Ofatumumab for the treatment of chronic lymphocytic leukemia

Over the last few years, several new monoclonal antibodies (mAbs) directed against lymphoid cells have been developed and investigated in chronic lymphocytic leukemia (CLL). Two mAbs have been demonstrated to have the most important clinical value in patients with CLL – rituximab, which targets the CD20 antigen, and alemtuzumab, which is active against the CD52 antigen. Recently, several new mAbs have been explored and evaluated in preclinical studies and in early clinical trials. One of these is ofatumumab (HuMax-CD20™), a fully human monoclonal immunoglobulin (Ig)$G_{1-\kappa}$ antibody targeting a CD20 molecule on B cells. Ofatumumab specifically recognizes an epitope encompassing both the small and large extracellular loops of CD20, distinct from the epitope recognized by rituximab. Ofatumumab, in comparison with rituximab, shows superiority in complement-dependent cytotoxicity of B cells, and does not induce cell death of tumor B cells by apoptosis. In a Phase I/II study, ofatumumab demonstrated significant depletion of $CD19^+/CD5^+$ CLL cells, and showed a favorable safety profile in previously treated CLL patients. A recent study demonstrates the effectiveness of ofatumumab in patients with fludarabine-refractory CLL. Ofatumumab potentially represents an active treatment option with clinical benefit for patients with very poor prognosis who have exhausted standard treatment options. In December 2004, the US FDA granted Fast Track status for the use of ofatumumab in therapy of CLL in patients who failed treatment with fludarabine.

KEYWORDS: chronic lymphocytic leukemia ▪ CLL ▪ leukemia ▪ monoclonal antibody ▪ ofatumumab ▪ therapy

Tadeusz Robak
Medical University of Lodz, Department of Hematology, Pabianicka 62 , Lodz, 93-513, Poland
Tel.: +48 426 895 191
Fax: +48 426 895 192
robaktad@csk.am.lodz.pl

Chronic lymphocytic leukemia (CLL) is a clonal disease characterized by proliferation and accumulation of small CD5-positive B cells. It is the most common adult leukemia in Europe and North America, with an annual incidence rate of 3–5 cases per 100,000 [1,2]. The disease is generally perceived as indolent but incurable, affecting predominantly elderly people. The median age at diagnosis is 65–70 years, with only approximately 20% of patients aged below 55 years. Clinical staging systems proposed in the early 1980s by Binet *et al.* [3] and Rai *et al.* [4] are still the most common and validated prognostic factors in patients with CLL. However, these systems do not allow identification of patients in early stages who are likely to progress, and those in whom the disease will remain stable for many years.

Within the past few years, several biological markers, including serum markers, immunoglobulin heavy chain variable region (IgV_H) mutation status, some cytogenetic abnormalities, P53 mutations, cell membrane expression of CD38 and ζ-associated protein-70 (ZAP-70) have become important prognostic factors [5,6]. Several serologic parameters such as thymidine kinase (TK), β2-microglobulin (β2M) and soluble CD23 (sCD23) also provide valuable information regarding disease progression and survival.

Current treatment options in chronic lymphocytic leukemia

The management of CLL patients is determined by the stage and activity of the disease. There is no evidence that cytotoxic therapy has beneficial effects in patients with the indolent form of the disease [7]. However, patients with symptomatic and/or progressive disease should be immediately treated [8]. Widely accepted guidelines for the initiation of chemotherapy in CLL patients have been proposed by the National Cancer Institute Sponsored Working Group (NCI-WG) [8,9]. According to these guidelines, the criteria for the initiation of therapy may not be identical for routine clinical practice and for patients included in clinical trials.

In the past 20 years, the purine nucleoside analogs (PNAs) fludarabine (FA), cladribine (2-CdA, 2-chlorodeoxyadenosine) and pentostatin (DCF, 2'-deoxycoformycin) have been introduced for treatment of CLL [10]. Significantly higher overall response (OR), complete response (CR) and longer progression-free survival (PFS) in patients with CLL treated with FA or 2-CdA have been confirmed in randomized, multicenter trials, and more recently in meta-analysis [11–15]. However, the median survival time did not differ between patients treated

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with PNA and alkylating agents. Combination therapies with PNA and cyclophosphamide (CY) are more active than monotherapy in terms of OR, CR and PFS [16–19]. However, higher overall response OR, CR and PFS do not translate to longer overall survival.

