Novel targeted treatment strategies for refractory chronic lymphocytic leukaemia

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Abstract: Patients with refractory chronic lymphocytic leukaemia (CLL) still have an unfavourable prognosis and novel treatment strategies are necessary, preferably adjusted to the patient's individual situation. Refractoriness is no longer limited just to fludarabine (F) but extends to F-combinations with other chemotherapeutic agents (e.g. cyclophosphamide [C] and FC) and antibodies (e.g. rituximab [R], FR, and FCR). Also, refractoriness to alemtuzumab is an increasing problem. New pharmacological developments provide promising approaches. This review focuses on novel therapies such as monoclonal antibodies and small molecules aiming at specific targets of the CLL cell in the refractory situation. Approved therapeutic regimens will be presented as well as investigational approaches. An overview of completed and current clinical trials is offered.

Keywords: CLL, refractory, monoclonal antibodies, small molecules

Introduction

Although much progress has been made on the treatment of chronic lymphocytic leukaemia (CLL) over the last few decades, this disease is still incurable. For more than 20 years the purine analogue fludarabine (F) has shown efficiency in CLL therapy. In 1989 the results of a clinical trial on fludarabine as monotherapy in pretreated CLL patients were published. The response data and toxicity profile were very promising [Keating *et al.* 1989]. At that time there were no hints as to which patients would respond to fludarabine and which would not. Today the combination of fludarabine, cyclophosphamide and rituximab (FCR) is the gold standard for the first-line treatment of CLL patients without relevant comorbidities. Mainly two clinical trials contributed to this progress: long-term results of a phase 2 trial in 300 untreated patients showed an overall response rate (ORR) of 95%, 72% complete remission (CR) and 23% partial remission (PR). The median follow up was 6 years. Six-year overall and failure-free survival was 77% and 51% [Tam *et al.* 2008]. The phase III CLL8 trial of the German CLL Study Group (GCLLSG) compared the former standard FC to the immunochemotherapeutic regimen FCR. The CR rate was significantly higher for the immunochemotherapy group (44% *versus* 22%, $p < 0.001$), progression-free survival (PFS) was

improved (65% *versus* 45% at 3 years, $p < 0.001$), and for the first time also extended overall survival (87% *versus* 83% at 3 years, $p = 0.01$) was achieved [Hallek *et al.* 2010].

Refractory CLL

Patients who did not achieve at least a PR or who progressed within 6 months to the last therapy are considered refractory [Hallek *et al.* 2008]. In CLL, refractoriness is mostly related to purine analogue regimens, especially F-based regimens, even though more recently we have to face also refractoriness to additional agents such as the CD52 antibody alemtuzumab. So far the mechanisms of treatment refractoriness in CLL are only partially understood. It is important to underline that stem cell transplantation (SCT) could be indicated in the patient population targeted by this review. However, SCT, similarly to other 'novel' agents such as bendamustine is somewhat outside the scope of this review (focused on novel targeted therapies) and can by no means comprehensively covered due to space limitations.

Analysis of the genetic background of CLL patients revealed an association between F refractoriness and alterations in the short arm of chromosome 17 where *TP53* is located, also called the

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'guardian of the genome'. Fludarabine treatment results in an activation of the p53-mediated apoptotic pathway. In a multivariate analysis of 100 patients with chronic B-cell leukemias (B-cell chronic lymphocytic leukaemia [B-CLL], B-cell prolymphocytic leukaemia [B-PLL] and Waldenström's macroglobulinemia) deletion of the *TP53* gene was the strongest prognostic factor for survival. Importantly, this genetic alteration also predicted nonresponse to purine analogue treatment [Dohner *et al.* 1995]. An analysis of *TP53* mutations was recently performed on a large cohort of 375 patients who were recruited in a randomized prospective trial on F *versus* fludarabine plus cyclophosphamide (FC) as first-line treatment (the CLL4 trial of the GCLLSG). Data confirmed the *TP53* mutation's poor impact on the prognosis regardless of the presence of a 17p deletion. Median PFS (23.3 *versus* 62.2 months) as well as overall survival (29.2 *versus* 84.6 months) were significantly decreased in patients with *TP53* mutation compared with those without the mutation (Figure 1) [Zenz *et al.* 2010].

Monoclonal antibodies

Rituximab

The IgG1 monoclonal anti-CD20 antibody rituximab has a chimeric structure: its light- and heavy-chain variable regions are murine, the constant regions of human origin. Rituximab is well established in the treatment of B-cell lymphomas and part of the gold standard of CLL first-line therapy FCR. Its strength in CLL is in combination rather than single treatment. The use of rituximab in CLL therapy is reviewed in detail by Jaglowski and Byrd [Jaglowski and Byrd, 2010].

FCR has not only been examined in primary therapy but also in the refractory situation. A total of 177 pretreated patients were included in a recent study: 82% of them had fludarabine as prior treatment, 21% were refractory to fludarabine. ORR was 73%, CR was achieved in 25%, nodular PR in 16% and PR in 32% of the cases. In the group of fludarabine refractory cases, ORR was 58%. In the multivariate analysis treatment failure was associated with fludarabine refractoriness [Wierda *et al.* 2005].

