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Chronic lymphocytic leukaemia – a new revolution in treatment strategies?

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A B S T R A C T

Chronic lymphocytic leukaemia (CLL) is the most frequently diagnosed type of leukaemia in the Western world. The majority of patients are > 65 years of age at diagnosis and the disease follows an extremely variable clinical course, with overall survival (OS) times ranging from months to decades. Recent advances in molecular biology and immunophenotyping have allowed the stratification of patients into specific risk groups. This could provide the basis for evaluating different treatment options for each group. Dramatic advances in the available treatment options for CLL have occurred alongside improvements in our understanding of the pathobiology of CLL. The introduction of immunotherapy such as rituximab to the treatment armamentarium has transformed the treatment of CLL, improving the outlook for both newly diagnosed and relapsed patients, in terms of extending progression-free survival and probably OS. This review aims to outline the advances that we have made in both the diagnosis and treatment of CLL, and to consider the benefits of these advances from the patient's perspective.

Introduction to chronic lymphocytic leukemia

Chronic lymphocytic leukaemia (CLL) occurs largely in the elderly. The overall median survival of patients with CLL is about 10 years, but with a wide range in duration. This disease has an extremely variable clinical course. While in some patients CLL runs an indolent clinical course and does not significantly impact upon life expectancy, in others the leukaemia exhibits aggressive behaviour and survival following diagnosis may be less than 2–3 years.[1] Although traditional treatment approaches lead to remission in some cases, almost all patients relapse and there is a clear need for better-informed treatment decisions and improved treatment strategies.

In recent years, important discoveries have been made which help to explain the heterogeneous nature of CLL. The discovery of molecular markers enables us to more accurately predict disease course. In addition to improvements in our

knowledge of the biology of CLL, the treatment of CLL has also improved dramatically. The development of novel agents, including rituximab, has increased the range of therapeutic options within the treatment armamentarium and has resulted in marked improvements in patient outcomes for both newly diagnosed and relapsed patients. Studies currently underway reflect our attempts to determine how best to combine these agents in order to further improve outcomes for our patients.

Biology and prognostic markers: what do we know about chronic lymphocytic leukaemia and what is relevant in the clinic today and tomorrow?

Considering the variable prognosis and absence of a curative therapy, the management of patients with CLL cannot be effectively planned without considering the patient's prognosis. Clinical staging techniques, such as the Rai and Binet systems, remain the 'gold standard' for assessing prognosis in patients with CLL.[2,3] Patients with low-risk CLL (Rai stage 0; Binet stage A) have a median survival approaching 15 years, those with intermediate-risk disease (Rai stage I or II; Binet stage B) have a median survival of 5–7 years and most patients with high-risk disease (Rai stage III or IV; Binet stage C) have a life expectancy of less than 3–4 years (Figure 1).[1] Clinical staging requires simply a physical examination and a blood count, and these techniques have been corroborated by a number of studies. They do, however, have some limitations; for example, some patients with CLL may demonstrate an indolent disease course that is not effectively identified by clinical staging alone. Conversely, other patients with CLL may demonstrate an aggressive disease course, similar to that observed in diffuse large B-cell lymphomas.[1]

The ability to accurately predict disease course would be a major advantage, particularly when considering treatment options for the ~80% of patients classified as having early-stage (Rai 0 or Binet A) disease at diagnosis. However, the application of disease staging and other clinical prognostic factors only serves to highlight the biological diversity of CLL, as the prognosis of any individual patient ultimately depends upon the complex relationship between the characteristics of the patient (age, gender, co-morbidity, performance status) and the disease (burden, kinetics and biology of the tumour), together with the sensitivity of the disease to treatment. Recent research has provided us with an insight into a number of biological prognostic markers that may enable us to more accurately predict disease course and thus make more efficient therapeutic choices.

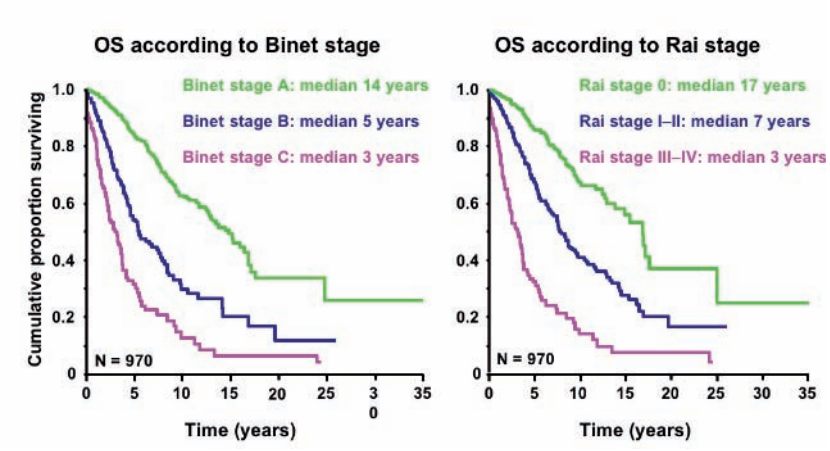

Figure 1. Overall survival according to disease stage in CLL patients. (*This research was originally published in Hematology. Montserrat E. Hematology 2006;279-284. © the American Society of Hematology).*