Over the last few years, several monoclonal antibodies (mAbs) and immunotoxins have been investigated in clinical trials in patients with CLL [20–24]. There are two antibodies that have the most important clinical value in patients with CLL at present. The first is the human–mouse antibody rituximab (Rituxan®, Mabthera®) that targets the CD20 antigen [23]. The second is alemtuzumab (Campath-1H), a humanized form of a rat antibody active against CD52 [24]. O'Brien *et al.* treated CLL patients with an initial dose of rituximab 375 mg/m^2, which was then increased to a fixed dose of between 500 and 2250 mg/m^2 once-weekly for 4 weeks [25]. The OR rate was 36% and ranged between 22 and 75%. All responses were partial responses (PR). Median time to progression in responded patients was 8 months, with the longest remission duration being 15+ months. In the study performed by Byrd *et al.*, 83 previously treated CLL patients were treated with different doses of rituximab (250–375 mg/m^2) three-times weekly for 4 weeks [26]. The OR rate was 45% (3% CR and 42% PR). Responses were noted in all groups of patients, including those with bulky lymphadenopathy and those for which alkylator and/or FA-based therapy had failed.

Alemtuzumab is a humanized therapeutic mAb that recognizes the CD52 antigen, expressed on normal and neoplastic lymphocytes, monocytes and natural killer cells. In 2001, alemtuzumab was approved in the USA and Europe to treat patients with CLL refractory to FA. Alemtuzumab is highly active in previously treated patients with CLL [27,28]. The effectiveness of this mAb in patients with CLL resistant to conventional treatment was first reported in 1997 by Osterborg *et al.* [27]. They found an OR rate of 43% in 29 patients, with a CR in 4% of patients with relapsed or refractory CLL. The median duration of response was 12 months. In 36% of patients, a CR was obtained in the bone marrow, and in 32% splenomegaly completely resolved. However, resolution of lymphadenopathy was observed only in 7%, and bulky lymphadenopathy did not respond to therapy.

Several reports have confirmed significant activity of alemtuzumab in relapsed or refractory CLL. Keating *et al.* [28] investigated the efficacy and safety of alemtuzumab in 93 patients with relapsed or refractory CLL exposed to alkylating agents, and having failed previous FA therapy. The OR rate was 33%, including a CR of 2% and PR of 31%. The median response duration was 8.7 months. Overall median survival was 16 months and median survival for responders was 32 months. The results of other studies in smaller groups of previously treated CLL patients have also been published. In different studies, the OR rate ranged from 31 to 60%, and the CR rate from 0 to 31% [29–32]. In the majority of studies, anti-tumor effects of alemtuzumab were more significant in blood and bone marrow than in lymph nodes. Alemtuzumab has also been investigated as first-line therapy in CLL. In a pilot study reported in 1996 by Osterborg *et al.* [33], nine patients with progressive, previously untreated CLL were included. Alemtuzumab was administered subcutaneously or intravenously. The OR rate was 89%, which included CR in three and PR in five patients.

In 2002, a prospective randomized Phase III trial (CAM 307) comparing high-dose chlorambucil with alemtuzumab in front-line treatment of progressive CLL was commenced [34]. In this trial, 279 patients were randomized, 149 patients received alemtuzumab 30 mg three-times per week for up to 12 weeks, and 148 patients received chlorambucil 40 mg/m^2 every 28 days for a maximum of 12 months. PFS was superior for alemtuzumab, with a 42% reduction in risk of progression or death ($p = 0.0001$), and median time to alternative treatment of 23.3 months compared with 14.7 months for chlorambucil ($p = 0.0001$). The overall response rate was 83% with alemtuzumab, including 24% CR, and 55% with chlorambucil, including 2% CR ($p < 0.0001$). Moreover, elimination of minimal residual disease occurred in 31% (11 of 36 of complete responders to alemtuzumab and none to chlorambucil). Adverse events were similar in both arms, with the exception of infusion-related and CMV events for alemtuzumab. Recent analysis indicates that CMV reactivation occurred in 15–25% of alemtuzumab-treated refractory or relapsed CLL. At present, aggressive anti-infective prophylaxis is a mandatory procedure.