Pentostatin is supposed to be less myelosuppressive than fludarabine. In a phase II study

the combination of rituximab with cyclophosphamide and pentostatin was examined in 46 patients of whom 32 had pretreated CLL. In the CLL cases ORR was 75%, CR rate was 25%; 75% of fludarabine refractory patients responded. Infections of grade 3 or 4 occurred in 28% of the cases. All in all, this regimen was well tolerated. About 72% of the patients received six cycles of therapy at full dose [Lamanna *et al.* 2006].

In vitro synergistic effects of a combination of fludarabine, cytarabine and cisplatin [Li *et al.* 1997; Wang *et al.* 1991] led to a phase I/II study for patients with Richter syndrome or fludarabine refractoriness. Thirty fludarabine refractory patients were included of whom 33% responded; 7% of CR was achieved in this group. Adverse events were mainly grade 3 or 4 neutropenia and thrombocytopenia [Tsimberidou *et al.* 2008].

The combination of rituximab with HD-methylprednisolone has been tested in 14 patients with refractory disease. ORR was 93% (all but one patient responded), CR rate was 36%, and the safety profile well tolerable [Castro *et al.* 2008]. Another 14 heavily pretreated patients (13 of whom with fludarabine) were treated with this combination. Again, the ORR was 93%, but only two CR and one nodular PR were achieved. The only patient with 17p-deletion did not respond. Infectious complications, especially opportunistic infections (candida, aspergillus, viral infections) were the most serious adverse events. Eight patients died during the study [Dungarwalla *et al.* 2008]. All in all rituximab in combination with HD-methylprednisolone seems to be effective but is associated with serious infectious risks.

In a phase II study the combination with hyper-CVXD (cyclophosphamide, vincristine, liposomal daunorubicine, dexamethasone) showed activity but also high toxicity in 30 cases of Richter syndrome or fludarabine refractoriness. Nineteen patients with refractory CLL were treated: 37% of them achieved either a CR (one patient) or a PR (six patients). The toxicity profile was unfavourable: 11 patients died during the first 2 cycles mainly of systemic fungal and bacterial infections. Myelosuppression was the most common adverse event [Tsimberidou *et al.* 2003].

Figure 1. *TP53* mutations are associated with poorer outcome in chronic lymphocytic leukaemia patients. Blue: without *TP53* mutation; yellow: with *TP53* mutation.

(a) Progression-free survival at a median follow up of 52.8 months (without mutation: 62.2 months, with mutation: 23.3 months).

(b) Overall survival (without mutation: 84.6 months; with mutation: 29.2 months).

Meanwhile new anti-CD20 antibodies are under development or have already entered clinical trials: the type I CD20 antibody ofatumumab and the type II antibody GA-101. Type I anti-CD20 antibodies such as rituximab or ofatumumab lead to redistribution of the CD20 antigen into lipid rafts. They exert complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), but low direct apoptosis induction. Type II antibodies such as the novel antibody GA-101 do not fix complement. They act via direct cell death induction through homotypic aggregation and mediate ADCC (Figure 2).

Alemtuzumab

The humanized monoclonal IgG1 antibody alemtuzumab is directed against the CD52

antigen on mature lymphocytes. It was approved in the USA and EU for the treatment of fludarabine refractory CLL in 2001. Since 2008 this drug is also approved for first-line treatment of patients who are ineligible for fludarabine containing therapies, i.e. those with 17p deletion or *TP53* mutation.

A large multicentre trial in the USA and Europe of intravenous alemtuzumab administration showed better survival data for patients with fludarabine refractory CLL compared with historic cohorts. A total of 93 patients who were fludarabine refractory were enrolled in that prospective noncomparative phase II trial. ORR was 33%, CR was achieved in 2%; 7% of the patients had a CR except for cytopenia. The most

Figure 2. Modes of cell death induction by monoclonal antibodies.

1. Direct cell death induction: binding of the monoclonal antibody triggers intracellular signalling followed by programmed cell death.
2. Antibody-dependent cellular cytotoxicity (ADCC): effector cells, like NK cells or macrophages, bind via their Fc γ -receptor to the Fc portion of the monoclonal antibody fixed to the target cell's surface. Cytokine release then leads to apoptosis in the target cell.
3. Complement-dependent cytotoxicity (CDC): binding of the monoclonal antibody initiates the complement cascade which results in the membrane attack complex and therefore cell lysis.

common adverse events were infusion related. At least one episode of infection occurred in 55% of the patients. About 27% developed grade 3 or 4 infections. Myelosuppression with transient cytopenia was seen in most of the patients. The median survival of patients who had failed purine analogue therapy was increased compared with historical controls [Keating *et al.* 2002].