A number of serological parameters, such as β_2-microglobulin (β_2M), thymidine kinase (TK) and soluble CD23, have been shown to predict disease prognosis in CLL independently of disease staging.[4–6] Higher than normal levels of β_2M at presentation are associated with adverse prognostic features, and higher serum levels have been observed in CLL patients with a shorter survival.[4] Similarly, serum TK and soluble CD23 levels correlate with tumour mass and the proliferative activity of CLL cells, thus predicting disease progression even in early-stage disease.[5,6]

Recent research has also provided an insight into molecular predictors of CLL progression and survival. Certain chromosomal aberrations may be strongly predictive of survival, with deletions in 17p and 11q being particularly indicative of poor prognosis, while patients with a deletion in 13q as a single anomaly have an excellent prognosis.[7] Despite these advances, the techniques employed in assessing chromosomal aberrations require standardisation and additional confirmatory studies before they can be of any practical use in the clinic.

The most important molecular predictor identified to date is the demonstration that approximately 50% of patients present with somatic hypermutations in the rearranged variable regions of the immunoglobulin heavy chains (IgV_H). Studies have revealed a more aggressive form of CLL with rapid disease progression and shorter survival in patients with unmutated V_H genes compared with those with mutated V_H genes.[8,9] The prognostic significance of V_H mutational status is independent of clinical stage and cytogenetic abnormalities and has been verified by several groups. However, determining V_H mutational status requires DNA sequencing, making it both time consuming and expensive, and thus impossible to employ on a routine basis. This has resulted in many attempts to identify an easily measurable surrogate marker for V_H mutational status that could be used to assess prognosis in CLL patients.

CD38 expression was the first surrogate marker which was found to correlate with V_H mutational status.[8] However, the relationship between CD38 and V_H is not absolute, and there have been suggestions that CD38 expression can vary over time.[9,10] Rosenwald and colleagues examined the genetic signature of CLL and identified a small number of genes that allow the separation of mutated and unmutated CLL; the most specific of these is a gene that encodes for a 70 kD zeta-associated protein (ZAP-70).[11] Both this study and a study conducted by our own group have revealed a strong correlation between ZAP-70 expression and V_H mutational status.[12]

In conclusion, the recent advances in assessing prognosis for CLL patients mean that it is not difficult to envisage a future where clinical stages are complemented, if not replaced, by the measurement of biological markers. Further large prospective trials are needed before these advances can become a reality for standard practice.[13] However, these new developments have the potential to revolutionise the practical management of CLL.

What is the rationale for using rituximab in chronic lymphocytic leukaemia?

The use of the CD20-specific monoclonal antibody rituximab has significantly improved patient responses in both indolent and aggressive $CD20^+$ B-cell haematological malignancies.[14–17] CD20 is a 33–37 kDa, non-glycosylated, tetraspan transmembrane phosphoprotein molecule that is expressed on the surface of virtually all B cells, both normal and malignant. CD20 is primarily involved in the regulation of transmembrane calcium conductance, cell cycle progression and

B-lymphocyte proliferation.[18] Within the cell membrane, CD20 is resident in specialised microdomains known as lipid rafts, where it is co-located with the B-cell receptor and the glycosyl-phosphatidyl-inositol-linked complement defence proteins, CD55 and CD59 on the exterior of the cell, and Src-family kinases on the interior side – these lipid rafts probably function as signal transduction platforms (Figure 2). CD20 also probably functions as a store-operated calcium channel that replenishes intracellular calcium stores that are depleted following ligation of the B-cell receptor.[18,19]

Rituximab binds to CD20 with nanomolar affinity.[20] The antibody binds to a conformationally dependent and discontinuous epitope comprised of (170)ANPS(173) and (182)YCYSI(186) contained within a 44 amino acid extracellular loop with both segments brought into steric proximity by a disulphide bond between cysteine residues 167 and 183.[21] Cross-linking of CD20 by antibodies such as rituximab induces rapid redistribution in the plasma membrane in lipid rafts where it may modify cell function, induce apoptosis and allow effector recruitment for antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).[22] Although the exact mechanism of action of rituximab remains unknown, ADCC and CDC are thought to be important effector mechanisms.[23] However, the binding of CD20 by rituximab and the redistribution of lipid rafts probably modifies the signal transduction, calcium flux and complement defence capacity that enhances, and acts synergistically, with the immunological effector mechanisms.