In patients with CLL deletion, 17p13 is an independent prognostic factor identifying patients with rapid disease progression and short survival time in multivariate analysis [35,36]. The deletion 17p affecting the *TP53* gene has been associated with failure after treatment with alkylating agents, purine analogs and rituximab [37–39]. Lozanski *et al.* who treated 36 patients with FA refractory B-cell CLL (B-CLL) with

alemtuzumab, including 15 patients with *p53* mutations or deletion, reported clinical responses in six patients (40%) with *p53* mutations, deletion or both [29]. Subsequently, interim analysis of the CLL2H trial [40] and the study of Osuji *et al.* [41], confirmed the efficacy of alemtuzumab in patients with p53 abnormalities. Stilgenbauer *et al.* reported the responses (CR or PR) in seven of 13 (53.8%) cases with 17p abnormalities [40]. Similarly, Ossuji *et al.* observed OR in four out of eight (50%) patients with *p53* deletion [41]. Thus, alemtuzumab might be a more rational initial treatment choice for patients with these abnormalities.

Monoclonal antibodies and chemotherapy have synergistic activity in patients with CLL [42,43]. Several recent reports suggest that in patients with CLL, rituximab combined with PNA can increase the OR and CR rates and prolong PFS as compared with PNA or rituximab alone, with acceptable toxicity [44–50]. The addition of rituximab to a variety of chemotherapy regimens for the treatment of patients with CLL has yielded promising results in Phase II and III trials.

The combination of rituximab with FC (R-FC regimen) demonstrated particularly high rates of overall response, CR, PFS and overall survival in previously untreated and relapsed/refractory CLL [47,48]. In order to validate this concept, the German CLL study group (GCLLSG) initiated a multicenter, multinational Phase III trial, CLL8, to evaluate the efficacy and tolerability of R-FC versus FC for the first-line treatment of patients with advanced CLL [44]. In this study, 817 patients were randomly assigned to receive six courses of either FC or R-FC. At the time of analysis (June 2008) the median observation time was 25.5 months. The OR rate was significantly higher in the R-FC arm (95%) compared with the FC arm (88%) ($p = 0.001$). The CR rate of the R-FC arm was 52% as compared with 27.0% in the FC arm ($p < 0.0001$). PFS was 76.6% at 2 years in the R-FC arm and 62.3% in the FC arm ($p < 0.0001$). There was a trend for an increased overall survival rate in the R-FC arm (91 vs 88% at 2 years; $p = 0.18$).

In the REACH trial, 552 relapsed or refractory patients from 17 countries were randomized (1:1) to receive either R-FC or FC [48]. A median of one prior treatment had been administered, consisting of single-agent alkylator therapy (66%), purine-analogs (16%) or combination treatments (cyclophosphamide, doxorubicin, vincristine and prednisolone [CHOP], cyclophosphamide, vincristine, prednisone [COP], F-containing, 18%). Patients with prior FC combination treatment or prior rituximab were not eligible. The median observation time was 25 months. The primary end point of PFS was significantly prolonged by a median of 10 months in the R-FC arm (30.6 months), compared with FC (20.6 months; $p = 0.0002$). Secondary end points showed similar results. Overall response rate was higher for R-FC versus FC (70 vs 58%; $p = 0.0034$), due to superior CR rates (24 vs 13%; $p = 0.0007$). Grade 3/4 adverse events were higher in the R-FC arm (80%) versus FC (74%), but serious adverse events were similar (50 vs 48%, respectively). Grade 3/4 neutropenia and febrile neutropenia were only marginally increased for R-FC (42 and 15%) versus FC (40 and 12%, respectively), and the same was seen for thrombocytopenia (R-FC 11% vs FC 9%). Grade 3/4 infections (R-FC 18%, FC 19%) were similar, and there was no difference in bacterial, viral or fungal infections between the two arms. Grade 3/4 anemia was slightly increased in the FC arm (R-FC 2%, FC 5%).