The CLL2H trial of the GCLLSG showed that subcutaneous administration of alemtuzumab is as effective as intravenous infusion in fludarabine refractory CLL. The ORR was 34% with 4% of CR (Figure 3). Importantly, side effects of infusion reactions were markedly reduced. Efficiency was the same in all genetic subgroups, notably in 17p and 11q deletion, *TP53* mutation and unmutated immunoglobulin heavy chain variable (IGHV) status [Stilgenbauer *et al.* 2009]. The CLL2O trial of the GCLLSG and FCGCLL/MW aims to achieve a higher remission rate by adding high-dose dexamethasone (40 mg) to subcutaneous alemtuzumab and to prolong remission duration and survival by either allogeneic SCT or alemtuzumab maintenance. An interim analysis of 50 patients who completed induction therapy with either F-refractory disease or 17p-deletion (untreated or at relapse) showed an

ORR of 47% for the F-refractory cohort. No patient in this cohort achieved a complete remission. Estimated overall survival at 12 months was 54%. At a median follow up at 41.9 weeks, 2 patients died due to progressive disease and 5 due to infection [Stilgenbauer *et al.* 2010].

Ofatumumab

Ofatumumab is a fully human IgG1 antibody. Like rituximab it is a type I monoclonal antibody and targets the CD20 antigen on the surface of B-lymphocytes. Nevertheless ofatumumab binds to an epitope that is distinct from rituximab: the small extracellular loop and the N-terminal region of the large extracellular loop of CD20. Its location is closer to the membrane which may contribute to stronger CDC [Teeling *et al.* 2006, 2004].

Two clinical trials were essential for the approval of ofatumumab in refractory CLL by the US Food and Drug Administration (FDA) in 2009. In a multicentre dose-escalating study, 33 patients with relapsed or refractory CLL were included. The maximum tolerated dose was not reached; 2000 mg weekly doses were well tolerated. Infusion-related events such as transient rigor, pyrexia, increased sweating, fatigue or rash occurred mostly during the first

Figure 3. Genomic aberrations do not influence overall survival or time to treatment failure in chemotherapy-refractory chronic lymphocytic leukaemia after alemtuzumab treatment. Blue: 17p deletion ($n=31$); yellow: 11q deletion ($n=20$); grey: all other subgroups ($n=49$).

(a) Median overall survival: 18.3, 22.7 and 18.6 months (log-rank $p=0.661$).

(b) Median time to treatment failure: 5.8, 6.8 and 5.4 months (log-rank $p=0.842$).

administration of ofatumumab. Infectious complications were seen in 51% of the cases, most of them were of grade 1 or 2. One infectious interstitial lung disease was fatal. Neutropenia or thrombocytopenia was seen in 15% of the patients. The cohort treated with the highest dose achieved a remission rate of 50%. There were no CR [Coiffier *et al.* 2008].

An interim analysis of a large international multi-centre trial [ClinicalTrials.gov identifier: NCT00349349] also showed that ofatumumab

is well tolerated and clinically active in the refractory situation. The analysis was based on the data of 59 patients with fludarabine and alemtuzumab refractory CLL (FA-ref) and 79 patients with fludarabine refractory CLL with bulky disease (BF-ref) where alemtuzumab is supposed to be less effective. The ORR was 58% for the FA-ref group and 47% for the BF-ref group (Figure 4). One CR was achieved in a patient with bulky disease. Importantly rituximab pretreatment did not have an influence on the response rates. There, the ORR was 54% in the FA-ref patients

Figure 4. Overall survival in a phase II trial of ofatumumab as a single agent.
(a) Overall survival in fludarabine- and alemtuzumab-refractory patients (FA-ref).
(b) Overall survival in fludarabine-refractory patients with bulky disease (BF-ref).

and 44% in the BF-ref cases. Distinct clinical improvement was achieved in patients with baseline thrombocytopenia or anemia and decreased Eastern Cooperative Oncology Group (ECOG) performance status. Adverse events were similar to those of the dose-escalating study cited above: frequently infusion-related events and infectious complications. The ORR in 17p-deleted cases was lower [Wierda *et al.* 2010].

GA-101

The structure of GA-101 is quite different from conventional monoclonal anti-CD20 antibodies. It is a humanized IgG1 monoclonal antibody with a glyco-engineered Fc region. As a type II antibody GA-101 induces direct cell death (caspase-independent apoptosis) rather than CDC. The glyco-engineering of the Fc region leads to

increased binding to the Fc receptor of immune effector cells such as natural killer (NK) cells or macrophages and to enhanced ADCC [Mossner *et al.* 2010].

