Expert Opinion

Ofatumumab, a second-generation anti-CD20 monoclonal antibody, for the treatment of lymphoproliferative and autoimmune disorders

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Background: Lymphoproliferative and autoimmune disorders share monoclonal dysregulation and survival advantage of B-lymphocytes. Thus, therapies directed towards eliminating B-cells will play an important role as CD20 is exclusively expressed in B-lymphocytes and its modulation by monoclonal antibodies such as rituximab has improved outcomes in lymphoproliferative and autoimmune disorders. Ofatumumab is a new, fully human anti-CD20 antibody and has been shown to be effective and safe, but its role in these conditions is still unclear. *Objectives*: To describe the preclinical and clinical data available on ofatumumab for the treatment of lymphoproliferative and autoimmune disorders. *Methods*: An extensive search of published articles and abstracts on preclinical and clinical studies with ofatumumab was undertaken. *Conclusions*: Ofatumumab is a second-generation anti-CD20 antibody that has been demonstrated to be safe and efficacious in patients with lymphoproliferative and autoimmune disorders. Ofatumumab is fully human, attaches to a newly identified epitope and shows lower off-rates and improved complement-dependent cytotoxicity. Initial data present ofatumumab as an attractive agent with lower rates of infusion-related events than rituximab. Ongoing Phase III trials in patients with follicular lymphoma, chronic lymphocytic leukemia and rheumatoid arthritis are ongoing, and Phase II trials in patients with aggressive lymphoma and multiple sclerosis are also under development.

Keywords: autoimmune disorders, CD20, chronic lymphocytic leukemia, lymphoma, monoclonal antibody, multiple sclerosis, non-Hodgkin's lymphoma, ofatumumab, rheumatoid arthritis

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1. Introduction

B-lymphocytes play an important role in the development of lymphoproliferative and autoimmune disorders (LPDs and AIDs, respectively). Briefly, LPDs are characterized by a monoclonal proliferation of malignant B- or T-lymphocytes, in which a combination of increased proliferation, survival advantage and/or decreased apoptosis can be observed [1]. In a similar fashion, AIDs are characterized by B-lymphocyte dysregulation, which translates into increased activation and development of uncontrolled self-recognition properties [2]. Furthermore, there is a bidirectional association between these disorders, since patients with LPDs often present with AIDs (e.g, lymphoma patients can develop autoimmune hemolytic anemia) [3] and patients who suffer from AIDs have an increased risk of developing LPDs (e.g., the risk of developing lymphoma in patients with

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systemic lupus erythematosus is 7 times higher than in the general population) [4]. Based on this evidence, drugs directed towards eliminating the malignant or autoimmune clones of B-lymphocytes, such as anti-CD20 monoclonal antibodies, can be of great therapeutic value in these disorders.

Non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) represent two distinct subcategories of malignant LPD, which originate from clonal proliferation of malignant lymphocytes. NHL is the most predominant adult hematologic malignancy in the USA with 66,000 anticipated cases in 2008 [5]; 85% of these cases will arise from a malignant B-lymphocyte. The most common subtypes are diffuse large B-cell (DLBCL) and follicular lymphoma (FL). CLL, on the other hand, is the most prevalent LPD in the USA with 95,000 cases [6]. CLL is characterized by the accumulation of neoplastic $CD5^+/CD19^+$ B-lymphocytes. Fifty per cent of cases will be largely asymptomatic and will not need therapy. DLBCL is considered a curable disease; but FL and CLL are incurable with current standard therapies. AIDs comprise a constellation of conditions, such as rheumatoid arthritis (RA) and multiple sclerosis (MS). RA has an annual incidence of 30 cases per 100,000 population; more than a million Americans live with RA [7]. This condition, which is characterized by the development of painful, swollen joints and the presence of anticyclic citrullinated peptide antibodies, can be disabling and has a great impact on quality of life and productivity as the joint damage becomes permanent with time, decreasing mobility. MS is the most common autoimmune demyelinating disease of the central nervous system, affecting more than 300,000 people in the USA [8]. It is twice as common in females as in males, with a peak incidence at the age of 35 years but does not affect lifespan substantially [9]. The most common form of MS is relapsing-remitting MS (RRMS), characterized by unpredictable relapses followed by either complete, partial or no neurological recovery.