FC ± rituximab is now the standard first-line therapy in younger patients with symptomatic and/or progressive CLL. Moreover, several studies have confirmed significant activity of these agents in relapsed or refractory CLL. Management decisions are more difficult in frail, elderly patients because of the apparent increase in toxicity of PNAs, especially in combination with CY and rituximab. In this patient population, chlorambucil is still accepted as the first-line treatment [51–53]. Chlorambucil, with or without steroids, gives the initial response rate of 60–90%, with a CR in up to 20% of all patients, depending on the dose and response criteria [52]. Other alkylating agents have been less extensively investigated than chlorambucil, and mainly in combination therapy. CY has a similar activity to chlorambucil, and is usually used when chlorambucil is poorly tolerated. CY was more frequently used in combination therapy with vincristine and prednisone (COP, CVP) or with doxorubicin, vincristine and prednisone (CHOP) [51].

Bendamustine is an agent with alkylating and purine-like qualities [54]. This agent seems to have a low cross-resistance with alkylating agents and FA. Various doses and treatment schedules have shown significant efficacy and acceptable toxicity in previously treated patients. The dose of 70 mg/m^2 on days 1 and 2 every 4 weeks is suggested in heavily pretreated and treatment-refractory patients [54,55]. Recently, the safety and efficacy of bendamustine were also confirmed in an open-label, randomized, comparative trial [56].

In March 2008, bendamustine was approved by the US FDA for the treatment of previously untreated patients with CLL. However, efficacy of this agent, relative to first-line therapy with FA and other first-line therapies than chlorambucil, has not been investigated.

Novel mAbs potentially useful in CLL

Over the last few years, several new mAbs directed against lymphoid cells have been developed and investigated in preclinical studies and clinical trials [43]. Some of these are highly active in chronic lymphoid malignancies and are potentially useful in the treatment of CLL.

New mAbs directed against CD20 include the human mAb ofatumumab (HuMax CD20™), GA-101 and IMMU-106 (hA20) [57–59]. These agents are highly cytotoxic against B-cell lymphoid cells, and are evaluated in lymphoid malignancies.

GA-101 (Hoffman La Roche, Basel, Switzerland) is a novel third-generation, humanized anti-CD20 IgG1 mAb [57,60]. GA-101 has a significantly increased antibody-dependent cytotoxicity (ADCC), reduced complement-dependent cytotoxicity (CDC) and exhibits superior caspase-independent apoptosis induction in comparison with rituximab [60]. In this Phase I/IIa study, GA-101 was administered as a single agent to 24 patients with $CD20^+$ malignant disease for whom no therapy of higher priority was available [57]. Patients have been treated with GA-101 at doses from 50 to 2000 mg. Antibody has shown a similar safety profile to rituximab, and promising efficacy in this difficult-to-treat patient population. Based on this data, GA-101 mAb is a promising therapeutic agent for CD20-positive B-cell lymphoid malignances, including CLL.

IMMU-106 (hA 20; Immunomedics, Inc., NJ, USA) is a new humanized anti-CD20 mAb evaluated to elucidate its action as an anti-lymphoma therapeutic [59,61,62]. The mechanism of cytotoxicity of IMMU-106 is similar to rituximab and includes direct apoptosis, ADCC and CDC. A Phase I/II dose-escalation study of IMMU-106 was performed in 55 patients with relapsed/refractory non-Hodgkin lymphoma (NHL), including 37 follicular lymphomas (FLs) and 18 other B-cell lymphomas [59]. A total of 39 patients with at least 12 weeks follow-up had one or more responses evaluated by the Cheson's criteria, with all CR occurring in FL and one with marginal zone lymphoma (MZL). These results may indicate that IMMU-106 is at least as effective as rituximab, and further studies in B-cell lymphoid malignancies should be undertaken.

