Treatment options for high-risk chronic lymphocytic leukaemia

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Ther Adv Hematol
(2011) 2(3) 147–159
DOI: 10.1177/2040620711404469

***Abstract*:** Chronic lymphocytic leukaemia (CLL) is the most common form of leukaemia in the Western world. The natural history of CLL is extremely variable with a survival time from initial diagnosis that ranges from 2 to more than 20 years. Understanding the clinical diversity and allowing the subclassification of CLL into various prognostic groups not only assists in predicting future outcome for patients, but also helps to direct treatment decisions. Chlorambucil and fludarabine were the standard therapy for CLL for decades. Randomized studies have reported superior overall response and progression-free survival (PFS) for fludarabine compared with alkylator-based therapy and for the fludarabine–cyclophospamide (FC) combination over fludarabine alone. More recently the addition of rituximab to the FC regimen (R-FC) has shown significant improvement in overall response, PFS and overall survival compared with FC alone. However, there are patients for whom this regimen still provides less satisfactory results. Within the above studies CLL patients who have some of the poorer prognostic markers, such as unmutated *IgVH* genes and/or high beta-2 microglobulin (B2M), and those who fail to achieve a minimal residual disease (MRD) negative remission are likely to have a shorter PFS compared with those without these features. Various strategies have been explored to improve the outcome for such patients. These include the addition of agents to a frontline R-FC regimen, use of consolidation and consideration of maintenance.

The only group that can be clearly identified pretreatment for whom conventional fludarabine-based therapies produce significantly inferior response rates, PFS and overall survival are the patients who harbour a genetic fault; deletion or mutation or a combination of deletion and mutation of tumour protein p53 (*TP53)*. *TP53* inactivation is a less common finding at first treatment but becomes much more common in fludarabine-refractory patients. Alemtuzumab and high-dose corticosteroids have been shown to be effective in this group of CLL patients. Trials combining these two agents have shown improved responses, particularly for those patients with bulky nodal disease for whom alemtuzumab alone may be insufficient. Since the duration of responses remains relatively short, suitable patients should be considered for allogeneic stem cell transplantation according to the European Group for Blood and Marrow Transplantation (EBMT) guidelines. Furthermore, there are a number of other new treatments on the horizon, including humanized antibodies directed against novel targets and small-molecule inhibitors.

***Keywords*:** CLL, high risk, treatment, prognosis

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Introduction

Chronic lymphocytic leukaemia (CLL) is a neoplasm of small monomorphic CD19+ B-lymphocytes in the peripheral blood, bone marrow, lymph nodes and spleen, usually coexpressing CD5 and CD23. It is more common in Europe than in Asia [Oscier *et al.* 2004]. The median age at diagnosis is between 65 and 70 years but 20–30% of patients are less than 55 years old at diagnosis [Oscier *et al.* 2004]. The median survival varies between 5 and 10 years [Brenner *et al.* 2008] and is independent of whether patients present above or below 50–55 years; but younger patients are more likely to die of CLL-related causes [Oscier *et al.* 2004]. Not all patients will require treatment but well established criteria

have been developed by the International Workshop on CLL (IWCLL) to determine those who do. This includes patients with Binet stage C disease as well as patients with stage A or B disease with features of disease progression [Hallek *et al.* 2008]. The choice of therapy is affected by the patient's own wishes, their age, their performance status and the number of comorbidities.

Defining high-risk CLL

High-risk CLL would generally be regarded as the subgroup of patients who require treatment for progressive disease but also show features suggesting that they are expected to have a poorer outcome than average. Both disease and patient factors influence this predicted outcome.

Disease factors

Clinical parameters as well as several laboratory tests are used to predict the natural course of the disease at diagnosis and following treatment [Grever *et al.* 2007]. Clinical stage, age and gender have long been recognized to influence survival. More recently, genomic aberrations (chromosome 17 and 11), immunoglobulin mutation status (*IgVH*), ZAP-70 and CD38 expression have provided further information in relation to the speed of progression following diagnosis [Morilla *et al.* 2008; Rassenti *et al.* 2008; Krober *et al.* 2002; Oscier *et al.* 2002]. Combinations of these various prognostic indices have been used to create nomograms which can more accurately predict clinical outcome in CLL [Wierda *et al.* 2007].

