Postępy Nauk Medycznych, t. XXIV, nr 6, 2011

*Tadeusz Robak[1], Paweł Robak[2]

Chronic lymphocytic leukemia: prognosis and treatment

Przewlekła białaczka limfocytowa: rokowanie i leczenie

Departments of Hematology[1] and Experimental Hematology[2], Medical University of Łódź and Copernicus Memorial Hospital
Head of Department: prof. dr hab. med. Tadeusz Robak

S u m m a r y

Chronic lymphocytic leukemia (CLL) is a clonal lymphoid disease characterized by the proliferation and accumulation of small CD5/CD19/CD23-positive lymphocytes in the blood, lymph nodes, spleen, liver and bone morrow. Clinical staging systems proposed in the early 1980s by Binet and Rai have been the longest used for prognostic scoring in patients with CLL. Within the past few years, several biological markers, including serum markers, immunoglobulin heavy chain variable region (IgV_H) 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. Chemotherapy is usually not indicated in the early and stable disease. Widely accepted guidelines for the initiation of chemotherapy in CLL patients have been proposed by the National Cancer Institute Sponsored Working Group (NCISWG). Chlorambucil (Chl), with or without prednisone, was used for many years in the first line treatment of patients with advanced and progressive CLL. More recently, purine nucleoside analogs (PNA), have been introduced and chlorambucil is not longer the leading standard everywhere. Subsequently, higher efficacy of the fludaraine and cyclophosphamide (FC) protocol than fludaraine alone has been confirmed in a phase III trials of treatment naive patients with advanced CLL. Cladribine (2-CdA) in combination with cyclophosphamide (CC regimen) has been also investigated in patients with previously untreated CLL in phase II and III trials. The results of a randomized study comparing the activity and toxicity of CC versus FC in previously untreated progressive or symptomatic CLL indicate that both combinations give similar efficacy and toxicity. Currently rituximab combined with FC or CC is becoming the first-line choice for younger patients.

Key words: CLL, prognosis, treatment, chlorambucil, purine nucleoside analogs, monoclonal antibodies, hematopoietic stem cell transplantation

S t r e s z c z e n i e

Przewlekła białaczka limfocytowa (CLL) jest klonalną chorobą limfoidalną charakteryzującą się proliferacją i akumulacją małych limfocytów Bofenotypie CD5+/CD19+/CD23+ we krwi obwodowej, węzłach chłonnych, wątrobie, śledzionie i szpiku kostnym. Klasyfikacje kliniczne opracowane w latach osiemdziesiątych przez Rai'a i Bineta są do dziś przydatne w ustalaniu rokowania chorych na CLL. Ponadto, w ostatnich latach wprowadzono szereg nowych, biologicznych czynników prognostycznych, np. stan mutacji genów immunoglobulinowych (IgV_H), anomalie cytogenetyczne, ekspresję ZAP-70 i szereg markerów surowiczych, w tym stężenie kinazy tymidynowej (TK), β2-microglobuliny (β2M) i rozpuszczalnego receptora CD23. Leczenie CLL nie jest zwykle wskazane we wczesnym, bezobjawowym okresie choroby. Chemioterapia lub chemioimmunoterapia jest natomiast stosowana w progresywnej, objawowej CLL. Przez wiele lat lekiem z wyboru był chlorambucyl. Obecnie jest on stosowany głównie u starszych pacjentów z współistniejącymi innymi chorobami. U młodszych chorych stosuje się analogi puryn, fludarabinę lub kladrybinę, w skojarzeniu z cyklofosfamidem i rytuksymabem. U opornych chorych może być celowe leczenie alemtuzumabem lub ofatumumabem.

Słowa kluczowe: CLL, rokowanie, leczenie, chlorambucyl, analogi nukleozydów pyrynowych, przeciwciała monoklonalne, przeszczepianie komórek krwiotwórczych

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 (IgV_H) 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 IgV_H genes was 8 years, compared with approximately 25 years for patients with mutated IgV_H 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 IgV_H 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 IgV_H 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 thymidin 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 $< 30 \times 10^9$/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 chlorambucill 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/m^2 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/m^2 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 month, $p < 0.0001$). 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'-deoxycoformycin) 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 immunoglobulin 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/m^2 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/m^2-375 mg/m^2) 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/m^2 which was then increased to a fixed dose of between 500 and 2250 mg/m^2 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 = 0.0034), due to superior CR rates (24% vs. 13%, p = 0.0007). Grade 3/4 adverse events were higher in the R-FC arm (80%) vs. FC (74%), but serious 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%).