Epratuzumab (Immunomedics, Inc.) is a humanized anti-CD22 mAb currently in clinical trials for the treatment of NHL and autoimmune disorders [63–65]. Epratuzumab is selectively active against normal and neoplastic B cells. The single-agent activity of epratuzumab was assessed in Phase I/II trials in patients with NHL [65]. Overall, 24% of patients showed an objective response in the diffuse large B-cell lymphoma (DLBCL) group. However, there was no objective response among the group of 12 patients with small lymphocytic lymphoma (SLL). Other studies demonstrated higher efficacy of epratuzumab in combination with rituximab. However, further studies are needed to elucidate the role of epratuzumab in the treatment of CCL.

Lumiliximab (anti-CD23 mAb) is a genetically engineered macaque–human chimeric anti-CD-23 $IgG_{1-\kappa}$ mAb [66]. This mAb binds specifically to CD23 and induces ADCC, CDC and apoptosis. The CD23 antigen is a cell-surface protein considered one of the most useful markers for identifying CLL [67]. In a Phase I study, the safety, efficacy and pharmacokinetics of lumiliximab were evaluated in 46 patients with refractory/relapsed CLL [68]. Reductions in absolute lymphocyte counts and lymphadenopathy were observed in 42 of 46 (91%) patients. This study has shown that lumiliximab is clinically active and safe in pretreated patients with CLL.

Galiximab (B 7.1) (Biogen Idec Inc., CA, USA) is a macaque–human chimeric anti-CD80 monoclonal antibody with human IgG1 constant region and macaque variable region, structurally indistinguishable from human antibodies [69]. Promising results have been obtained in the *in vitro* and *in vivo* studies evaluating the anti-tumor activity of galiximab in lymphoma models, especially if this mAb was combined with chemotherapeutic agents, such as FA and doxorubicin [70,71].

CD40, a member of the TNF receptor super family is highly expressed in a variety of B-cell malignancies including CLL [72]. A fully human anti-CD40 mAb HCD122 (Abgenix, Inc., CA, USA), formerly known as CHIR-1212, has been recently selected based on its inhibition of CD40L-induced biological sequelae [73]. HCD122 can mediate ADCC *in vitro* and has antiproliferative and anti-tumor activities as a single agent in CLL, multiple myeloma and NHL [74]. Recently, Byrd *et al.* reported a Phase I trial of HCD122 in 14 patients with relapsed/refractory CLL after FA treatment [75]. This study has shown that HCD122 is safe and well-tolerated up to the 3 mg/kg dose

level. SGN-40 is another anti-CD40 IgG1 mAb that induces cytotoxicity against CLL cells [76]. SGN-40 induces apoptosis and mediates ADCC against $CD40^+$ NHL B-cell lines, contributing to *in vivo* anti-tumor activity observed in human lymphoma xenograft models.

Ofatumumab: mechanism of action & pharmacokinetics

Ofatumumab is an IgG_{1-K} human anti-CD20 mAb that binds human B cells [58,77–80]. Ofatumumab is produced with a recombinant murine cell line (NSO) using standard mammalian cell cultivation and purification technologies [81]. The NSO cell line has been transfused with a GS vector carrying the antibody genes derived from the human anti-CD20 hybridoma cell line (2F2) generated via transgenic mouse technology [80,81]. HCo7 and KM mice were immunized with human CD20-transfected NS/0 cells [82]. For priming, mice received 1×10^7 CD20 transfected cells in complete Freund adjuvant (CFA) intraperitoneally, and were boosted with cells in phosphate-buffered saline. After development of positive CD20 antibody titer, mice received a final intravenous boost of 0.5×10^7 cells 3 days prior to fusion [82]. The molecular weight of the antibody is 149 kDa as determined by mass spectrometry [81].

Ofatumumab specifically recognizes a distinct epitope encompassing both the small and large extracellular loops, and binds specifically to the amino acid residues 159, 163 and 166 in the large extracellular loop of the CD20 molecule. The epitope is therefore distinct from the binding site recognized by other CD20 mAbs, such as rituximab [81,83]. Rituximab had an absolute requirement for alanine and proline at position 170 and 172, respectively, within the large extracellular loop of CD20. In contrast, ofatumumab recognizes a novel epitope located N-terminally of this motif in the small extracellular loop of CD20 that is not bound by rituximab. The recognition of this epitope seems to be closely linked with C1q capture and CDC potency [83]. In *in vitro* experiments, ofatumumab was able to lyse a range of rituximab-resistant targets, such as primary CLL cells, in the presence of human plasma or unfractionated blood [81]. The close binding proximity of ofatumumab to the cell membrane likely results in highly efficient complement deposition on B-cell membranes, without high levels of systemic release of activated complement components [83].