The value of these parameters in predicting outcome following novel chemo-immunotherapy regimens is more controversial. The raised beta-2 microglobulin level (B2M), white blood cell count (WBC) and lactate dehydrogenase (LDH) at the time of initiating treatment with rituximab–fludarabine–cyclophosphamide (R-FC) have been associated with inferior outcomes [Tam *et al.* 2008]. According to a recent report by Lin and colleagues only *IgVH* emerged as a strong determinant of remission duration [Lin *et al.* 2009a] after treatment with R-FC. These results have been confirmed in the randomized study of FC *versus* R-FC which also showed shorter progression free-survival (PFS) for patients with unmutated (UM) *IgVH* even after the addition of rituximab to FC [Hallek *et al.* 2010].

Chromosomal abnormalities may be the single most important prognostic marker in CLL since they identify subgroups of patients who respond very poorly to conventional chemotherapy and have inferior survival [Dohner *et al.* 2000]. Fluorescence *in-situ* hybridization (FISH) can identify genomic aberrations in approximately 80% of CLL cases [Stilgenbauer *et al.* 2002; Dohner *et al.* 2000] and the most frequent aberrations are deletions in 13, 11 or 17 and trisomy 12 [Krober *et al.* 2002; Stilgenbauer *et al.* 2002; Dohner *et al.* 2000]. Deletion 17p, which involves the loss of the tumour suppressor gene *TP53,* is associated with the poorest outcome, followed by del(11q), whilst del(13q) is associated with favourable survival [Dohner *et al.* 2000]. Deletion of *TP53* is frequently associated with mutation of the remaining allele, but a further 5% of the patients will have a *TP53* mutation without loss of the normal allele [Zenz *et al.* 2008; Byrd *et al.* 2006]. *TP53* inactivation also leads to higher expression of the mutant p53 protein which can be detected in tissue sections by immunohistochemistry [Martin *et al.* 2010]. The cut off value for the clinical relevance of *TP53* inactivation is more debatable. Oscier and colleagues have shown the significant level is 10% while Tam and colleagues reported this to be 25% [Oscier *et al.* 2010; Tam *et al.* 2009]. Patients with inactivated *TP53* by mutation or deletion show *in vivo* and *in vitro* resistance to standard chemotherapy regimens using alkylating agents or purine analogues and to rituximab based combination chemo-immunotherapy [Grever *et al.* 2007].

TP53 inactivation is detected in 7–8% of CLL patients needing treatment; however, this figure is higher in Binet stage C patients and rises during the disease course reaching a value of 35–50% in relapsed/refractory patients [Zenz *et al.* 2008; Lozanski *et al.* 2004; Sturm *et al.* 2003; Cordone *et al.* 1998]. Importantly, the presence of *TP53* abnormalities does not always predict early disease progression and there is considerable heterogeneity in clinical course from diagnosis [Best *et al.* 2009; Tam *et al.* 2009].

Fludarabine is included in most of the frontline treatment protocols in CLL, and patients with *TP53* abnormalities have consistently shown poor response rates, PFS and overall survival with these regimens [Hallek *et al.* 2010; Oscier *et al.* 2010; Catovsky *et al.* 2007].

Quality of response to treatment, complete or partial response (CR *versus* PR) has long been shown to influence duration of remission and

survival outcome. Recent methodology allows a much more sensitive classification of remission based on the presence or absence of minimal residual disease (MRD). MRD has now been assessed prospectively by multiparameter flow cytometry or patient-specific polymerase chain reaction (PCR) in a number of clinical trials. Patients who achieve MRD negativity have a longer PFS independent of the treatment received [Hallek *et al.* 2010; Moreton *et al.* 2005].

Patient factors

The majority of CLL patients are over the age of 60 years. The number of comorbidities increases with increasing age. Thus, a significant proportion of patients are either not eligible to receive (e.g. because of poor renal function) the more intensive chemo-immunotherapy regimens or unable to tolerate the same dose schedules. This may compromise the ability to achieve deep long-lasting remissions.

