Chronic lymphocytic leukemia: prognosis and treatment

Przewlekła białaczka limfocytowa: rokowanie i leczenie

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a clonal disease characterized by proliferation and accumulation of small CD5 positive B-cells. It is the most common leukemia in the western world accounting for approximately 30% of all leukemias in Europe and North America with an incidence of 4:100,000/year (1). The disease is diagnosed most commonly in the elderly. The median age at diagnosis is 65-70 years with 80% of patients diagnosed over 60 years of age. The natural clinical course of CLL is highly variable and chemotherapy is usually not indicated in an early and stable disease. However,
patients with progressive and more advanced disease require treatment (2).

**PROGNOSTIC FACTORS**

Clinical staging systems proposed in the early 1980's by Binet et al. (3) and Rai et al. (4) are still the most common and validated prognostic factors in the patients with CLL. Within the past few years, several biological markers, including serum markers, immunoglobulin heavy chain variable region (IgVH) mutation status, some cytogenetic abnormalities, P53 mutations, cell membrane expression of CD38 and intracellular expression of zeta-associated protein-70 (ZAP-70), have become important prognostic factors (5). A correlation between the immunoglobulin gene mutational status and prognosis has shown that the median survival for stage A patients with unmutated IgVH genes was 8 years, compared with approximately 25 years for patients with mutated IgVH genes.

Deletion of 11q22-q23 and deletion of 17p13 are independent prognostic factors identifying patients with a rapid disease progression and a short survival time in a multivariate analysis (6). The deletion 17p affecting the TP53 gene has been associated with failure after treatment with alkylating agents, purine analogs, and rituximab (7). In contrast, deletion of chromosome band 13q14 is associated with a favourable outcome. Moreover, patients with trisomy 12 have a shorter survival than those with 13q deletion. Deletion of 17p and deletion of 11q predominate among advanced stages of CLL and among patients with unmutated IgVH genes. In patients with CLL deletion 17p13 is independent prognostic factor identifying patients with rapid disease progression and short survival time. Furthermore, ZAP-70 expression and CD38 expression on leukemic lymphocytes have been found to correlate with IgVH mutations and a shorter patient survival. Recent data may indicate that a prognostic score constructed using modified Rai stage, cellular CD38 and serum lactate dehydrogenase (LDH) significantly predict time to treatment failure and survival in patients at the time of diagnosis, and perform as well or better than models using the newer markers. The expression of ZAP-70 remains constant over the course of the disease as opposed to CD38. ZAP-70 expression can be evaluated using flow cytometric techniques, immunohistochemistry, western blotting or reverse transcriptase-polymerase chain reaction techniques. The cut-off to classify patients as ZAP-70 positive or ZAP-70 negative remains controversial and arbitrarily varies between 10% and 20%. Standardization of its measurement at the messenger RNA (mRNA) or protein level needs further investigation. Several serologic parameters such as thymidine kinase (TK), β2-microglobulin (β2M) and soluble CD23 (sCD23) provide also valuable information about the disease progression and survival.

**INDICATIONS FOR TREATMENT**

There is no evidence that cytotoxic therapy has beneficial effects in patients with the indolent form of the disease (9, 10). However, patients with symptomatic and/or progressive disease should be immediately treated.

Widely accepted guidelines for the initiation of chemotherapy in CLL patients have been proposed by the National Cancer Institute Sponsored Working Group (NCI-WG) (11, 12). According to these guidelines the criteria for the initiation of therapy may not be identical for routine clinical practice and for patients included in clinical trials. In the routine clinical practice, therapy should not be initiated in patients who have asymptomatic CLL, including those with Rai stage 0 or Binet stage A until disease progression or unless disease-related symptoms are evident. Laboratory results supporting deferred therapy include a non-diffuse pattern of bone marrow involvement, serum haemoglobin concentration > 13 g/dL, peripheral blood lymphocytes < 30 × 10⁹/L, and lymphocyte doubling time longer than 12 months. The criteria for treatment initiation include disease-related symptoms, especially fever, body weight loss and extreme fatigue, increasing bone marrow failure, autoimmune anaemia and/or thrombocytopenia responding poorly to corticosteroid treatment, massive or progressive splenomegaly and/or lymphadenopathy, progressive lymphocytosis and recurrent infections.