In a dose-finding phase I trial of relapsed or refractory CD20-positive B-CLL, GA-101 was administered in doses between 50 and 2000 mg on days 1, 8 and 22 every 3 weeks for 9 cycles. A total of 21 patients were included, 33% with high-risk cytogenetics and 70% with unmutated IGHV. ORR was 62% with one Complete Remission with incomplete blood count recovery (CRi), 7 PR and 5 stable disease (SD). GA-101 was well tolerated. Infusion-related reactions were the most frequent side effects, followed by haematological toxicity and infectious complications [Morschhauser *et al.* 2009]. Currently a

phase III study of bendamustine *versus* bendamustine plus GA-101 in rituximab refractory B-cell non-Hodgkin lymphomas (B-NHLs) is recruiting patients [ClinicalTrials.gov identifier: NCT01059630], as well as a phase II trial of GA-101 monotherapy compared with rituximab monotherapy in relapsed CD20-positive indolent B-cell lymphomas [ClinicalTrials.gov identifier: NCT00576758]. Untreated patients with comorbidities can be included into the GCLLSG's CLL11 trial [ClinicalTrials.gov identifier: NCT01010061] which compares GA-101 and chlorambucil *versus* rituximab and chlorambucil *versus* chlorambucil monotherapy.

Lumiliximab

Lumiliximab is a primatized anti-CD23 monoclonal antibody; it has macaque variable and human constant regions. The IgE receptor CD23 is expressed on the majority of CLL cells but only at low levels on other cells which provides quite a CLL-specific therapeutic option. *In vitro* experiments showed synergistic effects in combination with fludarabine and rituximab [Pathan *et al.* 2008].

A total of 46 patients with relapsed or refractory CLL were included in a recent phase I trial. Neither partial nor complete remissions were achieved. Some clinical benefit was observed with decreasing lymphadenopathy and lymphocytosis. Nevertheless this was a transient effect and not dose related. Lumiliximab was well tolerated [Byrd *et al.* 2007].

Based on the results of this phase I study and the preclinical data, a phase I/II trial was designed to examine the combination of lumiliximab with RFC in 31 patients. Doses of 375 and 500 mg/m² were used. Infectious complications were mostly of grade 1 or 2, three grade 3 infections were observed. A total of 48% of the patients completed all of the six cycles. ORR was 65% with 52% of CRs. PFS for all responders was 28.7 months. In comparison with the above cited study of FCR in refractory CLL [Wierda *et al.* 2005], the rate of complete remissions was double as high with 52% *versus* 25% [Byrd *et al.* 2009].

Bevacizumab (Avastin)

The humanized monoclonal IgG1 antibody bevacizumab (Avastin) is approved for the treatment of several solid tumour entities such as metastatic colon cancer and non-small cell lung cancer.

It binds to vascular endothelial growth factor (VEGF) and is an inhibitor of angiogenesis.

A multicentre phase II trial of bevacizumab in relapsed or refractory CLL has been completed [ClinicalTrials.gov identifier: NCT00290810]. Studies about the combination with fludarabine and rituximab [ClinicalTrials.gov identifier: NCT00845104] or pentostatin, cyclophosphamide and rituximab [ClinicalTrials.gov identifier: NCT00816595] or FCR [ClinicalTrials.gov identifier: NCT00448019] are recruiting patients.

TRU-016

TRU-016 is a humanized anti-CD37 SMIP protein. CD37 is expressed on normal and transformed B cells. *In vitro* studies showed more direct and NK-cell mediated cell killing compared with rituximab. Results of a phase I dose-escalating trial in 57 relapsed/refractory patients were presented at the ASH 2010. Nine dose levels from 0.03 up to 20 mg/kg were tested. The maximum tolerated dose was not reached. Adverse events were mild infusion reactions, neutropenia, idiopathic thrombocytopenic purpura (ITP) and presumed zoster. Two serious infusion reactions of grade 2 and 3 occurred. Seven PRs were achieved; response seemed to be dependent on the number of prior therapies. No responses were achieved in patients with more than two prior therapies although a reduction in lymphocyte counts was reported in 67% of those cases [Furman *et al.* 2010]. Two clinical trials are currently open for recruitment: a phase I/Ib study in pretreated patients [ClinicalTrials.gov identifier: NCT00614042] and a phase I/II study about the combination with bendamustine [ClinicalTrials.gov identifier: NCT01188681].

Small molecules

Flavopiridol

Flavopiridol (alvocidib) is a cyclin-dependent kinase inhibitor. Cyclin-dependent kinases are involved in the regulation of the cell cycle, transcription and mRNA processing. *In vitro* studies in CLL cells showed p53-independent apoptosis induction [Byrd *et al.* 1998], downregulation of the antiapoptotic molecules Mcl-1 and XIAP [Kitada *et al.* 2000] and reduced RNA synthesis via phosphorylation inhibition of the C-terminal domain of RNA polymerase II [Chen *et al.* 2005].

The results of a first phase I trial of flavopiridol in refractory or relapsed CLL were disappointing. Thirteen patients were included and treated with a 24-hour continuous infusion every 2 weeks. Doses were 80 up to 140 mg/m². There were no remissions seen [Flinn *et al.* 2005].