Given that levels of CD20 expression are relatively low in CLL when compared with non-Hodgkin's lymphoma and normal B-lymphocytes, the rationale for using rituximab in CLL may appear unclear. Furthermore, selective loss of CD20 antibodies may occur by down-modulation or by removal by FcR-expressing effector cells, a phenomenon referred to as CD20 shaving,[24–26] or blockade by circulating antigen. Preclinical data suggest that rituximab may sensitise CLL cells to chemotherapy and has a synergistic effect with a number of chemotherapeutic agents.[27] Some of these chemosensitisation

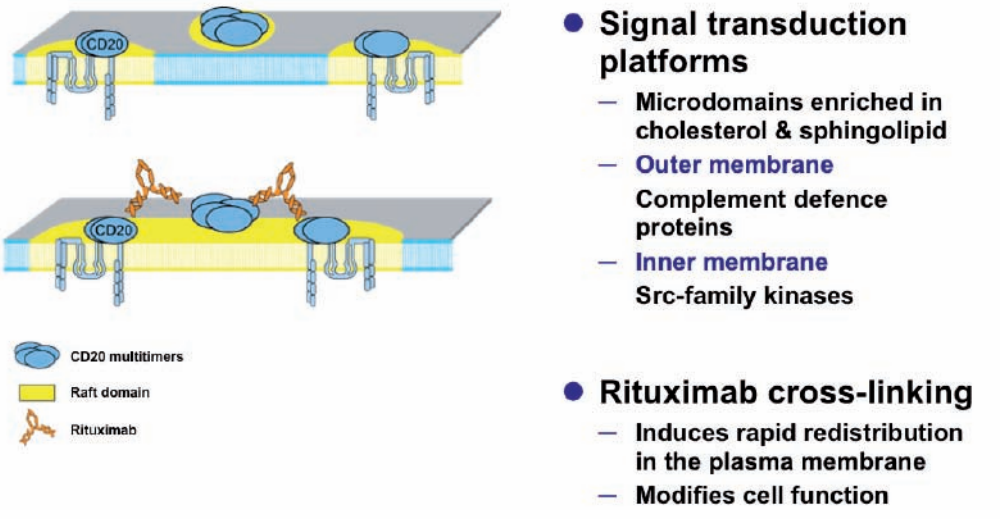

Figure 2. CD20 structure and function. *(Cragg MS, et al. Curr Dir Autoimmun 2005; 8:140–174. Reproduced with permission from S Karger AG, Basel).*

effects occurred even with incomplete saturation of the antigen.[28] Secondly, rituximab has been shown to act synergistically with macrophages to promote ADCC killing of CLL cells.[29] In addition, alternative dosing strategies such as dose-intense single-agent rituximab therapy have demonstrated improved responses, and higher objective responses have been observed with rituximab dose escalation.[30–32] Finally, there is increasing evidence from clinical studies documenting that the addition of rituximab to fludarabine,[33] or to the combination of fludarabine plus cyclophosphamide, such as in the study conducted by Keating and colleagues, produces high complete response (CR) rates in previously untreated CLL patients, and improves the efficacy of rituximab in CLL.[34]

In summary, while the mechanism of action of rituximab is only partially understood, this antibody is clearly effective therapy for CLL, and this is particularly true when rituximab is concurrently administered with other forms of chemotherapy. This results from a range of synergistic actions that include redistribution of CD20 within lipid rafts with altered signal transduction and calcium flux, induction of apoptosis and enhancement of immunological effector mechanisms. Clinical evidence is also mounting that documents the synergistic effects in the clinical setting with the use of the drug in combination chemotherapy to enhance and optimise its activity, thus improving patient outcomes.

First-line treatment of chronic lymphocytic leukaemia: what new developments are to come?

The first-line management of CLL has been revolutionised by the development of successful chemotherapy combinations and, more importantly, by the addition of monoclonal antibodies to these regimens. The development of fludarabine, deoxycoformycin and 2-chlorodeoxyadenosine (2-CDA) demonstrated the potency of the purine analogues as single agents. The ability of these agents to inhibit DNA repair has led to the rational development of combinations of purine analogues and alkylating agents. In patients with CLL, overall response rates (ORRs) to first-line chemotherapy range from 70–90%, with CRs achieved in < 10% of patients treated with chlorambucil and ~35% of patients treated with fludarabine-based regimens. A series of randomised clinical trials conducted in Europe and the United States have demonstrated the superior activity of combinations of fludarabine and cyclophosphamide (FC) compared with fludarabine alone;[35,36] similar results have been seen with 2-CDA combinations,[37] a difference which is significant in all studies. However, it should be noted that no survival benefits were observed with 2-CDA combinations.

Rituximab has shown clinical activity in CLL, both as monotherapy and in combination with chemotherapy. Significant clinical benefits have been observed in patients receiving monotherapy with increased doses of rituximab.[32] Concurrent administration of chemotherapy and rituximab has been demonstrated to be superior to single-agent fludarabine followed in both groups by rituximab in a Cancer and Leukemia Group B (CALGB) study.[38] Rituximab has also demonstrated efficacy when combined with the FC regimen (FCR) and FC plus mitoxantrone (R-FCM), resulting in high complete and partial remission rates, and long progression-free survival (PFS) and overall survival (OS).[39,40]

The MD Anderson Cancer Center (MDACC) study of FCR utilised a protocol whereby 375 mg/m^2 rituximab were administered