Standard treatment of CLL

Alkylating agents and purine nucleoside analogues have been the cornerstones of CLL treatment for several decades [Catovsky *et al.* 2007; Robak *et al.* 2000; Saven *et al.* 1995; Hansen *et al.* 1988; Montserrat *et al.* 1985]. Chlorambucil is still widely used in the treatment of CLL, particularly in elderly patients. It is well tolerated but induces only low complete response rates [Catovsky *et al.* 2007]. Fludarabine monotherapy gives better overall response and complete response rates when compared with chlorambucil [Catovsky *et al.* 2007; Rai *et al.* 2000; Johnson *et al.* 1996; Keating *et al.* 1991]. However, the impact on survival is more controversial with one study reporting improvement in overall survival for fludarabine compared with chlorambucil after long followup [Rai *et al.* 2009] whilst others have shown no survival benefit [Eichhorst *et al.* 2009; Catovsky *et al.* 2007]. The combination of fludarabine and cyclophosphamide (FC) has been assessed in three large randomized phase III trials [Catovsky *et al.* 2007; Flinn *et al.* 2007; Eichhorst *et al.* 2006], showing superior overall response rates and PFS for FC compared with fludarabine alone, but with no significant difference in overall survival. As reported by Oscier and colleagues three risk groups could be identified based on chromosomal abnormalities, *IgVH* and B2M [Oscier *et al.* 2010]. The worst prognostic group was defined by the presence of 10% *TP53* deletion, confirming that the combination of FC is an unsuitable treatment for this subgroup of patients who are unlikely to respond well and who also may suffer severe haematological toxicity [Kay *et al.* 2007].

Tam and colleagues assessed the response to R-FC of 300 previously untreated patients and showed an overall response rate of 95% with 72% complete responses [Tam *et al.* 2008]. This regimen has also been compared with FC in a prospective randomized trial of first-line treatment for advanced CLL [Hallek *et al.* 2010]. According to these recently published results overall response, PFS and overall survival rates were significantly better for R-FC compared with FC [Hallek *et al.* 2010]. Robak and colleagues have also reported the advantage of chemo-immunotherapy (R-FC) compared with chemotherapy alone (FC) in patients with previously treated CLL. With a median followup of 25 months the R-FC group showed significant improvement in overall response, complete response and PFS rates compared with FC [Robak *et al.* 2010]. However, even with the addition of rituximab to first or subsequent therapy, the patient subgroup with *TP53* inactivation has a lower response rate and poorer PFS and overall survival.

Treatment of patients with high-risk CLL

Treatment objectives will vary according to different risk groups (Table 1); single-agent as well as combination chemo-immunotherapy has been used with limited success in each group.

Patients with shorter remissions

Despite the prolonged remissions now being achieved for most patients following chemo-immunotherapy (65% at 3 years) [Hallek *et al.* 2010], some 20% of patients will still relapse within 3 years and alternative strategies need to be sought if the outcome for this group is to be improved. Previously the subgroup with del(11q) was known to have shorter remissions. This has largely been corrected by the addition of rituximab to FC. For patients receiving R-FC there is still a PFS disadvantage for those with unmutated *IgVH*. This group of patients may benefit from a more intensive induction regimen. The Barcelona group have added mitozantrone to R-FC showing a very high overall response with 82% complete response and 46% reaching MRD negative status [Bosch *et al.* 2009]. However, this regimen was still unable to induce good responses in the group with *TP53* deletion. The MD Anderson

Table 1. Objectives of treatment in different CLL patient sub-groups.