In 1997, the advent of rituximab (Rituxan®, Genentech, South San Francisco, CA, USA), a chimeric anti-CD20 monoclonal antibody (MAb), dramatically altered the foundation for the treatment of NHL. Rituximab is approved as both first-line treatment for aggressive and indolent subtypes of NHL (DLBCL and FL, respectively), and for relapsed or refractory, indolent or follicular, CD20-positive NHL. Clinical data reported by multiple investigators demonstrated increased response rates and prolonged survival times with rituximab in combination with chemotherapy in patients with DLBCL [10-12] and FL [13,14]. Similarly, the clinical benefits of rituximab can be seen in patients with CLL when added to other chemotherapeutic agents or, to a lesser degree, as single agent [15,16]. Of note, rituximab is not yet approved by the FDA to treat CLL but it is the most commonly used MAb for this condition. The treatment for RA is based in nonspecific modulation of B-cells by corticosteroids, methotrexate (MTX) and anti-TNFα MAb. Rituximab obtained FDA approval for the treatment of RA after anti-TNFα failure in 2006 based on a Phase III study showing that the combination of rituximab and MTX improved responses in comparison to MTX alone based on American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) response criteria [17]. The dosing regimen for RA is different than those used to treat lymphoma. For MS, multiple therapies are available, such as interferon, glatiramer acetate, mitoxantrone and natalizumab, a humanized anti-CD74 MAb. The use of rituximab in MS is limited to small trials but with hopeful results [18].

The premise of MAb activity centers upon modulating new functional receptors in malignant or autoimmune cells to augment therapeutic efficacy. The actual mechanisms of action of MAb are unknown but, theoretically, their function is elicited by inducing complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and/or direct apoptosis [19]. Nonetheless, inherent limitations arise with rituximab as resistance ensues in patients with low-grade NHL and CLL and responses are less potent and shorter in duration [20,21]. Similarly, the efficacy of second-line therapy with rituximab is unclear in DLBCL patients who failed rituximab as front-line therapy [10]. Moreover, rituximab has been linked to life-threatening infusion reactions, hepatitis B reactivation, tumor lysis syndrome, severe mucocutaneous reactions and progressive multifocal encephalopathy [22,23]. Thus, it is paramount to discover and implement a new generation of MAb as a viable treatment entity to achieve long-term remission while decreasing the rate of therapy-related adverse events.

2. Synthesis

Ofatumumab (HuMax-CD20; GlaxoSmithKline, Collegeville, PA, USA and Genmab, Copenhagen, Denmark) is an IgG1, fully human, second-generation anti-CD20 MAb with a molecular weight of approximately 150 kDa. Ofatumumab was produced by immunizing HCo7 and KM mice with a murine cell line (NS/0) transfected with human heavy- and light-chain genes. The hybridoma was created by fusing B-cells from immunized mice and NS/0 cells. CD20-specific IgG1-producing hybridomas were then sequenced and subcloned. Unique features to ofatumumab compared with rituximab are a different binding site, prolonged release time from the target site and stronger CDC activity. Ofatumumab is now under clinical development for indolent and aggressive NHL, CLL, RA and MS with promising results.

3. Preclinical development

Elegant work from Teeling and colleagues [24] defined a series of three fully human anti-CD20 MAbs (ofatumumab, 7B8 and 11B8) with distinct characteristics. The first two were designated as type I anti-CD20 MAbs (rituximab-like), given their ability of translocating and concentrating CD20 molecules into detergent-insoluble lipid rafts and inducing CDC. The

MAb 11B8 was designated as type II (tositumomab-like) given its greater ability of eliciting ADCC and apoptosis. By separately using plasma and polymorphonuclear and mononuclear cells as 'effector fractions', ofatumumab was able to elicit cell killing when incubated with plasma alone in the absence of effector cells, suggesting ofatumumab to be a strong CDC inducer. Furthermore, the CDC effect was blunted after heat-induced complement inactivation. The addition of polymorphonuclear fraction mildly increased cell-killing rates but addition of mononuclear fraction did not, suggesting ofatumumab is a weak ADCC inducer. As part of the same experiment, CLL tumor cells, which are relatively rituximab-resistant given their low CD20 expression, were exposed to ofatumumab demonstrating cell-killing properties with plasma alone; rituximab showed no activity. Confirming this finding, rituximab and ofatumumab were effective against SU-DHL4 cells, which are characterized by high CD20 expression but, after exposure to Raji cells, which show low CD20 expression but increased expression of complement regulatory proteins (i.e., CD55 and CD59), only ofatumumab demonstrated activity. In addition, rituximab showed similar on-rates (maximal levels of binding in less than 15 min) but faster off-rates than ofatumumab in Ramos and Daudi cell lines. By means of radiolabeled immunoglobulin in DOHH cells, ofatumumab showed 70% binding after 3 h of incubation compared with approximately 30% binding of rituximab. After 6 h of exposure, rituximab-induced CDC levels were lower than ofatumumab (50% vs 90%, respectively) suggesting delayed off-rates were associated with maintenance of the CDC activity.