Response	Patients, n	(%)	
CR	217	(72)	95%
Nodular PR	31	(10)	
PR	37	(12)	
No response	13	(4)	
Early death	2	(1)	

Figure 3. Response to FCR according to National Cancer Institute Working Group (NCIWG) criteria. *(Keating MJ, et al. Blood 2005; 106:Abstract 2118).*

during the first treatment cycle, increasing to 500 mg/m^2 for cycles 2–6.[34] This study demonstrated a 72% complete remission rate using National Cancer Institute Working Group (NCIWG) criteria in 300 patients with CLL (Figure 3).[39] In addition, there was a strong correlation between response to therapy and OS in patients treated with FCR, with 99% of patients who achieved a CR still alive at 48 months. The median survival has not been reached and is projected to be greater than 7 years. Historical comparison of these FCR results with previous chemotherapy-alone clinical trials demonstrates a survival advantage in multivariate analysis. Many patients with this regimen are minimal residual disease-negative. The key prognostic factors have been age and the pre-treatment β_2M level. Rai stage or Binet stage no longer have an impact on survival. A large, multicentre, multinational, randomised controlled trial conducted by the German CLL Study Group comparing FCR with FC, has recently completed enrolment and will hopefully yield early results in 2008.

While FCR has significantly improved outcomes for younger patients with CLL, the survival of patients over the age of 70 years has not been impacted by this combination. Thus, patients over the age of 70 years have been assigned to alternative treatment strategies, such as rituximab plus granulocyte macrophage colony-stimulating factor. Treatment strategies for younger patients are often defined by measuring their β_2M level. Moreover, the addition of mitoxantrone in higher-dose rituximab has also been explored in younger patients, but preliminary analysis indicates that there does not appear to be an advantage to this approach. More recently, alemtuzumab, which has been shown to be highly effective at clearing disease from bone marrow, has been added to FCR (CFAR) and is being explored in patients < 70 years with twice the upper limit of normal β_2M level.[41] Looking to the future, FCR will now be combined with additional monoclonal antibodies, e.g., the CD23 antibody lumiliximab, in a variety of clinical trials. In addition, a soon to be started Phase II clinical study in the UK will examine the potential benefits of combining rituximab with chlorambucil.

The development of immunochemotherapy strategies for patients with CLL has dramatically impacted upon patient outcomes, extending both PFS and OS. New concepts of targeting minimal residual disease, together with consolidation and maintenance strategies, are now evolving. The challenge remains to accurately determine the impact of these novel approaches on OS, to integrate these results with information derived from molecular prognostic markers and to further identify novel targets relevant to the treatment of CLL. This approach will allow the application of novel therapies and paradigms in a disease- and risk-specific manner, and ultimately result in a consolidated approach to the management of the entire course of the disease.

Relapsed chronic lymphocytic leukaemia and novel directions for rituximab

Despite major treatment advances, CLL remains an incurable disease, with most patients relapsing and eventually becoming refractory to therapy. At present, the molecular mechanisms

that underlie the development of treatment resistance are poorly understood, but p53 mutations and deletions become more frequent as the disease progresses, rising from 7% at diagnosis to almost 50% at the development of fludarabine-refractory disease.[7,42,43] Most worryingly, once patients become refractory to alkylating agents and nucleoside analogues, median survival is less than 1 year.[44]

The management of relapsed CLL is dependent upon a number of factors including previous therapy, type and duration of response to previous therapy, time from last therapy and the age and performance status of the patient. A number of alternative treatment strategies have been explored in an attempt to improve outcomes for relapsed patients. One of these strategies is the use of rituximab-containing immunochemotherapy. Although rituximab as single agent has only modest activity when used at standard doses in patients with previously treated CLL, ORRs are increased to 40–45% when it is administered using higher, or more frequent, dosing schedules.[32,45] For example, in a rituximab dose escalation study conducted by O'Brien and colleagues, an ORR of 40% was observed in patients with CLL. Interestingly, this response was shown to be dose dependent (OR: 22%, 43% and 75% [p = 0.03] for low-dose, intermediate-dose, and high-dose, respectively).[32] In addition, the combination of rituximab and various chemotherapy regimens has been explored. For example, in previously treated CLL patients, FCR resulted in an ORR of 73%, with 25% of patients achieving a CR, and molecular remissions were observed in one-third of patients who achieved a CR.[46] Furthermore, the significant OS benefit of FCR over FC or fludarabine ± prednisolone has been shown in a retrospective analysis (Figure 4).[47] The REACH trial, an open-label, multicentre, randomised, comparative Phase III study to evaluate the efficacy and safety of FCR versus FC alone in previously treated CLL patients is currently ongoing. This worldwide study aims to recruit 550 patients. Rituximab plus pentostatin and cyclophosphamide also conferred a survival advantage upon previously treated CLL patients without any additional toxicity due to the addition of rituximab to the regimen.[48]

In addition to the use of rituximab-containing salvage regimens in relapsed CLL, the feasibility of rituximab maintenance therapy has also been investigated. Several studies have already confirmed the benefits of rituximab maintenance therapy in follicular

