimens, it is important to bear in mind the technique used to determine MRD. For example, consensus PCR has a sensitivity of 1 in 10,000 leukocytes, whereas MRD Flow (gated four-color flow cytometry) has a sensitivity of 1 in 50–100,000 leukocytes.^{28,37} Published future research should therefore indicate the sensitivity level of the assay used to measure MRD negativity.

Alemtuzumab consolidation therapy

As the action of alemtuzumab is limited by bulky lymph nodes, some researchers have hypothesized that activity may be greater with less extensive disease. Following debulking therapy with fludarabine, Montillo *et al.*³⁸ administered subcutaneous alemtuzumab three times weekly to a maximum dose of 10 mg for 6 weeks. They found that 16 of 30 (53%) patients achieved a molecular response as measured by PCR (IgVH-negative) after treatment with alemtuzumab.

Similarly, the German CLL study group has recently reported on a phase III trial using alemtuzumab consolidation therapy.³⁹ CLL patients responding to initial chemotherapy with fludarabine alone or in combination with cyclophosphamide were randomized for treatment with alemtuzumab (30 mg intravenously three times a week for 12 weeks) or observation. Of 21 evaluable patients, 11 were randomized to alemtuzumab before the study was stopped due to severe infections in seven of 11 patients. At 6 months after randomization, two patients in the alemtuzumab arm had converted to CR, whereas three patients in the observation arm had progressed. After alemtuzumab treatment, five of six patients achieved a molecular remission in peripheral blood whereas all patients in the observation arm remained MRD-positive ($p=0.048$). At a median follow-up of 21.4 months, patients receiv-

ing alemtuzumab showed a significantly longer progression-free survival (no progression vs. a mean of 24.7 months; $p=0.036$). In conclusion, the data show that consolidation therapy with alemtuzumab is able to achieve molecular remissions and longer survival in patients with CLL.

Autologous and allogeneic SCT

MRD-negative responses have been observed following autologous and allogeneic SCT. The persistence of MRD in CLL patients appears to have different implications according to whether autologous²³ or allogeneic SCT has been performed.^{23,40–42} Provan *et al.*²¹ reported an association between the persistence of MRD and subsequent relapse with autologous SCT; however, delayed MRD clearance following allogeneic SCT may be indicative of a graft-versus-leukemia (GVL) effect.

Discussion: aims of the Workshop

In this symposium, it was shown that MRD-negative CR should be the goal of therapy in selected subsets of patients with CLL. MRD status seems a better predictor of response duration and survival than current response criteria. Therefore, the NCI response criteria may need some careful revisions, at least with regard to the conduct of clinical trials in the near future. MRD status should be included in the post-treatment work-up of patients treated, with the intent to achieve a long-lasting remission. It cannot be stressed enough that the evaluation of MRD needs to be harmonized between different laboratories and study groups to render the results comparable between different trials in the future.

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