In a second study by Teeling and colleagues [25] rituximab did not have activity unless there were at least 30,000 CD20 molecules per cell and did not achieve full cell lysis even in cells with the highest expression of CD20. By contrast, ofatumumab began showing activity at CD20 concentrations of 4500 molecules per cell and achieved full lysis of any cell line expressing more than 60,000 molecules per cell. Additionally, by using a new human anti-CD20 MAb, 2C6, with faster dissociation rates than rituximab, it was demonstrated that faster off-rates were not always associated to weaker CDC activity since 2C6 was a stronger CDC inducer than rituximab. The group postulated that ofatumumab induced stronger CDC by recognizing a different form of CD20 or a different epitope. Rituximab and other murine anti-CD20 MAbs bind to an epitope that contains an alanine residue in position 170 (A170) and a proline in position 172 (P172). By mutating these residues, binding of rituximab to CD20 was effectively blocked but the mutations failed to block the binding of ofatumumab. Pepscan epitope mapping and ELISA testing were used to assess the reactivity of the different anti-CD20 MAbs against the sequenced potential epitopes. As expected, rituximab bound to the A170/P172 epitope, but ofatumumab bound to peptides located in the small extracellular loop, N-terminal from A170/P172. It is likely that the greater proximity of the new epitope to the cellular membrane plays a role in achieving stronger CDC activity.

Beum and colleagues [26], using a spinning disk confocal microscopic analysis, were able to demonstrate the structural changes different lymphoma cell lines undergo after opsonization with rituximab and ofatumumab in the presence of complement. In Daudi cells, larger deposition of C3b was observed in the ofatumumab-opsonized cells followed by membrane 'blebbing'. After addition of normal human serum, thin protruding structures called 'streamers' were identified. Ofatumumab produced streamers in 114 s while they were seen after 418 s with rituximab. Membrane blebbing and streamer formation are directly associated with anti-CD20 MAb complement-induced cell death since addition of EDTA, which chelates Mg^{++} and Ca^{++} and blocks complement activation; and lack of C5 and C9 blunted death of nucleated cells. In ARH77 cells, a rituximab-resistant cell line with high levels of CD55 and CD59, C3b deposition, membrane blebbing, streamers and cell killing were observed with ofatumumab; rituximab was not able to promote CDC activity after several hours of incubation. Even in CLL cells, ofatumumab showed a higher ability of inducing streamer formation than rituximab, although the killing rate was similar for both MAbs owing probably to the small sample size.

Recently, Bleeker and colleagues [27] published a series of experiments directed to evaluate *in vivo* the dose requirement for activity of ofatumumab. Initially, using Daudi cells, EC_{50} values for ADCC and CDC were 0.02 and 0.13 ug/ml, respectively and maximal levels of ADCC (51% cell lysis) were obtained with 50% target saturation while maximal CDC levels (68% cell lysis) required full target saturation. *In vitro* target saturation was achieved at levels of 5 ug/ml and no further increase in CDC or ADCC was observed with higher levels of ofatumumab. To validate these findings, a xenograft model was constructed using female severe combined immunodeficient (SCID) mice, which were injected with luciferase-transfected Daudi cells allowing *in vivo* evaluation of tumor growth by bioluminescence. Ofatumumab was administered on day 5 after tumor induction as a single dose of 0.5 mg/kg. Control mice developed disease on day 13 and tumor continued growing until day 35; control mice developed clinical signs and were sacrificed. By contrast, the ofatumumab-treated mice developed disease with a delay of 3 – 4 weeks. To assess for dose–effect relationship, mice were treated with a 0.5-mg/kg single dose of ofatumumab 5 days and 14 days after tumor induction. Mice treated 5 days after tumor induction maintained an ofatumumab concentration of above 1 μg/ml throughout the experiment, while mice treated 14 days after tumor induction started at similar levels and these levels declined to 0.1 – 0.2 μg/ml by the end of the experiment, indicating that tumor burden may play a role in ofatumumab plasma concentrations. Accelerated tumor growth was observed when ofatumumab levels were below 0.4 μg/ml.