Recent laboratory studies confirmed previous observations that ofatumumab is more effective than rituximab at CDC and killing of the target cells [84–87]. Ofatumumab was more effective than rituximab in inducing CDC in nine of ten DLBCL tumor samples obtained from chemotherapy-refractory DLBCL patients [80]. In this experiment, the lethal dose (LD_{50}) for ofatumumab was 0.1 ± 2.8 µg/ml and 6.4 ± 4.9 µg/ml for rituximab. Moreover, ofatumumab-induced CDC of DLBCL cells was less sensitive to expression of complement defense molecules CD55 and CD59 than CDC induced by rituximab. Importantly, the sensitivity to ofatumumab-induced CDC appeared to also be independent of expression of Bcl-2 and XIAP. The real-time dynamics of complement activation promoted by ofatumumab and rituximab on CD20-positive Daudi and ARH77 has also been investigated [85]. Both mAbs activated complement and induced significant changes of the cells morphology, including rapid blebbing, and long string-like structures (referred to as streamers) were cast off the cells. Interestingly, ofatumumab readily promoted complement activation, C3b deposition and killing of ARH77 cells, whereas rituximab-mediated C3b deposition was lower, and killing was close to background levels in comparison with ofatumumab [87,88]. Maximum deposition of C3b fragments on ARH77 cells was observed more rapidly for ofatumumab (30 sec) than for rituximab (5 min) [86]. In line with this observation, binding of ofatumumab to ARH77 cells generated streamers five- to ten-fold more than was observed for cells opsonized with rituximab. In addition, ofatumumab is able to effectively induce ADCC [89].

Pharmacokinetic parameters were evaluated in patients with relapsed or refractory FL and CLL in Phase I/II studies [58,90,91]. There were four cohorts of patients with FL who received four weekly intravenous infusions of 300, 500, 700 or 1000 mg of ofatumumab [90]. The median C_{max} values per dose group were 129, 185, 380 and 610 µg/ml, respectively. The $t_{½}$ values were calculated as 447, 245, 322 and 621 h. Plasma clearance values were 9, 19, 10 and 7 ml/h/kg, with AUC values of 75,000, 51,000, 18,500 and 32,6000 h/µg/ml, respectively. A correlation between exposure (AUC and C_{max}) and clinical response to treatment at weeks 19 and 26 was not found. For other pharmacokinetic parameters ($t_{½}$, volume of distribution [Vz], clearance [Cl]), correlation to responses was only found for clinical response at week 26 and $t_{½}$ and Cl ($p < 0.05$) [90].

The pharmacokinetics of ofatumumab has also been investigated in CLL patients [58,91]. In this study, a significant corelation between time

to progression and time to the next anti-CLL therapy with AUC (p = 0.005 and p = 0.001, respectively) and Cl (p = 0.003 for both correlations) was found. Comparing the clinical pharmacokinetic data of ofatumumab and rituximab, it can be seen that ofatumumab appears to have a longer half-life. In a study of patients who received rituximab 375 mg/m^2 once-weekly for four doses over 22 days, rituximab serum concentration increased over the treatment course, with the median concentration being 464.7 µg/ml after the fourth infusion of antibody (the maximum concentration measured as 996.6 µg/ml) [89]. The half-life increased from 76.3 h after the first infusion to 205.8 h after the fourth infusion, alongside a decrease (fourfold) in the clearance (from 38.2 to 9.2 ml/h). From this, it can be seen that ofatumumab appears to have a longer half-life, although the doses of the antibodies vary and need due consideration for comparative purposes.