Chemoimmunotherapy 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 olone.

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/m^2 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/m^2 on day 1, 2-CdA at a dose of 0.12 mg/m^2 on days 2 through 4, and intravenous CY at a dose of 250 mg/m^2 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 ranged 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 font-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

40 mg/m^2 every 28 days for a maximum of 12 months. Progression free survival was superior for alemtuzumab with a 42% reduction in risk of progression or death ($p = 0.0001$) and median time to alternative treatment of 23.3 months compared with 14.7 months for chlorambucil ($p = 0.0001$). The overall response rate was 83% with alemtuzumab, including 24% CR, and 55% with chlorambucil including 2% CR ($p < 0.0001$). Moreover, elimination of minimal residual disease occurred in 31% (11 of 36 of complete responders to alemtuzumab and none to chlorambucil). Adverse events were similar in both arms, with the exception of infusion-related and CMV events for alemtuzumab. Recent analysis indicates that CMV reactivation occurred in 15-25% of alemtuzumab treated refractory or relapsed CLL. At present, aggressive anti-infective prophylaxis is a mandatory procedure.

OFATUMUMAB

Ofatumumab (HuMax-CD20; Arzerra™, Glaxo-SmithKline plc/Genmab A/S) is a second-generation, fully human, anti-CD20, IgG1 mAb (30). Ofatumumab recognizes a different CD20 epitope to rituximab, and has demonstrated a higher cytotoxic potential than rituximab. The close binding proximity of ofatumumab to the cell membrane likely results in highly efficient complement deposition on B-cell membranes, without high levels of systemic release of activated complement components. the Hx-CD20-406 phase III study was undertaken to evaluate the efficacy and safety of ofatumumab in patients with fludarabine- and alemtuzumab-refractory (FA-ref) CLL or patients with fludarabine-refractory CLL with bulky lymphadenopathy (BF-ref) who were less suitable for alemtuzumab treatment (31). In this study, patients received eight once-weekly infusions of ofatumumab followed by four once-monthly infusions during a 24-week period (dose 1, 300 mg; doses 2-12, 2000 mg). This analysis included 138 treated patients with FA-ref ($n = 59$) and BF-ref ($n = 79$) CLL. In this study the ORRs were 58% and 47% in the FA-ref and BF-ref groups, respectively. One CR was observed in the BF-ref group, and all other responses were partial responses (PRs). The ORRs among patients refractory to fludarabine combined with cyclophosphamide and rituximab (R-FC) were 50% and 44% in the FA-ref and BF-ref groups, respectively. Median PFS and overall survival times were 5.7 and 13.7 months in the FA-ref group, respectively, and 5.9 and 15.4 months in the BF-ref group, respectively. Median overall survival time was significantly longer among responding patients compared with nonresponders. There were no unexpected toxicities. Overall, infusion-related reactions were seen in 64% of patients in the FA-ref group and 61% of patients in the BF-ref group. Infections were observed in 67% of the patients. These results demonstrate promising efficacy of ofatumumab monotherapy in heavily pretreated patients with fludarabine-refractory CLL. In October 2009, the US FDA granted accelerated approval to ofatumumab for the treatment of patients with FA-ref CLL. The approval was based on a clinically meaningful and durable ORR observed in the above trial (Hx-CD20-406). In April 2010, the European Medicines Agency granted a conditional marketing authorization for ofatumumab, for the treatment of FA-ref CLL patients.