Patient Group	Objective	Therapeutic Options
Poor prognostic markers (*IgVH*, ZAP 70, CD38, B2M, 11 q del)	Prolong PFS	Intensify first line treatment: FCMR, CFAR.
MRD + at end of induction		Consolidation and/or maintenance
TP53 deletion/mutation	Overcome chemoresistance	CamPred, flavopiridol, lenalidomide, HDACi, RICSCT
Elderly/comorbidity	Improve tolerability/efficacy	F-FC 'light' Replace FCR e.g. with bendamustine + R, lenalidomide, new antibodies (ofatumumab, GA101)

CamPred, alemtuzumab + high-dose methylprednisolone; CFAR, cyclophosphamide, fludarabine, alemtuzumab, rituximab; R-FC, rituximab, fludarabine and cyclophosphamide; FCMR, fludarabine, cyclophosphamide, mitozantrone, rituximab; HDACi, histone deacetylase inhibitor; PFS, progression free survival; RICSCT, reduced intensity conditioned stem cell transplant.

Cancer Centre (MDACC) has designed a regimen adding alemtuzumab to R-FC for high-risk patients defined by high B2M levels. This population had a high proportion of patients with *TP53* deletion. A higher overall response (complete response rate overall was 70% and 57% for *TP53* deletion cases) was achieved compared with historical controls with these features treated with R-FC alone [Parikh *et al.* 2009]. However, PFS was still significantly shorter for those patients with *TP53* abnormalities.

The other group who can be identified as likely to progress earlier are those who still have detectable disease at the end of treatment. This group may benefit from a consolidation or maintenance approach. Alemtuzumab consolidation in those patients achieving a good partial response or a MRD positive complete response is able to improve response in 50–70% of patients [Wierda *et al.* 2010a; Montillo *et al.* 2006]. In the only randomized trial PFS was significantly better for the group receiving alemtuzumab consolidation compared with observation alone, with median followup of 24 months [Schweighofer *et al.* 2009]. However, there continue to be concerns regarding safety and this approach should not be undertaken outside a clinical trial [Lin *et al.* 2009b; Osterborg *et al.* 2009].

Patients with TP53 abnormalities

DNA damage leading to activation of the *TP53* pathway plays a pivotal role in apoptosis induction in response to conventional chemotherapy. Agents with antitumour activity which is independent of this pathway are important in the treatment of the group of patients who have del/mutation of *TP53*.

Alemtuzumab. Alemtuzumab has been used successfully both in relapsed/refractory CLL and in the frontline setting [Cortelezzi *et al.* 2009; Stilgenbauer *et al.* 2009; Hillmen *et al* 2007; Osuji *et al* 2005; Byrd *et al.* 2004; Lozanski *et al.* 2004; Stilgenbauer and Dohner, 2002]. In a phase III randomized trial Hillmen and colleagues demonstrated that alemtuzumab is superior to chlorambucil as first-line treatment for CLL patients with better overall response, complete response and PFS [Hillmen *et al.* 2007]. Although there was a trend for better overall response and PFS in the alemtuzumab group for patients with *TP53* inactivation, this did not reach statistical significance due to the small numbers in the two treatment groups. In the German CLL 2H phase II trial Stilgenbauer and colleagues examined subcutaneous (SC) alemtuzumab treatment in fludarabine-refractory CLL. The overall response rate was 34% and, in contrast to other treatment studies, there was no significant difference in efficacy between the different genetic subgroups, including those patients with del*TP53* [Stilgenbauer *et al.* 2009]. A number of studies of alemtuzumab in refractory CLL (Table 2) have reported results for the subgroup with del*TP53* showing response rates of up to 50% for these patients [Lozanski *et al.* 2004; Osuji *et al* 2005;]. It therefore appears that this antibody has activity in CLL which is independent of the *TP53* pathway.

Steroids. The mechanism of apoptosis induction by glucocorticoids in CLL cells is not fully understood. It is has been demonstrated however, that this mechanism is independent of *TP53* activation status. There is a resurgent interest in the treatment of high-risk, particularly *TP53* inactivated, CLL, with glucocorticoids (Table 3). The efficacy of high-dose methylprednisolone (HDMP; 1 g/m^2 daily for 5 days) was assessed by Thornton and colleagues in the treatment of resistant CLL and showed an overall response rate of 55% with mean duration of response of 19 months [Thornton *et al.* 1999]. This regimen was also

Table 2. Alemtuzumab in the treatment of relapsed/refractory CLL: response in different risk categories.