Efficacy of ofatumumab in CLL patients

Ofatumumab has been investigated in a Phase I/II study in 33 CLL patients with refractory or relapsed diseases [59]. Median age was 61 years (range 27–82). Median time from diagnosis to ofatumumab therapy was 6.3 years (range 1.2–14). Median number of prior treatments was three (range 1–9) and 67% of the patients were Binet stage B. Three cohorts of patients with relapsed or refractory CLL received 4 weekly intravenous infusions of ofatumumab. The first infusion was 100, 300 and 500 mg in cohort A, B and C, and subsequent doses were 500, 1000 and 2000 mg, respectively. Maximum tolerated dose was not reached. All patients had significant reduction in leukemia cells. Moreover, several flow cytometry results showed that ofatumumab caused rapid and prolonged depletion of normal B lymphocytes in all patients. Their recovery to normal levels was not observed until 5–6 months after completion of therapy. The objective response rate was 50% (13 out of 26) in cohort C. All responses were a PR, except for one nodular partial response in cohort C. There was no CR of the 33 patients previously treated with rituximab (7), alemtuzumab (6) and/or FA (20). However, not all of these responses were sustained. At week 19, only nine patients were in sustained response. The median time to progression was 133 days, and the median duration of response was 112 days. Time to the next antileukemic therapy was 366 days [91]. This Phase I/II study indicates that ofatumumab is an active and well-tolerated agent in refractory/relapsed CLL.

Subsequently, an international pivotal trial of ofatumumab in patients with CLL refractory to both FA and alemtuzumab, or refractory to FA with bulky lymphadenopathy (>5 cm) has been undertaken (Hx-CD20-406 Phase III study) [92]. Patients with FA-refractory CLL and refractory to alemtuzumab or ineligible for alemtuzumab due to bulky lymphadenopathy remain difficult to treat, and outcomes with current salvage regimens remain poor. In addition, no standard treatments are available for this patient population [93]. Patients in the ongoing pivotal trial receive ofatumumab in 8 weekly infusions followed by 4 monthly infusions for a total of 12 planned doses (dose 1: 300 mg; doses 2–12: 2000 mg). The primary end point is OR rate assessed by an Independent Review Committee over a 24-week period. This interim analysis included 59 patients refractory to FA and alemtuzumab (FA and alemtuzumab refractory; FA-ref) and 79 patients refractory to FA with bulky lymphadenopathy (bulky FA-refractory; BF-ref). The preliminary results reported in the abstract form demonstrate promising efficacy of ofatumumab monotherapy in a heavily-pretreated patient population with FA-refractory CLL [92]. Full efficacy results from this trial are awaited.

Safety profile & side effects

The majority of side effects in CLL and NHL patients were related to the first infusion of the drug, and their number and severity decreased at each subsequent infusion [58,90]. These included grade 1–2 pruritus, dyspnea, rigors/chills, sweating, nausea, hypotension, urticaria, fatigue, pyrexia, headache and rash, and were relative to infusion. These probably resulted from the cytokine-release syndrome, which is a symptom complex associated with the use of many mAbs [94]. It results from the release of cytokines from cells targeted by the antibody. When cytokines are released into the circulation, systemic symptoms such as fever, nausea, chills, hypotension, tachycardia, asthenia, headache, rash, scratchy throat and dyspnea can result [94]. The severity of these symptoms can be partially reduced by the use of premedication with antihistamines and corticosteroids [94].

In 33 CLL patients receiving multiple doses of ofatumumab, the dose schedule was found to be well-tolerated in patients with CLL in doses up to 2000 mg [58]. There were a total of 246 adverse events reported in 27 patients, with 150 of these being considered related to treatment. There were 92% mild (grade 1–2) and