**ALKYLATING AGENTS**

For many years, chlorambucil has been considered the drug of choice in previously untreated patients. Higher response rates were obtained when higher doses of chlorambucil were used (13). Chlorambucil is still acceptable as the first line treatment of progressive CLL in frail, elderly patients with comorbidities, because of the apparent increase in toxicity of purine nucleoside analogs (PNAs) in this group of patients. The recent results of the German CLL study group (GCLLSG) support such a recommendation (14).

Bendamustine (Treanda; Cephalon), is a bifunctional alkylating agent composed of an alkylating nitrogen mustard group and a purine like benzimidazole ring. This agent seems to have a low cross-resistance with alkylating agents and fludarabine. Bendamustine have been investigated in several clinical trials (15). Various doses and treatment schedules have shown significant efficacy and acceptable toxicity in previously treated patients. The dose of 70 mg/m² on days 1 and 2 every 4 weeks is suggested in heavily pretreated and treatment-refractory patients. Bendamustine was also investigated in an open-label, randomized, comparative trial (16). Previously untreated patients were randomly assigned to receive either bendamustine (100 mg/m² i.v. on days 1 and 2) or chlorambucil (0.8 mg/kg p.o. on days 1 and 15). An overall response rate and a CR rate were significantly higher in patients treated with bendamustine. Moreover, the median duration...
of response was longer after treatment with bendamustine (21.7 months) than after chlorambucil (9.3 months, p < 0.0001). Other alkylating agents have been less extensively investigated than chlorambucil and mainly in combination therapy. Cyclophosphamide (Cy) has a similar activity to chlorambucil and is usually used when chlorambucil is poorly tolerated. Cyclophosphamide was more frequently used in combination therapy with vincristine and prednisone (COP, CVP) or with doxorubicin, vincristine and prednisone (CHOP) (10).

**PURINE NUCLEOSIDE ANALOGS**

In the past 20 years the purine nucleoside analogs (PNA) – fludarabine (FA), cladribine (2-CdA, 2-chlorodeoxyadenosine) and pentostatin (DCF, 2'-deoxycytodfomycin) have been introduced for treatment of CLL (10,13). Significantly higher OR, CR, and longer progression free survival (PFS) in patients with CLL treated with FA or 2-CdA have been confirmed in randomized, multicenter trials and more recently in meta-analysis. However, the median survival time did not differ between patients treated with PNA and alkylating agents. Combination therapies with PNA and CY are more active than monotherapy in terms of OR, CR and PFS. However, higher overall response (OR), CR and PFS do not translate for longer overall survival. Over the last few years several monoclonal antibodies (mAbs) and immunotoxins have been investigated in clinical trials in patients with CLL. Recently, we performed a randomized study that compared the activity and toxicity of 2-CdA and CY (CC programe) versus FA and CY (FC programe) in previously untreated progressive or symptomatic CLL (2). We did not observe any significant differences in CC and FC efficacy and toxicity across different patient prognostic subgroups (17).

**MONOCLONAL ANTIBODIES**

Over the last few years several monoclonal antibodies (mAbs) and immunotoxins have been investigated in clinical trials in patients with CLL (18). The most important clinical value in the patients with CLL have at present two antibodies. The first is human mouse antibody rituximab that targets CD20 antigen. The second is alemtuzumab, a humanized form of a rat antibody active against CD52.

**RITUXIMAB**

Rituximab (Rituxan, Mabthera, F. Hoffmann-La Roche) is an IgG1 kappa immunoglobulin containing murine light – and heavy-chain variable region sequences and human constant region sequences (18, 20). The Fab domain of the rituximab binds specifically to the CD20 antigen, expressed on normal and malignant B-lymphocytes. The Fc domain recruits immune effect or functions to mediate B-cell lysis in vitro and in vivo.