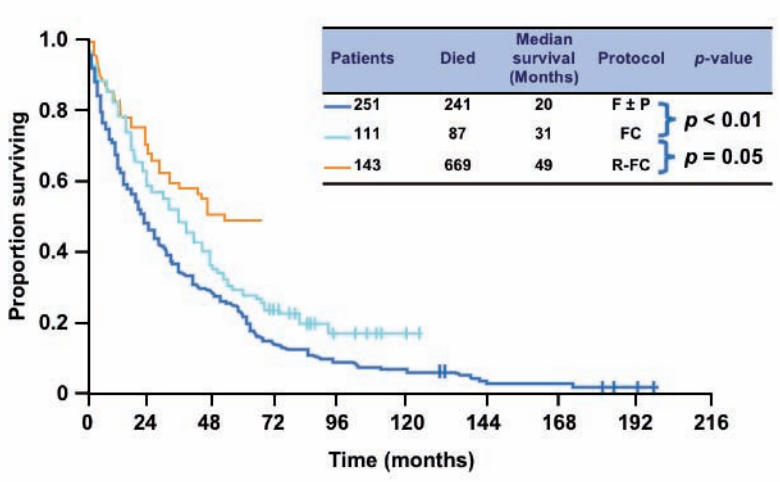

Figure 4: Median survival following treatment of relapsed CLL patients with FCR. *(Wierda W, et al. Cancer 2006; 106:337–345. Figure 1A of this article reproduced with the permission of John Wiley & Sons, Ltd.)*

lymphoma (FL).[49,50] The situation is less clear in the CLL setting. There are, however, some indications from a Phase II trial conducted by the Minnie Pearl Cancer Research Network in patients with CLL or small lymphocytic lymphoma that re-treatment of patients in remission with rituximab at 6-month intervals was well tolerated with a PFS of 18.6 months.[51] Another study conducted by Del Poeta and colleagues has also observed a significantly prolonged duration of response and improved outcomes in previously untreated CLL patients who received rituximab consolidation/maintenance therapy while in first remission, with a 67% PFS at 5 years.[52] Moreover, the benefits of rituximab maintenance therapy after rituximab combined with R-FCM are currently being investigated by an ongoing Spanish trial.

Some patients have poor prognostic features, which are unlikely to be overcome by standard therapy, such as the p53 dysfunction associated with either TP53 mutation/deletion or the inactivation of the p53-regulation machinery (mainly by the *ATM* gene). Alemtuzumab appears to be active in these patients;[42] however, allogeneic stem cell transplantation (alloSCT) should also be considered as a treatment strategy for eligible patients. A recent review published by a consensus panel aimed to identify situations where alloSCT might be considered as a preferred treatment option for patients with CLL, and concluded that this strategy is a reasonable approach for high-risk patients with adverse clinical course or poor biological features.[53]

The combining of different monoclonal antibodies has also been explored with the combination of rituximab and alemtuzumab producing promising outcomes (30% CR and 55% ORR) in patients with relapsed/refractory CLL.[54] Moreover, the addition of chemotherapy (fludarabine plus cyclophosphamide) to both antibodies has shown promising results in heavily pre-treated patients, with response rates of 57% in patients previously treated with FCR, 51% in fludarabine-refractory patients and 64% in patients with unfavourable cytogenetics.[41] Prospective randomised trials are currently underway to test the efficacy of newer monoclonal antibodies, such as the anti-CD23 antibody (lumiliximab), in combination with rituximab. In addition, a fully human anti-CD20 antibody (ofatumumab) is currently undergoing Phase II studies in CLL patients who are resistant to fludarabine and alemtuzumab. Similarly, agents such as flavopiridol,[55] Bcl-2 antisense (oblimersen)[56] and lenalinomide[57] have all been assessed in Phase II/III studies.

In conclusion, the future treatment of relapsed/refractory CLL patients will be based on immunochemotherapy. For selected patients, alloSCT may be the preferred treatment option. High-quality supportive and palliative care also needs to underlie the treatment of all patients with relapsed or resistant CLL.

Patient factors to consider in chronic lymphocytic leukaemia

With numerous chemotherapy regimens available and an increasing understanding of molecular prognostic markers in CLL, choosing when and how to treat individual patients has become a task that requires increasing skill and expertise. As previously discussed, CLL has an extremely variable clinical course and while immediate treatment will be required by those patients with high-risk, aggressive forms of the disease, treatment may be delayed in those patients exhibiting an indolent disease course. In addition, four key points should be considered when selecting the optimal treatment for an individual patient: the physical condition of the patient (independent of age), the prognostic risk factors/aggressiveness of the