Data concerning activity and toxicity of ofatumumab combined with fludarabine and cyclophosphamide (O-FC) in previously untreated patients with CLL were recently reported (32). Patients were randomized to receive ofatumumab, either 500 mg or 1000 mg, with fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 (FC), both daily for 3 days of each 4-week course. Patients received ofatumumab 300 mg with FC for the first course, and total treatment was six courses in both arms. In both groups, the first dose of ofatumumab was 300 mg. Data from 61 patients were available for response assessment. The CR rate was 32% for patients treated with a dose 500 mg and 50% for patients treated with a dose 1000 mg: the ORR was 77% and 73%, respectively. The median PFS has not been reached. The most common grade 3-4 AEs were infections observed in 11 patients, including febrile neutropenia in three patients in each group, and neutropenia in 29 patients. The encouraging activity of ofatumumab used as single agent and in combination with chemotherapy in patients with refractory and previously untreated B-cell lymphoid malignancies warrants its further investigation.

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.

The therapeutic use of PNA in older patients has demonstrated higher response rates and longer progression-free survival compared with chlorambucil.

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 neuotropenia) (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 Salles 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-CD23), epratuzumab (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 CEL TRANSPLANTATION

The exact role of hematopoietic stem cel 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

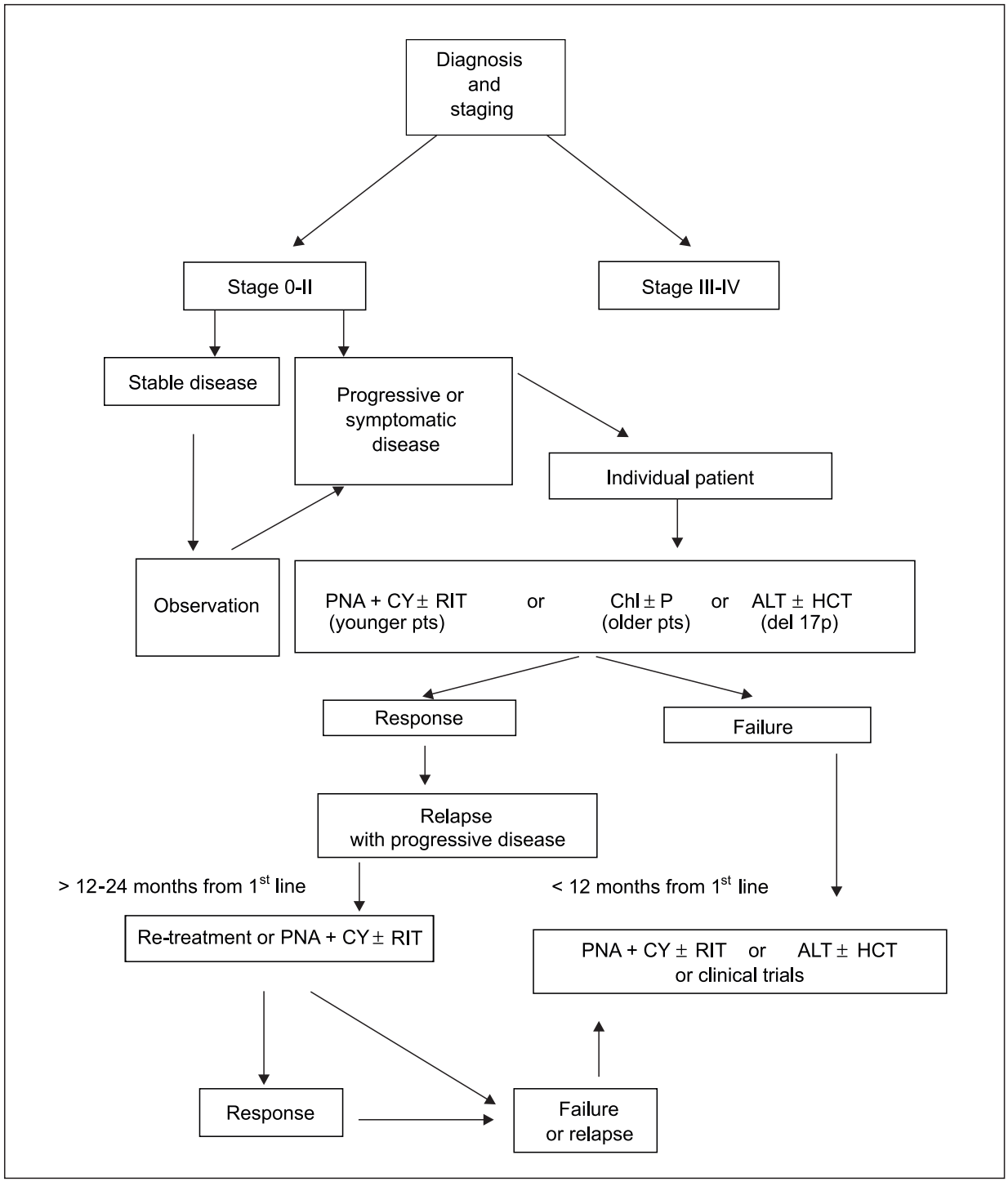

Fig. 1. Proposed treatment algorithm for CLL patients (modified from (10)).
Abbreviations: PNA – purine nucleoside analogs; CY – cyclophosphamide; RIT – rituximab; ALT– alemtuzumab; Chl – chlorambucil; P – prednisone; HCT – hematopoietic cell transplantation

overall survival (OS) rate was 63% in the RIC group as compared with18% 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.

BIBLIOGRAPHY

1. Jemal A, Siegel R, Xu J, Ward E: Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
2. Robak T, Jamroziak K, Robak P: Current and emerging treatments for chronic lymphocytic leukaemia. Drugs 2009; 69: 2415-49.
3. Binet JL, Auquier A, Dighiero G et al.: A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981; 48: 198-206.
4. Rai KR, Sawitsky A, Cronkite EP et al.: Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46: 219-34.
5. Montiilo M, Hamblin T, Hallek M et al.: Chronic lymphocytic leukemia: novel prognostic factors and their relevance for risk-adapted therapeutic strategies. Haematologica 2005; 90: 391-9.
6. Dohner H, Stilgenbauer S, Benner A et al.: Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343: 1910-6.
7. Byrd JC, Gribben JG, Peterson BL et al.: Select high-risk genetic features predict earlier progression after chemoimmunotherapy with fludarabine and rituximab in chronic lymphocytic leukemia:justification for risk-adapted therapy. J Clin Oncol 2006; 24: 437-43.