Study	Disease phase	*n*	ORR %	Duration (months)
Lozanski *et al.* [2004]	p53 mutation	15	40	8
	11q	11	27	N/A
Stilgenbauer *et al.* [2009]	Fludarabine refractory			
	17p	13	54	10
	11q	13	38	
	IgVH unmutated	27	52	
Cortelezzi *et al.* [2009]	Chemotherapy refractory unfavourable cytogenetics	10	60	N/A
Osuji *et al.* [2005]	p53 deleted	7	43	4.1

ORR, overall response rate.

Table 3. High-dose methylprednisolone with or without rituximab in the treatment of CLL.

Treatment Regimen	Pt No.	ORR%	CR%	PFS (median)	Study	Response in *TP53*
HDMP (1 g/m^2 × 5,q28d)	25	77	0	12 months	Thornton, 2003	50% (10 patients)
HDMP 1 g/m^2 × 5d (2-6 cycles)	12	83	25	Not achieved at 13 month F/U	Xu, 2010	
HDMP + R 1 g/m^2 × 5d + R weekly x 4 (median 1 cycle)	37	78	22		Bowen, 2007	56% (9 patients)
HDMP + R as above × 3 cycles	14	93	36	15 months	Castro, 2008	1 nodular PR
HDMP + R 1 g/m^2 × 5d + R 375 mg/m^2 d1 q 28 days	14	93	14	7 months	Dungarwalla, 2008	0% (1 patient)
HDMP + R 1st line 1 g/m^2 × 3 d	28	96	32		Castro, 2009	

CR, complete response rate; F/U, followup; HDMP, high-dose methylprednisolone; ORR, overall response rate; PFS, progression-free survival; PR, partial response; R, rituximab; Pt No., patient number.

effective in patients with *TP53* inactivation [Thornton *et al.* 2003]. Xu and colleagues also examined single agent HDMP in fludarabine-refractory CLL patients. In this study 12 patients refractory to fludarabine were treated with 2–6 cycles of HDMP at the same dose. The overall response rate was 83% and patients with adverse cytogenetic features, including *TP53* deletion, achieved similar response rates to patients who did not have such disease characteristics [Xu *et al.* 2010].

Combinations. Clinically, alemtuzumab is most effective in clearing disease from blood and bone marrow and less effective in the treatment of bulky nodal disease. In contrast, HDMP has the ability to reduce the size of very bulky adenopathy; although complete clearance of the bone marrow is unusual. There is therefore a good rationale for combining these two agents in order to achieve maximal disease response in CLL patients with *TP53* deletion. The combination of alemtuzumab and methyl prednisolone has been tested in the UK CLL206 trial for patients at either first or subsequent line of therapy who had a *TP53* deletion [Pettitt *et al.* 2009]. About half of the patients in the trial had received prior treatment (22/41) and the overall response rate was 86%. However 100% of the previously untreated patients responded. This trial has shown better response rates and PFS compared with other treatment modalities, such as FC and R-FC, in previously untreated CLL with *TP53* inactivation, but the median PFS is still disappointingly short at 19 months. Furthermore, the combination of alemtuzumab and HDMP was associated with higher grade 3–4 infection rates compared with FC, R-FC and alemtuzumab alone [Hallek *et al.* 2010; Hillmen *et al.* 2007]. In an attempt to further improve response rates and PFS the combination of alemtuzumab, dexamethasone and lenalidomide (Cam-Dex-Rev) induction followed by a randomization to lenalidomide maintenance or no further treatment in high-risk CLL is being assessed in a current UK phase II trial. Dexamethasone has been substituted for HDMP in the regimen to try and reduce the steroid-related side effects.