The precise mechanism of rituximab cytotoxicity remains unclear. However, several mechanism by which rituximab may be cytotoxic are suggested. These include complement-dependent cytotoxicity (CDC), which involves fixation of the complement by the Fc portion of immunoglobin and the subsequent activation of the complement cascade. Moreover, rituximab induces antibody-dependent cell mediated cytotoxicity (ADCC) in vitro. These two mechanisms are categorized into “immune-mobilizing” mechanisms or direct effects. However, accumulating evidence suggests that rituximab can also directly induce apoptosis.

Rituximab in conventional doses of 375 mg/m² weekly for 4 doses has rather low efficiency in CLL. In the study performed by Byrd et al. 83 previously treated CLL patients were treated with different doses of rituximab (250 mg/m²-375 mg/m²) 3 times weekly for 4 weeks (20). The OR rate was 45% (3% CR and 42% PR). Responses were noted in all groups of patients including those with bulky lymphadenopathy and those for which alkylator and/or FA based therapy had failed. However, some studies suggest that higher doses are more effective than standard doses, used routinely in other lymphoid malignancies O’Brien et al. treated CLL patients with an initial dose of rituximab 375 mg/m² which was then increased to a fixed dose of between 500 and 2250 mg/m² once weekly for 4 weeks (21). The OR rate was 36% and ranged between 22-75%. All responses were partial responses (PR). Median time to progression in responded patients was 8 months with the longest remission duration 15 + months.

**IMMUNOCHEMOTHERAPY**

Several recent reports suggest that in patients with CLL, rituximab combined with PNA can increase the OR and CR rates and prolonge PFS as compared with PNA or rituximab alone with acceptable toxicity. The addition of rituximab to a variety of chemotherapy regimens for the treatment of patients with CLL has yielded promising results in phase II and III trials (22-24). The combination of rituximab with FC (R-FC regimen) demonstrated particularly high rates of overall response, CR, PFS, and overall survival in previously untreated and relapsed/refractory CLL. In order to validate this concept the German CLL study group (GCLLSG) initiated a multicentre, multinational phase III trial, CLL8, to evaluate the efficacy and tolerability of R-FC versus FC for the first-line treatment of pts with advanced CLL (23). In this study 817 patients were randomly assigned to receive 6 courses of either FC of R-FC. The overall response rate was significantly higher in the R-FC arm (95%) compared to FC (88%) (p = 0.001). The complete response rate of the R-FC arm was 52% as compared to 27.0% in the FC arm (p < 0.0001). PFS was 76.6% at 2 years in the R-FC arm and 62.3% in the FC arm (p < 0.0001). There was a longer OS in the R-FC arm.

In the REACH trial 552 relapsed or refractory patients from 17 countries were randomized (1:1) to receive either R-FC or FC (24). A median of one prior
treatment had been administered, consisting of single-agent alkylator therapy (66%), purine-analogs (16%), or combination treatments (CHOP, COP, F-containing, 18%). Patients with prior FC combination treatment or prior rituximab were not eligible. Median observation time was 25 months. The primary endpoint PFS was significantly prolonged by median 10 months in the R-FC arm (30.6 months) compared to FC (20.6 months, \( p = 0.0002 \)). Secondary endpoints showed similar results. Overall response rate was higher for R-FC vs. FC (70% vs. 58%, \( p = 0.0007 \)). Grade 3/4 adverse events were higher in the R-FC arm (80%) vs. FC (74%), but severe adverse events were similar (50% vs. 48%, respectively). Grade 3/4 neutropenia and febrile neutropenia were only marginally increased for R-FC (42% and 15%) vs. FC (40% and 12%, respectively), the same was seen for thrombocytopenia (R-FC 11% vs. FC 9%). Grade 3/4 infections (R-FC 18%, FC 19%) were similar, and there was no difference in bacterial, viral, or fungal infections between the two arms. Grade 3/4 anemia was slightly increased in the FC arm (R-FC 2%, FC 5%).

**Chemoinmunotherapy combining rituximab with FA and cyclophosphamide is becoming the first-line choice for younger CLL patients.** Moreover, several studies have confirmed significant activity of these agents in relapsed or refractory CLL (25). In recent years, four drug combination therapies with R-FC and other agents including mitoxantrone, lumiliximab or alemtuzumab, have been investigated in CLL patients and showed that these regimens are highly effective in previously untreated CLL. However, at present it is unclear whether these regimens have an advantage over R-FC immunochemotherapy alone.