8. Inamdar KV, Bueso-Ramos CE: Pathology of chronic lymphocytic leukemia: an update. Ann Diagn Pathol 2007; 11: 363-89.
9. Dighiero G, Maloum K, Desablens B et al.: Chlorambucil in indolent chronic lymphocytic leukemia. French Cooperative Group on Chronic Lymphocytic Leukemia. N Engl J Med 1998; 338: 1506-1514.
10. Robak T: Recent progress in the management of chronic lymphocytic leukemia. Cancer Treat Rev 2007; 33: 710-28.
11. Cheson BD, Bennett JM, Grever M et al.: National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 1996; 87; 4990-7.
12. Hallek M, Cheson BD, Catovsky D et al.: International Workshop on Chronic Lymphocytic Leukemia. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008; 111: 5446-56.
13. Robak T, Kasznicki M: Alkylating agents and nucleoside analogues in the treatment of B-cell chronic lymphocytic leukemia. Leukemia 2002; 16: 1015-27.
14. Eichhorst BF, Busch R, Stilgenbauer S et al.: German CLL Study Group (GCLLSG). First-line therapy with fludarabine compared with chlorambucil does not result in a major benefit for elderly patients with advanced chronic lymphocytic leukemia. Blood 2009; 114: 3382-91.
15. Knauf W: Bendamustine in the treatment of chronic lymphocytic leukemia. Expert Rev Anticancer Ther 2009; 9: 165-74.
16. Knauf WU, Lissichkov T, Aldaound A et al.: Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol 2009; 27: 4378-84.
17. Robak T, Jamroziak K, Gora-Tybor J et al.: Comparison of cladribine plus cyclophosphamide with fludarabine plus cyclophosphamide as first-line therapy for chronic lymphocytic oeukemia: A phase III randomized study by the Polish Adult Leukemia Group (PALG-CLL3 Study). J Clin Oncol 2010; 28: 1861-9.
18. Robak T: Monoclonal antibodies in the treatment of chronic lymphoid leukemias. Leuk Lymphoma 2004; 45: 205-19.
19. Robak T: Alemtuzumab for B-cell chronic lymphocytic leukemia. Expert Rev. Anticancer Ther 2008; 8: 1033-51.
20. Byrd JC, Murphy T, Howard RS et al.: 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 2001; 19: 2153-64.
21. O'Brien SM, Kantarjian H, Thomas DA et al.: Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 2001; 19: 2165-70.
22. Keating MJ, O'Brien S, Albitar M et al.: Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol 2005; 23: 4079-88.
23. Hallek M, Fischer K, Fingerle-Rowson G et al.: Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet 2010; 376: 1164-74.
24. Robak T, Dmoszynska A, Solal-Céligny P et al.: Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol 2010; 28: 1756-65.
25. Robak T, Lech-Maranda E, Robak P: Rituximab plus fludarabine and cyclophosphamide or other agents in chronic lymphocytic leukemia. Expert Rev Anticancer Ther 2010; 10: 1529-43.
26. Robak T, Smolewski P, Cebula B et al.: Rituximab plus cladribine with or without cyclophosphamide in patients with relapsed or refractory chronic lymphocytic leukemia. Eur J Haematol 2007; 79: 107-13.
27. Keating MJ, Flinn I, Jain V et al.: Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002; 99: 3554-61.
28. Osterborg A, Fassas AS, Anagnostopoulos A et al.: Humanized CD52 monoclonal antibody Campath-1H as first line