Although rituximab monotherapy is not very effective in treating CLL there are several small studies assessing the combination of rituximab with steroids (Table 3) and with alemtuzumab. Castro and colleagues evaluated the efficacy of rituximab in combination with HDMP as frontline therapy as well as in fludarabine-refractory CLL patients. The overall response rate was 96% with a complete response rate of 32% in frontline treatment and 93% and 36% respectively in fludarabine-refractory disease [Castro *et al.* 2009, 2008]. Of note, a reduced steroid dose of 3 rather than 5 days was used in the frontline study. Dungarwalla and colleagues demonstrated the efficacy of the 5 day schedule of HDMP and rituximab in heavily pretreated CLL patients [Dungarwalla *et al.* 2008]. A total of 13 out of 14 patients in the trial had received fludarabine in the past and 6 patients had poor risk chromosomal abnormalities del(17p) or del(11q). The combination showed superior overall response rates (93%) compared with previous experience with HDMP alone (43%), including in the cytogenetic high-risk group. Similar results were reported by Bowen and colleagues showing five out of nine patients with *TP53* inactivation responding to rituximab–HDMP combination [Bowen *et al.* 2007]. Although both these trials showed promising results infectious complications remain a major obstacle in this type of treatment. According to a study by Frankfurt and colleagues, the combination of rituximab and alemtuzumab as first line therapy was well tolerated by elderly patients with overall response rates of 95% at 2-year followup [Frankfurt *et al.* 2009]. Faderl and colleagues assessed the efficacy of alemtuzumab and rituximab in 40 relapsed CLL patients. A total of 64% of the patients in the study were fludarabine refractory. Complete response was achieved in 18% of patients while the overall response rate was 53% [Faderl *et al.* 2010]. Other novel agents which may have activity in patients with *TP53* inactivation include flavopiridol, CAL101, lenalidomide and HDAC inhibitors (see below).

Patients with comorbidities

Age alone does not preclude patients from receiving more intensive combination regimens such as R-FC. Indeed, data from the trials of FC and R-FC demonstrate that older patients (>65/70 years) achieve the same benefit from combination chemotherapy or chemo-immunotherapy compared with single agents or FC alone as those in the younger age group [Catovsky *et al.* 2007]. However, it is important to highlight that some clinical trials included mostly younger and fitter patients and those results should not be applied to older and less fit patients [Hallek *et al.* 2010]. It is more common for older patients to require dose reductions or fail to complete the planned six cycles of treatment. For this reason investigators have assessed the value of reduced dose R-FC or R-FC 'light' in older patients showing that this can be equally efficacious and less toxic [Foon *et al.* 2009]. A study in the over 60 year age group showed no advantage for fludarabine over chlorambucil [Eichorst *et al.* 2009] and thus addition of rituximab or ofatumumab to chlorambucil is currently being evaluated in phase III trials. An alternative approach is to replace the 'more toxic' FC with other agents such as pentostatin, bendamustine or lenalidomide which may be better tolerated in this age group. It may be possible to deliver some of these therapies to those patients with comorbidities and a poorer performance status who may otherwise not be able to receive treatment.

Patients with refractory CLL

CLL patients who fail all conventional therapies have the highest risk of poor survival outcome, regardless of their other biological risk factors. This is the group for whom new therapies are badly needed. There are a large number of novel agents in preclinical and clinical development (Table 4). The challenge will be to develop the most rational combinations for their optimal use. Some of the available newer therapies are discussed below.

Table 4. Targeted novel agents with therapeutic potential in CLL.

Drug	Target
ABT-263	Bcl-2 family
obatoclax	
oblimersen	
forodesine	PNP
enzastaurin	PKCB
fostamatinib	Syk
dasatinib	SRC
AZD 2174	VEGFR
AEG 35156	XIAP
TRU-016 (SMIP)	CD37
AMD 3100 (Plerixifor)	CXCR4
PARP inhibitor	ATM (del11q)
PBOX	Tubulin

CLL, chronic lymphocytic leukaemia; SMIP, Small molecule inhibitory protein.