The protein binding capacity of flavopiridol in foetal bovine serum is much lower than in human serum. Therefore, the doses for clinical use had been underestimated. Considering pharmacokinetics the administration schedule was changed for the next phase I trial in refractory CLL (*n*=52). Flavopiridol was administered as a 30-minute loading dose followed by a 4-hour continuous infusion. Doses ranged from 30 to 50 mg/m² with loading doses of 30–40 mg/m². Tumour lysis syndrome became a problem in 30 cases and caused one death. The most frequent adverse events were neutropenia, diarrhoea and cytokine release syndrome. A total of 40% of the patients achieved a PR, the median PFS was 12 months. Also patients with high-risk cytogenetics (17p or 11q deletion) responded [Phelps *et al.* 2009]. In a phase II study prophylactic dexamethasone was added on each treatment day to prevent the cytokine release syndrome. The cycle length was reduced well as the number of treatments per cycle. This improved the tolerability of the regimen [Lin *et al.* 2009]. An interim analysis of a phase II study of flavopiridol showed clinical responses in patients with fludarabine refractory CLL. A total of 108 patients were treated; using the NCI-96 criteria for response assessment, 31% achieved a partial response. Also patients with high-risk cytogenetics such as the 17p or 11q deletion had a benefit. No complete responses were seen. The most serious adverse events were infections (32%), tumour lysis syndrome (19%), diarrhoea (17%) and febrile neutropenia (15%). Three cases of death seemed to be related to flavopiridol treatment (colitis, pseudomonal sepsis and tumour lysis syndrome) [Lanasa *et al.* 2010]. Analysis of 112 patients of a phase I and phase II trial of single-agent flavopiridol in relapsed or refractory patients revealed no significant difference in the overall survival; also patients with high-risk cytogenetics show a high ORR. Nevertheless the estimated PFS by 24 months was lower in patients with poor risk cytogenetics (17p, 4%, and 11q, 5%, *versus* other cytogenetics, 24%) [Woyach *et al.* 2010].

SNS-032

Another Cdk-inhibitor is SNS-032 which inhibits Cdk2, Cdk7 and Cdk9 [Conroy *et al.* 2009]. *In vitro* studies showed more potent inhibition of RNA synthesis and cell death induction by SNS-032 than by flavopiridol. Levels of Mcl-1 and XIAP were reduced whereas Bcl-2 was not affected [Chen *et al.* 2009]. Results of a phase I study in patients with advanced CLL and multiple myeloma were published in 2010. Nineteen patients with CLL were included. All of them were heavily pretreated, 26% were fludarabine refractory. The dose-limiting toxicity for CLL patients was tumour lysis syndrome, the maximum tolerated dose was 75 mg/m² and myelosuppression was the most frequent toxicity of grade 3 or 4. One patient achieved a reduction of more than 50% in measurable disease (without improvement in haematological parameters), whereas one patient had stable disease. All in all, clinical activity was limited even though *in vitro* biomarker analyses showed inhibition of Cdk7 and Cdk7, CLL cell apoptosis and decreases in Mcl-1 and XIAP levels [Tong *et al.* 2010].

One trial of intravenous SNS-032 in patients with advanced (not necessarily refractory) B-lymphoid malignancies is currently ongoing but not recruiting [ClinicalTrials.gov identifier: NCT00446342].

Lenalidomide

Lenalidomide is well established in the treatment of multiple myeloma and showed therapeutic efficiency in MDS. This immune-modulating drug (IMiD) is a structural analogue of thalidomide. It is supposed to be more potent than thalidomide and to bear less side effects (fatigue and neuropathy) but has also teratogenic properties. Its mode of action is not fully understood. Direct cell killing has not been detected in CLL cells so far. As an immune modulating drug lenalidomide causes activation of T and NK cells and therefore indirect cell death induction [Corral *et al.* 1999]. It inhibits cytokines such as tumour necrosis factor alpha (TNF α), VEGF and interleukin 6 (IL-6) [Chaulet *et al.* 2011; De Luisi *et al.* 2011]. A comprehensive overview of IMiD therapy in CLL is provided by Awan and colleagues [Awan *et al.* 2010].

In clinical trials a typical side effect of lenalidomide is quite common: the tumour flare reaction. The tumour flare reaction can mimic disease

progression and is characterized by a sudden increase in lymph node size, painful lymphadenopathy, bone pain, rash and pyrexia. In most cases it can be dissolved by nonsteroidal anti-inflammatory drugs or steroids.

Two phase II trials showed the efficacy of lenalidomide also in refractory CLL: 45 patients were included and treated with 25 mg p.o. on 21 days of a 28-day cycle. A total of 51% of the patients were refractory to fludarabine. The ORR was 47% with a CR rate of 9%. Fatigue (83%) and tumour flare reaction (58%) were the most common nonhaematological adverse events. Myelosuppression was a common treatment-related toxicity [Chanan-Khan *et al.* 2006].

In the second study 44 patients with relapsed or refractory CLL were enrolled. A dose-escalating schedule was chosen with a starting dose of 10 mg for 28 days and dose escalation in steps of 5 mg per cycle up to 25 mg. An ORR of 32% was achieved with a CR rate of 7%, nodular PR in 2% and PR in 23%. A total of 39% of the patients had progress during the treatment. Myelosuppression was the most common adverse event. No tumour lysis syndrome was observed, but tumour flare reactions occurred in 12% of the cases [Ferrajoli *et al.* 2008].