treatment in chronic lymphocytic leukemia. Br J Haematol 1996; 93: 151-3.
29. Hillmen P, Skotnicki A, Robak T et al.: Alemtuzumab compared with chlorambucil as first line therapy for patients requiring treatment for chronic lymphocytic leukemia. J Clin Oncol 2007; 25: 5616-23.
30. Robak T: Ofatumumab, a human monoclonal antibody for lymphoid malignancies and autoimmune disorders. Curr Opin Mol Ther 2008; 10: 294-309.
31. Wierda WG, Kipps TJ, Mayer J et al.: Ofatumumab as single-agent CD20 immunotherapy in fludarabine-refractory chronic lymphocytic leukemia. J Clin Oncol 2010; 28: 1749-55.
32. Wierda WG, Kipps T, Dürig J et al.: Chemoimmunotherapy with ofatumumab, fludarabine and cyclophosphamide (O-FC) in previously untreated patients with chronic lymphocytic leukemia. Blood 2011 (In press).
33. Eichhorst B, Goede V, Hallek M: Treatment of elderly patients with chronic lymphocytic leukemia. Leuk Lymphoma 2009; 50: 171-8.
34. Foon KA, Boyiadzis M, Land SR et al.: Chemoimmunotherapy with low-dose fludarabine and cyclophosphamide and high dose rituximab in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol 2009; 27: 498-503.
35. Robak T: Novel drugs for chronic lymphoid leukemias: mechanism of action and therapeutic activity. Curr Med Chem 2009; 16: 2212-34.
36. Robak T, Robak E: New anti-CD20 monoclonal antibodies for the treatment of B-Cell lymphoid malignancies. BioDrugs 2010; 25: 13-25.
37. Tam CS, Khouri I: The role of stem cell transplantation in the management of chronic lymphocytic leukaemia. Hematol Oncol 2009; 27: 53-60.
38. Sorror ML, Storer BE, Sandmaier BM et al.: Five-year follow-up of patients with advanced chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. J Clin Oncol 2008; 26: 4912-20.
39. Peres E, Braun T, Krijanovski O et al.: Reduced intensity versus full myeloablative stem cell transplant for advanced CLL. Bone Marrow Transplant 2009; 44: 579-83.
40. Dreger P, Corradini P, Kimby E et al.: Indication for allogenic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia 2007; 21: 12-7.

otrzymano/received: 24.03.2011
zaakceptowano/accepted: 11.05.2011

Adres/address:
*Tadeusz Robak
Department of Hematology
Medical University of Łódź
Ciołkowskiego 2 Str., 93-510 Łódź
phone: (42) 689-51-91, fax: (42) 689-51-92
e-mail: robaktad@csk.umed.lodz.pl