19 grade 3–4. There were 10 serious adverse events in nine patients throughout the study being treatment related: grade 3 herpes zoster (n = 1), grade 4 neutropenia (n = 2), infectious interstitial lung disease (n = 1; fatal at week 4) and grade 3 hepatic cytolysis (n = 1; after the first infusion, this patient had increased liver enzymes before therapy and completely recovered after 3 days of therapy). The serious adverse events that were considered to not be drug-induced included angina pectoris (n = 1), carotid artery stenosis (n = 1), grade 3 pneumonia (n = 1), grade 2 sinusitis (n = 1) and grade 2 hemolytic anemia (n = 1). There were 17 (51%) patients who had infections, 88% of these grade 1–2. Of these, the most frequent was nasopharyngitis (n = 8, with 1 being grade 3). Other nonrelated hematological toxicities included two additional neutropenias and two worsenings of thrombocytopenias to grade 3 [58]. In addition, CLL patients receiving up to 2000 mg ofatumumab were negative for the presence of human antihuman antibodies [48]. Furthermore, no dose-limiting toxicities were reported, with up to 2000 mg ofatumumab. No patient tested developed antibodies to ofatumumab. In the preliminary data from the 406 Hx-CD20-406 Phase III study, the safety profile of ofatumumab appeared to be consistent with the prior Phase I/II study, with no unexpected toxicities [92]. Infusion-related reactions were common. These events generally subsided with subsequent infusions. Further results from this study are awaited. Contraindications to the use of ofatumumab have not yet been established.

Conclusion & future perspective

Ofatumumab is a novel, second-generation, fully human monoclonal antibody recognizing different epitopes of CD20 antigen than broadly used rituximab. Currently available Phase I/II clinical trials data suggest that this antibody is effective and relatively well-tolerated in CLL, and its efficacy appears to be comparable or better to that of rituximab used alone in patients with this leukemia. In addition, the preliminary results from the Hx-CD20-406 Phase III study suggest promising activity of ofatumumab in patients with FA-refractory disease. This mAb potentially represents an active treatment option with clinical benefit for patients with very poor prognosis who have exhausted standard treatment options. Ofatumumab is well-tolerated with no unexpected toxicities. Similarly to rituximab, the majority of related adverse events occurred at the first infusion, and the number of adverse events decreased at each subsequent infusion. However, its advantage over rituximab seems to be lack of the development of antibodies against ofatumumab in the treated patients, and higher cytotoxic potential against lymphoid cells due to superior CDC activity, at least in the *in vitro* studies.

In contrast to rituximab, data concerning activity and toxicity of ofatumumab combined with chemotherapy in CLL are not yet available. It should be noted that immunochemotherapy with rituximab is currently often used in clinical practice, and there are no data on the combination of ofatumumab and chemotherapy. However, relatively low immunogenicity and significant activity of ofatumumab in lymphoid

Executive summary

Mechanism of action of ofatumumab

- Ofatumumab is an $IgG_{1-\kappa}$ human mAb anti-CD20 reacting with human B cells.
- Ofatumumab specifically recognizes a CD20 epitope localized in the small extracellular loop distinct from the site of epitope recognized by rituximab.
- Ofatumumab is significantly more active than rituximab in complement-dependent cytotoxicity of target cells and has demonstrated equally effective antibody-dependent cytotoxicity *in vitro* as compared with rituximab.

Clinical efficacy of ofatumumab

- In a Phase I/II study in 33 chronic lymphocytic leukemia (CLL) patients with refractory or relapsed diseases, all patients had significant reduction in leukemia cells, and the objective response rate was 44%.
- In the Hx-CD20-406 Phase III study in patients with CLL refractory to fludarabine and alemtuzumab (FA-ref) or refractory to fludarabine with bulky lymphadenopathy (BF-ref), preliminary data suggests promising efficacy with high response rates.

Safety & tolerability of ofatumumab

- Data on the toxicity and safety of ofatumumab were reported in a Phase I/II study in patients with relapsed or refractory CLL. The majority of all adverse events were observed on the day of the first infusion. The most common infusion-related symptoms were transient rigors, pyrexia, fatigue, rash and increased sweating.
- Hematologic toxicities were rarely observed.
- The maximum tolerated dose of ofatumumab has not been reached in the studies performed so far.
- Contraindications to the use of ofatumumab have not yet been established.

malignancies indicate that this agent should be further investigated in combination with alkylating agents and/or purine nucleoside analogs to elucidate its potential advantage over rituximab in immunochemotherapy of these disorders. The encouraging single-agent activity in patients with refractory CLL warrants further investigation of ofatumumab in earlier disease settings, in combination with other agents and as maintenance therapy. Such data should come from direct comparison of ofatumumab and rituximab in well-designed, randomized, multicenter Phase III studies.

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