We investigated efficacy and toxicity of the combined therapy consisting of rituximab and 2-CdA (RC protocol) or 2-CdA, CY and rituximab (RCC protocol) in patients with refractory or relapsed CLL (26). The RC regimen consisted of a 6-hour infusion of rituximab 375 mg/m² on day 1 and 2-hour infusion of 2-CdA 0.12 mg/kg on days 2-6. The RCC protocol consisted of rituximab at a dose of 375 mg/m² on day 1, 2-CdA at a dose of 0.12 mg/m² on days 2 through 4, and intravenous CY at a dose of 250 mg/m² per day on days 2 to 4. The RC/RCC courses were repeated at 4-week intervals. Forty-six patients entered the study. Three patients (6.5%) achieved a CR and 31 (67%) patients achieved a PR. According to the particular regimen, the overall response rate was obtained in 12 (67%) patients treated with RC and in 22 (78%) treated with RCC. Hypersensitivity to rituximab occurred in 16 (33%) patients, mostly during the first infusion of the drug. Grade 3/4 neutropenia was seen in six (13%) patients, grade 3/4 thrombocytopenia in three (9%) patients, and grade 3/4 infections were observed in 10 (28%) patients.

**Combinations of high dose methyl prednisolone (HDMP) with rituximab were investigated in previously untreated and relapsed/refractory patients with CLL (25).** HDMP and rituximab were well tolerated and had promising activity when used in combination to treat patients with fludarabine-refractory CLL. These results demonstrate that in combination with rituximab it is a useful treatment strategy in refractory CLL including patients with p53 abnormalities. Further studies of this regimen in controlled trials are warranted.

**ALEMTUZUMAB**

Alemtuzumab (MabCampath, Genzyme) is an unconjugated recombinant DNA-derived humanized IgG1 mAb directed against the CD52 antigen. The CD52 antigen is expressed on normal and neoplastic lymphocytes, monocytes and natural killer cells but not on hematopoietic stem cells. In 2001, alemtuzumab was approved in the US and Europe to treat patients with CLL refractory to FA. **Alemtuzumab is highly active in previously treated patients with CLL (19, 27, 28).**

The effectiveness of this mAb in patients with CLL resistant to conventional treatment was first reported in 1997 by Osterborg et al. (28). They found an OR rate of 43% in 29 patients with a CR in 4% of patients with relapsed or refractory CLL. The median duration of response was 12 months. In 36% of patients a CR was obtained in the bone marrow and in 32% splenomegaly completely resolved. However, resolution of lymphadenopathy was observed only in 7% and bulky lymphadenopathy did not respond to therapy. Several reports have confirmed significant activity of alemtuzumab in relapsed or refractory CLL. Keating et al. (27) investigated the efficacy and safety of alemtuzumab in 93 patients with relapsed or refractory CLL exposed to alkylating agents and having failed previous FA therapy. The OR rate was 33% including CR of 2% and PR of 31%. The median response duration was 8.7 months and overall median survival was 16 months. The results of other studies in smaller groups of previously treated CLL patients have also been published. In different studies, the OR rate range from 31% to 60% and the CR rate from 0% to 31%. In the majority of studies, antitumor effects of alemtuzumab were more significant in blood and bone marrow than in lymph nodes. Alemtuzumab has been also investigated as first-line therapy in CLL.

In a pilot study reported in 1996 by Osterborg et al. (28) nine patients with progressive, previously untreated CLL were included. Alemtuzumab was administered subcutaneously or intravenously. The OR rate was 89% which included CR in three and PR in five patients. In 2002, a prospective randomized phase III trial (CAM 307) comparing high-dose chlorambucil with alemtuzumab in front-line treatment of progressive CLL was commenced (29). In this trial, 279 patients were randomized, 149 patients received alemtuzumab 30 mg 3 times per week for up to 12 weeks, and 148 patients received chlorambucil.
The therapeutic use of PNA in older patients has demonstrated higher response rates and longer progression-free survival compared with chlorambucil.

TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA IN ELDERLY PATIENTS

CLL is predominantly a disease of the elderly, with a median age at diagnosis of 65-70 years. However, the definition of a cut-off point for a patient to be considered elderly is an important issue. The majority of epidemiological studies and clinical trials use a cut-off point of 65 or 70 years to select the elderly population for this leukemia, but more than half of the patients who require therapy are older than 70 years of age. Advanced age has consistently been associated with a poor prognosis in patients with CLL, predominantly due to the frequent occurrence of comorbid conditions (2). Such concerns may result in a less aggressive therapeutic approach being chosen for all elderly patients. However, in a group of elderly patients of the same chronological age, some will be fitter than others. Older adults without severe co-morbidities and with normal age-adjusted renal function appear to tolerate chemotherapy well and with similar toxicity profiles to younger adults. Furthermore, recent randomised trials suggest that quality of life parameters and responses to therapy are not age-dependent. Therefore, uniformly selecting a less aggressive approach for all elderly patients may not be justified, and the optimum approach for treating elderly patients with progressive CLL has yet to be established.
However, first line therapy with purine analogues has failed to demonstrate significant increase in overall survival compared with chlorambucil in randomized phase II trials (14, 33). Adding CY to fludarabine for first line therapy increases its efficacy, although this approach is associated with a moderately higher toxicity compared with F monotherapy. R-FC is becoming the first-line choice for younger patients, but has until now been less frequently used for refractory or relapsed elderly CLL patients, due to the risk of myelosuppression. Reducing the dose of fludarabine and CY while increasing the dose of rituximab (RFC-lite) has recently demonstrated good efficacy (70% complete response) combined with improved tolerability (12% grade 3-4 neutropenia) (58).

As an alternative, rituximab combined with pentostatin and CY (RPC) appears to be a better tolerated regimen than R-FC for elderly patients (34).

However, elimination of cyclophosphamide from the R-FC regimen is also an option. Management decisions are more difficult in frail, elderly patients with co-morbidities because of the apparent increase in toxicity of PNAs, especially in combination with cyclophosphamide and rituximab. In this patient population, chlorambucil is still accepted as the first-line treatment. However, uniformly selecting a less aggressive approach for all elderly patients may not be justified and the optimum approach for treating elderly patients with progressive CLL has yet to be established.

**NOVEL DRUGS**

At present, available therapies are only partially efficient in patients with CLL and there is an obvious need to develop better strategies and new, more specific and active drugs. For the last twenty years, significant progress in molecular and cellular biology has resulted in a better characterization and understanding of the biology and prognosis of CLL. These achievements provided new opportunities for the development of innovative, more effective therapies in this disease. Over the last few years, several new mAbs directed against lymphoid cells have been developed and investigated in preclinical studies and clinical trials (35).

GA-101 (RO5072759) is a novel third generation mAb different from rituximab Salies GA, Morschhauser F, Cartron G et al. A phase I/II study of RO5072759 (GA101) in patients with relapsed/refractory CD20+ malignant disease. GA-101 has been derived from humanization of the parental B-Ly1 mouse antibody and subsequent glycoengineering using GlycoMab technology (36). Compared to rituximab, GA-101 binds with high affinity to the CD20 epitope and, as a result, induction of ADCC is 5-100 times greater than rituximab. It also exhibits superior caspase-independent apoptosis induction than rituximab. In the phase I/IIa study GA-101 was administered as a single agent to 24 patients, at doses from 50 mg to 2000 mg. The antibody has shown a similar safety profile to rituximab and promising efficacy in patients with CLL and other CD20+ malignant disease, for who no therapy of higher priority was available.

The results of recent preclinical and clinical studies suggest that in patients with CLL monoclonal antibodies with other target than CD20 can be useful in the treatment of this disease. These treatments include lumiliximab (anti-CD29), spratuzumab (anti-CD22), apolizumab (anti-MHC-II), galiximab (anti-CD80) and anti-CD40 monoclonal antibodies and TRU-016, small modular immunopharmaceutical (SMIP), a humanized fusion protein derived from key domains of an anti-CD37 antibody.