Bendamustine. Bendamustine is a drug with combined purine analogue and alkylating properties which has been assessed as a single agent and in combination with rituximab in several clinical trials. Rouè and colleagues showed that bendamustine is effective in *TP53* inactivated CLL cell in *in vitro* studies [Rouè *et al.* 2008]. Knauf and colleagues reported results of a phase III trial comparing bendamustine with chlorambucil in previously untreated CLL patients [Knauf *et al.* 2009]. Bendamustine showed an overall response rate of 68% compared with 48% in the chlorambucil group. Both complete response rate and PFS were better with bendamustine compared with chlorambucil. However, the results in this trial for the chlorambucil group were inferior to those reported in other frontline trials. The efficacy of bendamustine in combination with rituximab (BR) has been evaluated as first- and subsequent-line therapy for CLL by Fischer and colleagues. The combination (BR) was given as 28 day cycles for up to 6 courses. The overall response rate was 91% with a complete response rate of 33% in the frontline trial [Fischer *et al.* 2009] and 77% overall response rate (15% complete response) in the relapsed study [Fischer *et al.* 2008]. As yet there is insufficient data to determine whether or not bendamustine-based therapy will be effective in patients with *TP53* inactivation.

New anti-CD20 monoclonal antibodies. Ofatumumab is a fully humanized type I monoclonal antibody that has been shown to be effective in B-cell malignancies [Coiffier *et al* 2008]. In a phase II trial, ofatumumab showed promising results in fludarabine- and alemtuzumab-refractory CLL, the response rate was 58% [Lemery *et al.* 2010; Wierda *et al.* 2010b], including an overall response rate of 41% in those patients with del(17p). Also there was a response rate of 47% in fludarabine-refractory patients with bulky lymphadenopathy (>5 cm), although only 14% of these patients who had del(17p) achieved a response. Furthermore, responses were relatively short-lived (4–5 months). The effect of ofatumumab in combination with fludarabine and cyclophosphamide is being evaluated in current trials.

GA101 is a humanized type II antibody against CD20 and has shown superior efficacy compared with classical type I antibodies like rituximab in B-cell depletion in xenograft mouse models and in animal studies [Mossner *et al.* 2010]. GA101 has been assessed in phase I/II clinical trials and shown a similar safety profile to rituximab [Robak, 2009]. However, it is premature to recommend the use of this antibody until more data is available from clinical trials. Currently GA101 is being evaluated in a phase III trial for CLL patients considered unfit for R-FC, where the combination of chlorambucil with either GA101 or rituximab is being compared with chlorambucil alone as first-line therapy.

Lenalidomide. Targeting the tumour microenvironment is a new strategy in cancer treatment. Immunomodulatory drugs (IMIDs) are attractive as they modulate the tumour and the microenvironment [Chanan-Khan and Porter, 2006]. Lenalidomide is a thalidomide derivative with antineoplastic and antiangiogenic properties. The exact mechanism of action of this drug is unknown although it has shown significant efficacy in multiple myeloma and 5q- myelodysplastic syndrome. It lacks direct cytotoxicity against CLL cells and the action appears to be immune mediated as it alters cytokine levels and stimulates T and NK cells. Ferrajoli and colleagues assessed the efficacy of lenalidomide in relapse/refractory CLL. The overall response rate was 31% in patients with high-risk cytogenetics (deletions 17p and 11q) and 25% in patients with fludarabine-refractory disease [Ferrajoli *et al.* 2008]. Sher and colleagues reported similar results in patients with high-risk cytogenetics (deletions 17p and 11q). In this study the overall response rate was 38% with 19% achieving a complete response [Sher *et al.* 2010]. Chanan-Khan and colleagues reported the efficacy of lenalidomide in relapsed/refractory CLL in a phase II trial. A total of 51% of patients in the trial were fludarabine-refractory who showed overall response rates of 47% with complete response rates of 9% [Chanan-Khan *et al.* 2006].

Flavopiridol. Flavopiridol is a cyclin-dependent kinase inhibitor with antitumour activity independent of the *TP53* pathway [Pepper *et al.* 2003, 2001; Byrd *et al.* 1998]. It has been shown to be effective in inducing apoptosis in CLL cells in *in vitro* studies. Byrd and colleagues have evaluated the effect of Flavopiridol in a cohort of patients with high-risk cytogenetics and bulky lymphadenopathy. The partial response rate in this cohort of patients was 45% [Byrd *et al.* 2007]. In addition according to a report by Lin and colleagues the overall response rate was 57% in CLL patients with *TP53* deletion treated with single agent flavopiridol [Lin *et al.* 2009c].