ABT-263

ABT-263 also called navitoclax is an orally bioavailable small-molecule BH3-mimetic. Like the proapoptotic BH3-only proteins, it binds to the antiapoptotic members of the Bcl-2 family Bcl-2, Bcl-XL and Bcl-w and prevents their binding to the proapoptotic molecules Bax and Bak. Therefore, the mitochondrial apoptotic pathway is activated.

In most *in vitro* experiments ABT-737 a structure analogue of ABT-263 was used which is not orally bioavailable and metabolically unstable. In primary CLL cells, ABT-737 induced apoptosis at an EC₅₀ of 7 nM after 4 hours of incubation [Vogler *et al.* 2008]. In whole blood assays ABT-263 had an EC₅₀ of more than 10 µM after 4 hours of incubation, whereas in human serum free assays the EC₅₀ was less than 1 µM. In human plasma the agent is highly bound to albumin [Vogler *et al.* 2010]. This fact has to be considered for the planning of clinical trials with ABT-263.

The interim analysis of a phase I/IIa trial of ABT-263 in 21 patients with relapsed or refractory CLL showed an ORR of 33%. No CRs were achieved but PRs were confirmed radiographically in two patients, eight patients had a stable disease and two were progressive. To prevent thrombocytopenia caused by Bcl-XL inhibition, a loading dose of 100 mg was administered for 7 days followed by a continuous dose of up to 300 mg daily for 21 days. The most common adverse events were diarrhoea (52%), nausea (44%), vomiting (24%), fatigue (24%), thrombocytopenia (20%) and neutropenia (12%) [Roberts *et al.* 2009].

This trial is still recruiting [ClinicalTrials.gov identifier: NCT00481091], another one for refractory CLL is going to test the combination of ABT-263 with FCR or BR [ClinicalTrials.gov identifier: NCT00868413].

AT-101

AT-101 is another BH3 mimetic. It is an enantiomer of gossypol (R(–)-gossypol) which binds to the BH3 motif of Bcl-2, Bcl-xL and Mcl-1. Gossypol is extracted from cotton plant seeds. *In vitro* studies showed that gossypol as well as AT-101 induce cell death in primary CLL cells. Concentrations used were quite high at 10 and 30 µM for gossypol and 20 µM for AT-101 [Balakrishnan *et al.* 2009, 2008].

One phase II study of AT-101 as a single agent in patients with relapsed or refractory B-NHL has already been completed [ClinicalTrials.gov identifier: NCT00275431], as well as a trial of AT-101 in combination with rituximab in relapsed or refractory CLL [ClinicalTrials.gov identifier: NCT00286780]. Results have not yet been published. Currently one study of AT-101 in combination with lenalidomide in relapsed or refractory CLL is open for recruitment [ClinicalTrials.gov identifier: NCT01003769].

Talabostat mesylate (PT-100)

Talabostat is an orally bioavailable dipeptidyl peptidase inhibitor with antineoplastic and haematopoiesis stimulating properties. It inhibits the activation of fibroblast activation protein (FAP) which is essential for development, growth and metastasis of epithelial tumours [Kraman *et al.* 2010] but is also found in the stroma of lymphoid tissue. In xenograft models of human CD20 + B-cell lymphoma talabostat increased the cytotoxic activity of rituximab [Adams *et al.* 2004].

Talabostat interacts with bone marrow stromal cells and stimulates haematopoiesis via increased cytokine production (granulocyte colony stimulating factor [G-CSF], IL-6 and IL-11) [Jones *et al.* 2003]. *In vitro* studies in primary CLL cells showed apoptosis induction at least in a subset of primary CLL samples [Danilov *et al.* 2005]. A phase II trial is currently studying the potential of combined talabostat and rituximab in CLL relapsed after fludarabine pretreatment or in the F-refractory situation [ClinicalTrials.gov identifier: NCT00086203].

Obatoclox

The Bcl-2 inhibition capacity of the small-molecule obatoclox is broader than that of ABT-263. Apart from Bcl-2, Bcl-XL and Bcl-w it also binds to Mcl-1 which is known to be overexpressed in CLL. *In vitro* Mcl-1 can cause resistance to the Bcl-2 inhibitor ABT-737 which can be overcome by obatoclox [Nguyen *et al.* 2007].

In a phase I study in 26 patients with advanced CLL and fludarabine pretreatment, one PR was achieved. The maximum tolerated dose was 28 mg/m² over 3 hours every 3 weeks. Haemoglobin levels and platelet counts were improved; lymphocyte counts were reduced in 18 of 26 patients. Dose-limiting adverse events were infusion related neurologic symptoms such as somnolence, ataxia and euphoria [O'Brien *et al.* 2009b]. Obatoclox seems to be attractive for combination therapy with its modest single-agent activity and tolerable safety profile.