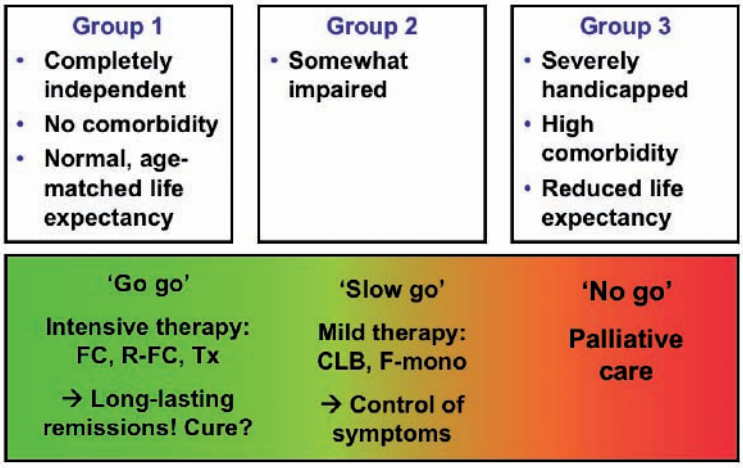

Figure 5. Classification and treatment of CLL patients using comprehensive geriatric assessment. *(Balducci L & Extermann M, Oncologist 2000; 5:224–2387).*

disease, the stage of the disease and patient preference.[58] For example, the Comprehensive Geriatric Assessment (CGA)[59] classifies patients on the grounds of patient fitness and presence of comorbidities, thus allowing us to make informed treatment decisions for individual CLL patients, ranging from intensive therapy for those who are capable of completely independent living, to palliative care for those who are severely handicapped and/or have a high level of comorbidities (Figure 5).

The assessment of health-related quality of life (HRQOL) has become one of the major endpoints, besides OS, within clinical trials in haematology and oncology. Despite CLL being the most common form of leukaemia in the Western world, HRQOL has rarely been evaluated within any of the large clinical trials that have so far been conducted, hence the impact of chemotherapy on HRQOL in CLL patients remains unclear.[60] A study conducted in patients younger than 65 years by the German CLL Study Group suggests that fludarabine-based treatment moderately improves HRQOL.[61] Given the relative lack of information currently available, the assessment of HRQOL should be a priority endpoint for future clinical trials.

Although the standard of care for first-line treatment of CLL can vary between centres, it usually involves the combination of an alkylating agent and a purine analogue. Phase II studies suggest that rituximab combined with fludarabine-based therapies represents a significant advance in therapy for CLL, and it is possible that, by optimising combination therapy we will be able to induce long-term remissions (10–15 years) and consequently approach the possibility of a curative regimen. Any agent being added to fludarabine and cyclophosphamide needs to have minimal additional toxicity as this regimen can be difficult for some patients to tolerate. Given the ability of rituximab to sensitise CLL cells to the actions of cytotoxic agents[27] and its low toxicity,[39,46] there has been a growing trend towards including rituximab in clinical practice.

Several studies have confirmed that FCR is well tolerated at doses of 500 mg/m^2, following an initial dosage of 375 mg/m^2 during the first cycle of chemotherapy. However, it remains to be determined whether the FCR regimen should be administered to elderly or frail patients, because it may induce prolonged neutropenia and infections. The tolerability profile of rituximab suggests that rituximab in combination with chlorambucil

may become an alternative approach for those patients who are unable to tolerate more intense combination chemotherapy. This hypothesis awaits confirmation by further prospective trials.

The side effects of repeated induction can be difficult to live with, which would make rituximab maintenance therapy an attractive possibility if we are able to prolong PFS or OS. Prolonged exposure to rituximab in FL is not associated with any cumulative toxicity or additional side effects compared with observation.[49] However, given the relatively modest activity of rituximab as a single agent in CLL,[31] this strategy needs to be confirmed by the ongoing trials in this disease.

Conclusions

The treatment of CLL has come a long way over the last few years. Novel molecular prognostic markers allow us to more accurately predict the likely disease course in any individual patient. In addition, while CD20 expression by CLL cells may be low, the synergism observed between rituximab and chemotherapeutic agents has significantly improved patient outcomes. By exploring different combinations of chemotherapeutic agents and monoclonal antibodies we are beginning to see further improvements in PFS and OS for newly diagnosed patients. Further trials will enable us to continue exploring novel combinations and thus determine the optimal therapy for each patient, based on their own individual prognosis. Relapse is, unfortunately, inevitable for too many patients; we hope that the exploration of novel treatment combinations in this setting will ultimately improve outcomes for this patient group too. Finally, we have examined the patient factors that must be considered when designing these treatment regimens. HRQOL is also an important consideration, which requires full evaluation in future clinical trials. However, therapies such as rituximab that do not add further toxicity to the treatment regimen are essential for improving outcomes without increasing adverse events.

To conclude, we have seen a revolution in both our understanding of, and treatments for CLL over the last few years. Genuine improvements in patient outcomes have been observed, and the continued drive to improve our knowledge should, in the future, lead to further advances and improvements for our patients.