Except for mAbs, several other agents have been explored and have shown promise in treating CLL. These treatments include immunomodulating agents, agents targeting the antiapoptotic bcl-2 family of proteins, antisense oligonucleotides and other agents. Immunomodulating agents are a new class of drugs that change expression of various cytokines and that costimulate immune effector cells. Lenalidomide (Revlimid) is an immunomodulating agent with possible antiangiogenic properties, that may also modulate cytokine activity in tumor microenvironment. Lenalidomide is orally available and has significant activity in CLL. However, in higher doses this agent has considerable toxicity and the optimal dosing schedule needs to be defined in future trials.

**STEM CELL TRANSPLANTATION**

The exact role of hematopoietic stem cell transplantation (HSCT) in the standard management of CLL patients is still undefined. HSCT has been utilized mainly for patients with high-risk CLL or for those who did not respond to standard therapies (37). Autologous HSCT following induction therapy has the potential for achieving molecular remission in a significant proportion of patients. However, relapses are inevitable and there is no evidence of plateau for an overall and event-free survival. Moreover, the optimal timing for autologous HSCT and optimal mobilization as well as conditioning therapies have not yet been defined. Currently, in the majority of US and European centres, this strategy has been abandoned as being both overly toxic and inadequately effective. Unfortunately, this therapy is associated with high transplant related mortality (TRM). In addition, allogenic HSCT for CLL is limited because of the lack of suitable donors for elderly patients, who constitute the majority at risk for developing this disease.

The development of reduced-intensity conditioning (RIC) regimens has improved the tolerability and reduced the TRM of allogenic HSCT in CLL with a preserving graft-versus-leukaemia effect (38). In addition, allogenic HSCT with RIC can overcome adverse cytogenetic risk. In a recent study, Peres et al. compared 21 CLL patients treated with RIC and 29 patients who received standard conditioning (39). The patients in both groups were similar in terms of the number of earlier therapies or adverse cytogenetics. The 5-year
The overall survival (OS) rate was 63% in the RIC group as compared with 18% in the standard group ($p = 0.006$). The primary cause of inferior survival in the standard conditioning recipients was TRM, which was 27% at day 100 for this compared with 14% in the RIC group ($p = 0.005$). These observations suggest a favourable outcome for advanced CLL patients who undergo a RIC regimen compared with standard myeloablative conditioning. However, the favourable role of RIC in patients with CLL should be confirmed by prospective studies. Recently, the EBMT transplant consensus regarding indications for allogenic HSCT in CLL has been reported by an international expert panel (40). The EBMT experts indicate that allogenic HSCT is a reasonable treatment option for younger patients with non-response or early relapse after having achieved a response with PNA-based combination, and patients with p53 abnormalities requiring treatment. However,
the optimum transplant strategies may vary according to distinct clinical situation.

CONCLUSIONS

Currently available therapies are only partially efficient, exposing obvious need to develop better strategies and new, more specific and active drugs. The approval of rituximab in combination with chemotherapy for use in both previously untreated and previously treated patients with CLL can be viewed as a substantial therapeutic advance. CLL is a heterogeneous disease with highly variable outcomes. These differences are accounted in part by genetic and molecular distinctions within clinical subgroups, as well as interactions with the microenvironment. It appears likely that treatments will need to be modified or tailored to subgroups of patients with CLL, based on intrinsic and extrinsic abnormalities and that multi-agent approach will be required. Recently, several new agents have been explored and have shown promise in treating this disease. In the opinion of the authors, introduction of the newer agents, especially mAbs, for therapy of CLL affirms the feasibility of developing drugs that can destroy even non-proliferating malignant cells. However, despite significant progress, CLL remains incurable disease and the introduction of new drugs and new therapeutic strategies is awaiting. Moreover, the elucidation of the ethiopathogenesis will probably result in the discovery of more specific molecular targets and the development of more specific and less toxic therapies for particular prognostic sub-groups of the disease. Future research should focus on the novel therapeutic strategies based on the molecular pathogenic mechanisms and the development of new targeted therapies.

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