Oblimersen

Oblimersen is a synthetic antisense oligonucleotide which targets the first six codons of the Bcl-2 mRNA. *In vitro* studies showed decreased Bcl-2 mRNA expression and synergistic effects with fludarabine [Marcucci *et al.* 2005], rituximab [Loomis *et al.* 2003] and alemtuzumab [Cotter *et al.* 2003]. In a phase I/II trial in patients with relapsed or refractory CLL modest single-agent activity was shown with a PR in 8% of the patients. Circulating lymphocytes were reduced by more than 50% in 50% of the cases. The maximum tolerated dose based on the phase I part of the study was 3 mg/kg/day. A cytokine release reaction within 24–48 hours during the first two cycles with high fever, hypotension and back pain was dose limiting [O'Brien *et al.* 2005].

A phase III trial compared the conventional FC-regimen with the combination of oblimersen

and FC in 241 patients. Oblimersen was intravenously administered at a dose of 3 mg/kg/day via continuous infusion for the first 7 days of a cycle of 28 days. Indeed the CR rate was significantly higher in the experimental arm (17% versus 7%, $p=0.025$). Also the duration of response was longer (>36 versus 22 months, $p=0.03$). The 5-year survival rate of the responders with complete or partial response were 47% in the experimental arm and 24% in the FC arm ($p=0.038$). Fludarabine-sensitive patients had the greatest benefit [O'Brien *et al.* 2009a].

HSP90 inhibitors

HSP90 plays an important role in the regulation of cell signalling, proliferation and survival. As an ATP-dependent chaperone, it regulates the folding, stabilization and activation of a broad range of proteins (so-called 'clients') involved in cell survival and proliferation, such as glucocorticoid receptors, transcription factors, kinases as well as the tumour suppressor protein p53 [Taipale *et al.* 2010; Picard, 2002]. The ansamycin antibiotic geldanamycin is an ATP-competitive inhibitor of HSP90. *In vitro* experiments showed selective toxicity of geldanamycin towards CLL and normal B cells at nanomolar concentrations, whereas T cells and CD34+ bone marrow haematopoietic progenitor cells were unaffected [Jones *et al.* 2004]. In another study in ATM/p53 mutated primary CLL cells, overexpressed mutated p53 was downregulated in response to HSP90 inhibition, whereas wild-type p53 was upregulated [Lin *et al.* 2008]. ZAP70 was shown to be another client of HSP90 that is degraded after HSP90 inhibition by the geldanamycin derivatives 17-AAG and 17-DMAG [Castro *et al.* 2005]. Synergistic effects were seen in primary CLL cells with the combination of rituximab and 17-AAG or 17-DMAG [Johnson *et al.* 2007]. HSP90 inhibitors may be of particular value for the treatment of TP53 mutated cases. 17-DMAG is cytotoxic to CLL cells, but not normal lymphocytes, and acts as an NFκB inhibitor by depletion of the HSP90 client IKK. This leads to decreased NFκB target gene transcription and caspase-dependent cell death [Hertlein *et al.* 2010]. Another novel HSP90 inhibitor, SNX7081, seems to be more potent than 17-AAG. SNX7081 sensitizes ATM/p53 mutated CLL cells to fludarabine treatment and overcomes fludarabine resistance *in vitro* [Best and Mulligan, 2010].

Table 1. Overview of clinical data for refractory chronic lymphocytic leukaemia: monoclonal antibodies.

Study	Total				Refractory				Median survival [months]	Median PFS [months]	Median survival [months]
	<i>n</i>	CR [%]	PR [%]	OR [%]	Median PFS [months]	Median survival [months]	<i>n</i>	CR [%]	PR [%]	OR [%]	
Wierda <i>et al.</i> [2005]	177	25	48	73			37 [21%]	5	43	59	
Lamanna <i>et al.</i> [2006]	32	25	47	75		44	8 (25%)	12.5	0	75	
Tsimberidou <i>et al.</i> [2008]	30						30 (100%)	7	27	33	
Castro <i>et al.</i> [2008]	14						14 (100%)	36		93	
Dungarwalla <i>et al.</i> [2008]	14	14			7	20	?				
Tsimberidou <i>et al.</i> [2003]	49	19	22	41		8.5	19 (39%)	5	32	37	
Keating <i>et al.</i> [2002]	93	2	31	33			45 (48%)	2	27	29	
Stilgenbauer <i>et al.</i> [2009]	109	4	30	34	7.7	19.1	109 (100%)	4	30	34	19.1
Stilgenbauer <i>et al.</i> [2010]	50						19	0		47	
Coiffier <i>et al.</i> [2008]	33	0	42	44			?				
Wierda <i>et al.</i> [2010]	138						59 (43%, FA-ref)			58	13.7
							79 (57%, BF-ref)	1	46	47	15.4
Morschhauser <i>et al.</i> [2009]	13	8	54	62			?				
Byrd <i>et al.</i> [2007]	46	0	0				25 (54%)	0	0		
Byrd <i>et al.</i> [2009]	31	52	13	65	19.3		3 (9.7%)				

CR, complete remission; PR, partial remission; ORR, overall response rate; PFS, progression-free survival; hyper-CVXD, cyclophosphamide, vincristine, liposomal daunorubicine, dexamethasone; s.c., subcutaneously; FA-ref, fludarabine- and alemtuzumab-refractory patients; BF-ref, fludarabine-refractory patients with bulky disease.