References

1. Montserrat E. New Prognostic Markers in CLL. Hematology Am Soc Hematol Educ Program 2006; 279–284.
2. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46:219–234.
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. Keating MJ, Lerner S, Kantarjian H, et al. The serum ,2 microglobulin (,2M) level is more powerful than stage in predicting response and survival in chronic lymphocytic leukemia (CLL). Blood 1998; 86:Abstract 2412.
5. Hallek M, Langenmayer I, Nerl C, et al. Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonsmoldering chronic lymphocytic leukemia. Blood 1999; 93:1732–1737.
6. Sarfati M, Chevret S, Chastang C, et al. Prognostic importance of serum soluble CD23 level in chronic lymphocytic leukemia. Blood 1996; 88:4259–4264.
7. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343:1910–1916.
8. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94: 1840–1847.
9. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999; 94: 1848–1854.
10. Montillo M, Hamblin T, Hallek M, et al. Chronic lymphocytic leukemia: novel prognostic factors and their relevance for risk-adapted therapeutic strategies. Haematologica 2005; 90:391–399.

11. Rosenwald A, Alizadeh AA, Widhopf G, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001; 194:1639–1647.
12. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med 2003; 348:1764–1775.
13. Binet JL, Caligaris-Cappio F, Catovsky D, et al. Perspectives on the use of new diagnostic tools in the treatment of chronic lymphocytic leukemia. Blood 2006; 107:859–861.
14. Marcus RE, Solal-Celigny P, Imrie K, et al. MabThera (rituximab) plus cyclophosphamide, vincristine and prednisone (CVP) chemotherapy improves survival in previously untreated patients with advanced follicular non-Hodgkin's lymphoma (NHL). Blood 2006; 108: Abstract 481.
15. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 2005; 106: 3725–3732.
16. Herold M, Haas A, Srock S, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interfon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology study. J Clin Oncol 2007; Apr 9, Epub ahead of print.
17. Feugier P, Van Hoof A, Sebban C, et al. Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 2005; 23:4117–4126.
18. Cragg MS, Walshe CA, Ivanov AO, et al. The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun 2005; 8:140–174.
19. Janas E, Priest R & Malhotra R. Functional role of lipid rafts in CD20 activity? Biochem Soc Symp 2005; 165–175.
20. Teeling JL, Mackus WJ, Wiegman LJ, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol 2006; 177:362–371.
21. Binder M, Otto F, Mertelsmann R, et al. The epitope recognized by rituximab. Blood 2006; 108:1975–1978.
22. Unruh TL, Li H, Mutch CM, et al. Cholesterol depletion inhibits src family kinase-dependent calcium mobilization and apoptosis induced by rituximab crosslinking. Immunology 2005; 116:223–232.
23. van Meerten T, van Rijn RS, Hol S, et al. Complement-induced cell death by rituximab depends on CD20 expression level and acts complementary to antibody-dependent cellular cytotoxicity. Clin Cancer Res 2006; 12:4027–4035.
24. Beum PV, Kennedy AD, Williams ME, et al. The shaving reaction: rituximab/CD20 complexes are removed from mantle cell lymphoma and chronic lymphocytic leukemia cells by THP-1 monocytes. J Immunol 2006; 176:2600–2609.
25. Kennedy AD, Beum PV, Solga MD, et al. Rituximab infusion promotes rapid complement depletion and acute CD20 loss in chronic lymphocytic leukemia. J Immunol 2004; 172:3280–3288.
26. Williams ME, Densmore JJ, Pawluczkowycz AW, et al. Thrice-weekly low-dose rituximab decreases CD20 loss via shaving and promotes enhanced targeting in chronic lymphocytic leukemia. J Immunol 2006; 177: 7435–7443.
27. Chow KU, Sommerlad WD, Boehrer S, et al. Anti-CD20 antibody (IDEC-C2B8, rituximab) enhances efficacy of cytotoxic drugs on neoplastic lymphocytes in vitro: role of cytokines, complement, and caspases. Haematologica 2002; 87:33–43.
28. Di Gaetano N, Xiao Y, Erba E, et al. Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line resistant to the cytotoxic activity of either drug alone. Br J Haematol 2001; 114:800–809.
29. Lefebvre ML, Krause SW, Salcedo M, et al. Ex vivo-activated human macrophages kill chronic lymphocytic leukemia cells in the presence of rituximab: mechanism of antibody-dependent cellular cytotoxicity and impact of human serum. J Immunother 2006; 29:388–397.
30. McLaughlin P, Grillo-Lopez AJ, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825–2833.
31. Huhn D, von Schilling C, Wilhelm M, et al. Rituximab therapy of patients with B-cell chronic lymphocytic leukemia. Blood 2001; 98:1326–1331.
32. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 2001; 19:2165–2170.
33. Byrd JC, Rai K, Peterson BL, et al. Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. Blood 2005; 105:49–53.
34. 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: 4079–4088.
35. Eichhorst BF, Busch R, Hopfinger G, et al. Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood 2006; 107:885–891.
36. Flinn IW, Neuberg DS, Grever MR, et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J Clin Oncol 2007; 25:793–798.
37. Robak T, Blonski JZ, Gora-Tybor J, et al. Cladribine alone and in combination with cyclophosphamide or cyclophosphamide plus mitoxantrone in the treatment of