However, four patients in the study developed severe tumour-lysis syndrome; some requiring haemodialysis.

CAL-101. CAL-101 is a selective inhibitor of the p110 delta isoform of phosphatidylinositol 3-kinase (PI3K) and has been shown to be effective in inducing apoptosis in primary CLL cells *in vitro* [Herman *et al.* 2010]. Clinical activity has been seen in relapsed or refractory CLL patients [Brown *et al.* 2009], including in those with *TP53* inactivation.

Histone deacetylase inhibitors. Substances with histone deacetylase (HDAC) inhibitor activity (valproic acid, suberoylanilide) have been shown to induce apoptosis and increase chemosensitivity to fludarabine [Stamatopoulos *et al.* 2010, 2009]. There are a number of new HDAC inhibitors which have been licensed for use in cutaneous T-cell lymphoma. However, these agents are yet to be properly evaluated in clinical trials in other haemopoietic malignancies, including CLL.

Stem cell transplant

CLL is not curable with currently available chemo-immunotherapy or novel small molecular agents. Allogeneic stem cell transplantation is the only potentially curable treatment option, but it is associated with significant mortality and morbidity [Dreger *et al.* 2003]. It is believed that reduced-intensity conditioned stem cell transplants (RISCT) lower the nonrelapse mortality but may still have the potential for cure by utilizing a graft *versus* leukaemia effect (GVL) [Delgado *et al.* 2009; Nishida *et al.* 2009; Ritgen *et al.* 2008; Dreger *et al.* 2005; Schetelig *et al.* 2003]. It has been reported that GVL can overcome adverse outcome factors, such as unmutated *IgVH* status, chromosomal abnormalities and ZAP-70 expression in refractory CLL [Khouri *et al.* 2007; Caballero *et al.* 2005; Moreno *et al.* 2005]. Dreger and colleagues have recently reported the long-term outcome of RISCT in patients with high-risk CLL. A total of 90 patients were followed up for a median of 46 months. This study showed 4-year event free survival of 42% and overall survival of 65% with nonrelapse mortality of 23% [Dreger *et al.* 2010]. Importantly, outcomes were the same whether or not patients had abnormalities of *TP53*. Non responders to standard treatment or early relapses (<12 months) after purine analogue-based therapy or autotransplants and patients with *TP53* mutation with treatment indications are reasonable candidates for RISCT according to EBMT guidelines [Schetelig *et al.* 2008; Dreger *et al.* 2007].

Conclusion

There have been major advances in the therapy of CLL over the past decade. The majority of fit patients with no high-risk disease features will achieve excellent remission rates and prolonged PFS with chemo-immunotherapy. Nevertheless, there remain a significant number of patients who will derive less benefit from such treatment or be unable to receive an intensive regimen. There is now much information about the biological and clinical characteristics of CLL which can be useful in predicting outcome but does not often provide the necessary information to specifically tailor therapy. It is possible to identify those patients who may have a shorter PFS (e.g. UM *IgVH*) and who may benefit from more intensive frontline regimens or consolidation/maintenance strategies. The only preselected treatment in CLL is for those with *TP53* deletion/mutation. This abnormality is clearly associated with the highest risk disease, poor response to most conventional agents, including chemo-immunotherapy combinations such as R-FC, and poor survival. These patients need to be treated with specifically designed regimens using p53-independent therapies such as alemtuzumab and steroids. Even in the absence of *TP53* inactivation patients who fail to respond to combination regimens or who relapse early (within 2 years of R-FC) will also have poor survival. Novel treatments, of which there are an increasing number, need to be explored in this group. Some of the new therapies target specific biology, such as the microenvironment (lenalidomide), the B-cell receptor and downstream signalling pathways, the genetics and the epigenetics. Suitable high-risk patients should also be considered for allogeneic transplantation.

Finally, and most importantly, all high-risk patients should be entered into clinical trials where possible in order to better characterize disease response to different therapies. This will enable more intelligent design of tailored treatment in the future.

Conflict of interest statement

None declared.

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