Table 2. Overview of clinical data for refractory chronic lymphocytic leukaemia: small molecules.

Study	Total						refractory					
	n	CR [%]	PR [%]	OR [%]	PFS [median, months]	Median survival [months]	n	CR [%]	PR [%]	OR [%]	PFS [median, months]	Median survival [months]
Phelps et al. [2009] Lin et al. [2009]	Flavopiridol											
	52		40		12.0		43 (83%)			40	12.4	
	64				8.6		38 (59%)					
Lanasa et al. [2010] Woyach et al. [2010]	Flavopiridol											
	113	0	31	31			106 (94%)					
	112			47	10	28	?					
Tong et al. [2010]	SNS-032											
	19	0	0	0			5 (26%)	0	0	0		
	Lenalidomide											
Chanani-Khan et al. [2006] Ferrajoli et al. [2008]	Lenalidomide											
	45	9	38	47			23 (51%)					
	44	7	25	32			12 (27%)					
Roberts et al. [2009]	ABT-263											
	21	0	10	33			?					
	Obatoclax											
O'Brien et al. [2009b]	Obatoclax											
	26						22 (85%)					
	Oblimersen											
O'Brien et al. [2005] O'Brien et al. [2009a]	Oblimersen											
	40		8				26 (65%)					
	120	17					69 (58%)					
Friedberg et al. [2008]	cyclophosphamide											
	Fostamatinib disodium											
	11		54									

CR, complete remission; PR, partial remission; ORR, overall response rate; PFS, progression-free survival.

HSP90 inhibitors have entered early clinical trials. One phase I study of 17-DMAG (alvespimycin) in relapsed CLL/SLL and B-PLL is currently recruiting [ClinicalTrials.gov identifier: NCT01126502], one in solid tumours and lymphomas is already completed [ClinicalTrials.gov identifier: NCT00088868]. Another phase I study of SNX5422 in refractory solid tumours and lymphomas is not recruiting but ongoing [ClinicalTrials.gov identifier: NCT00644072], a further study has already been completed, results are still pending [ClinicalTrials.gov identifier: NCT00647764].

CAL-101

CLL cells show an increased activity of phosphatidylinositol 3-kinase (PI3K). PI3Ks are involved in a great variety of cellular functions: cell growth, proliferation, differentiation, survival, migration and adhesion. The expression of the isoform PI3K-p110 δ is restricted to haematopoietic cells, and CLL cells show high expression levels. *In vitro* treatment with the PI3K-p110 δ inhibitor CAL-101 caused caspase-dependent apoptosis in CLL cells but not in T cells or NK cells [Herman *et al.* 2010].

Two clinical trials in the refractory situation are currently recruiting: a phase I dose-escalating study [ClinicalTrials.gov identifier: NCT00710528] and a phase I study about the combination of CAL-101 with bendamustine and either rituximab or ofatumumab [ClinicalTrials.gov identifier: NCT01088048].

Fostamatinib disodium

B-cell receptor survival signalling requires activation (phosphorylation) of SYK. Fostamatinib disodium is an orally bio-available inhibitor of SYK which acts ATP-competitive and inhibits the activation of mast cells, macrophages and B-cells. It has anti-inflammatory and immunomodulatory properties. In a phase I trial neutropenia was dose limiting. In a phase II study 68 patients with B-NHLs were enrolled, of whom 11 had relapsed or refractory CLL/SLL. Six PRs were achieved in the CLL/SLL group, two had SD [Friedberg *et al.* 2008]. Another phase I/II study has already been completed, results have not been published so far [ClinicalTrials.gov identifier: NCT00446095].

Conclusion

Refractory chronic lymphocytic leukaemia represents a clinical challenge. Novel treatment

approaches include antibody-based therapies as well as the use of small molecules, often in combination with established chemotherapeutic regimens. Approved for the refractory situation are the CD52 antibody alemtuzumab and, in the case of fludarabine and alemtuzumab refractoriness, the CD20 antibody ofatumumab. In the fludarabine refractory situation alemtuzumab treatment led to an overall response rate of 33%. The most promising and advanced new agents are the CDK inhibitor flavopiridol and the immunomodulating drug lenalidomide. Response rates of 30–50% were seen in single-centre experiences. A broad range of other novel drugs are still under development or in early phases of clinical trials. The results are eagerly awaited considering the still unfavourable prognosis of refractory CLL. Nevertheless new therapeutic regimens have to prove not only efficacy but also tolerability.

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Conflict of interest statement

None declared.

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