progressive chronic lymphocytic leukemia: report of a prospective, multicenter, randomized trial of the Polish Adult Leukemia Group (PALG CLL2). Blood 2006; 108:473–479.
38. Byrd JC, Peterson BL, Morrison VA, et al. Randomized phase 2 study of fludarabine with concurrent versus sequential treatment with rituximab in symptomatic, untreated patients with B-cell chronic lymphocytic leukemia: results from Cancer and Leukemia Group B 9712 (CALGB 9712). Blood 2003; 101:6–14.
39. Keating MJ, O'Brien S, Albitar M, et al. Extended follow-up of a chemo-immunotherapy regimen FCR (fludarabine, F; cyclophosphamide, C; and rituximab, R) as initial therapy for chronic lymphocytic leukemia (CLL). Blood 2005; 106:Abstract 2118.
40. Faderl S, Wierda WG, O'Brien S, et al. Fludarabine, cyclophosphamide, mitoxantrone plus rituximab (FCM-R) as frontline therapy for CLL: results of a phase 2 study. Blood 2006; 108:Abstract 2836.
41. Wierda WG, O'Brien S, Faderl S, et al. Combined cyclophosphamide, fludarabine, alemtuzumab, and rituximab (CFAR), an active regimen for heavily treated patients with CLL. Blood 2006; 108:Abstract 31.
42. Lozanski G, Heerema NA, Flinn IW, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 2004; 103: 3278–3281.
43. Thornton PD, Gruszka-Westwood AM, Hamoudi RA, et al. Characterisation of TP53 abnormalities in chronic lymphocytic leukaemia. Hematol J 2004; 5:47–54.
44. Keating MJ, O'Brien S, Kontoyiannis D, et al. Results of first salvage therapy for patients refractory to a fludarabine regimen in chronic lymphocytic leukemia. Leuk Lymphoma 2002; 43:1755–1762.
45. 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; 19:2153–2164.
46. Wierda W, O'Brien S, Wen S, et al. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol 2005; 23:4070–4078.
47. Wierda W, O'Brien S, Faderl S, et al. A retrospective comparison of three sequential groups of patients with recurrent/refractory chronic lymphocytic leukemia treated with fludarabine-based regimens. Cancer 2006; 106:337–345.
48. Lamanna N, Kalaycio M, Maslak P, et al. Pentostatin, cyclophosphamide, and rituximab is an active, well-tolerated regimen for patients with previously treated chronic lymphocytic leukemia. J Clin Oncol 2006; 24: 1575–1581.
49. Ghielmini M, Schmitz SF, Cogliatti SB, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood 2004; 103:4416–4423.
50. van Oers MH, Klasa R, Marcus RE, et al. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin's lymphoma, both in patients with and without rituximab during induction: results of a prospective randomized phase III intergroup trial. Blood 2006; 108:3295–3301.
51. Hainsworth JD, Litchy S, Barton JH, et al. Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase II trial of the Minnie Pearl Cancer Research Network. J Clin Oncol 2003; 21:1746–1751.
52. del Poeta G, del Principe MI, Buccisano F, et al. Consolidation and maintenance immunotherapy with rituximab improves progression-free survival within ZAP-70 positive chronic lymphocytic leukemia (CLL). Blood 2006; 108:Abstract 2824.
53. Dreger P, Corradini P, Kimby E, et al. Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia 2007; 21:12–17.
54. Faderl S, Ferrajoli A, Wierda W, et al. Continuous infusion/subcutaneous alemtuzumab (Campath-1H) plus rituximab is active for patients with relapsed/refractory chronic lymphocytic leukemia (CLL). Blood 2005; 106: Abstract 2963.
55. Byrd JC, Lin TS, Dalton JT, et al. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. Blood 2007; 109:399–404.
56. O'Brien S, Moore JO, Boyd TE, et al. Randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen sodium (Bcl-2 antisense) in patients with relapsed or refractory chronic lymphocytic leukemia. J Clin Oncol 2007; 25:1114–1120.
57. Chanan-Khan AA, Miller KC, Musial L, et al. Clinical efficacy of lenalidomide (revlimid) in patients with relapsed or refractory chronic lymphocytic leukemia (CLL): updated results of a phase II clinical trial. Blood 2006; 108:Abstract 306.
58. Hallek M. Chronic lymphocytic leukemia (CLL): First-line treatment. Hematology Am Soc Hematol Educ Program 2005; 285–291.
59. Balducci L, Extermann M. Management of cancer in the older person: a practical approach. Oncologist 2000; 5: 224–237.
60. Holzner B, Kemmler G, Kopp M, et al. Quality of life of patients with chronic lymphocytic leukemia: results of a longitudinal investigation over 1 yr. Eur J Haematol 2004; 72:381–389.
61. Eichhorst BF, Busch R, Obwandner T, et al. Health-related quality of life in younger patients with chronic lymphocytic leukemia treated with fludarabine plus cyclophosphamide or fludarabine alone for first-line therapy: a study by the German CLL Study Group. J Clin Oncol March 2007; Epub ahead of print.