To clarify further ofatumumab efficacy, cynomolgus monkeys were used as their CD20 molecule is similar to humans with only one amino acid difference. Three different dosing regimens of ofatumumab were used (1.25, 6.25 and 12.5 mg/kg), given daily for 4 days. B-cell depletion from peripheral blood was observed immediately after ofatumumab infusion. Initial B-cell recovery was seen at 29 and 56 days with low-dose and medium-/high-dose ofatumumab, respectively. Full B-cell recovery was seen after day 96 in the low-dose group and after 136 days in the medium-/high-dose groups. Lymphatic depletion of B-cells was also achieved with ofatumumab as germinal center atrophy was observed in mandibular and mesenteric lymph nodes 2 weeks after the final dose. The authors concluded that ofatumumab concentrations of 50 ug/ml were needed to induce full B-cell depletion and concentrations of 5 – 10 µg/ml were sufficient to sustain activity.

Ex vivo experiments showed ofatumumab was more effective than rituximab in eliciting CDC in DLCBL cell lines, SU-DHL4, SU-DHL5 and HT, and in cells from 10 refractory DLBCL patients [28]; the lethal doses for ofatumumab and rituximab were 0.1 ± 2.8 and 6.4 ± 4.9 µg/dl, respectively. Furthermore, the effect of ofatumumab was less sensitive to the expression of complement regulatory proteins, CD55 and CD59, than rituximab.

4. Clinical development

4.1 Non-Hodgkin's lymphoma

4.1.1 *Phase I/II*

Hagenbeek and colleagues reported results on a Phase I/II open-label, multicenter, dose-escalating clinical trial (NCT00092274) of ofatumumab in 40 CD20-positive patients with relapsed or refractory FL [29-31]. Ofatumumab was administered in four incremental doses of 300, 500, 700 or 1000 mg i.v. once weekly for 4 weeks (n = 10 in each cohort) and followed for 1 year. Before infusion, patients received oral acetaminophen and intravenous antihistamine; in case of grade 3 or higher adverse events (AEs), i.v. glucocorticosteroids were administered. Of the 40 patients, 15 had previous rituximab exposure either as monotherapy or part of combination therapy and, of these, 4 were refractory to rituximab. All patients were evaluated for safety parameters and 37 were included for efficacy assessment. By week 19, response rates of 63,33,20 and 50% were achieved in patients receiving 300, 500, 700 and 1000 mg, respectively. From week 19 to week 26, one additional response was noted in a patient receiving 1000 mg. Five patients achieved complete response (CR), 2 unconfirmed CR (CRu) and 9 partial response (PR); stable disease (SD) and progressive disease (PD) were observed in 18 and 3 patients, respectively. In patients previously treated with rituximab, 64% responded across dose groups, with three achieving CR, one CRu, five PR, four SD and one PD. Of the four patients who were rituximab-refractory, three responded to ofatumumab treatment (1 CR, 1 CRu and 1 PR). Rapid, significant and sustained B-cell depletion was observed in evaluable patients from all dose groups until approximately 20 weeks after final treatment, with a slow recovery measured to week 54 from initiation of treatment. Interim data indicated a decrease in median baseline B-cell count from 114×10^6 cells/l to 8×10^6 cells/l in 16 available patients 1 week after the first infusion; B-cells were not detectable in eight patients. Conversion to negative BCL-2 in peripheral blood was assessed in all dose groups with a 65% conversion rate in evaluable patients across dose groups. Based on Kaplan-Meier estimates, at a median follow-up of 9.2 months, the median time to progression for all patients was 8.8 months, the median time to progression for responders was 32.6 months and the median duration of response was 29.9 months. Four patients who did not progress were being monitored at the time of publication; median time to next anti-lymphoma therapy was not reached by 12 months.

In an ongoing Phase II, open-label, randomized clinical trial, 56 previously untreated patients with FL received ofatumumab combined with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP; NCT00494780). The two dose cohorts would receive 300 mg of ofatumumab at the first infusion, followed by five infusions of either 500 or 1000 mg of ofatumumab every 3 weeks, in combination with six cycles of CHOP. Patients would be monitored at 12 weeks following the final treatment, every 12 weeks until 2 years and then every 24 weeks until 5 years or initiation of alternative treatment. The primary end point was objective response from the initiation of treatment until 12 weeks after final treatment at 30 weeks. This trial is ongoing but not recruiting patients at the time of publication.

In a Phase II, open-label, non-randomized clinical trial, 75 patients with relapsed DLBCL who were not candidates for stem cell transplantation will receive an initial 300-mg infusion followed by seven weekly 1000-mg infusions of ofatumumab (NCT00622388). Patients would be monitored every 4 weeks after final treatment and every 12 weeks thereafter for 5 years or the initiation of an alternative treatment. The primary end point was objective response rate at 24 weeks after treatment initiation. At the time of this publication, recruitment was still ongoing.

4.1.2 *Phase III*

A multicenter, international Phase III study co-chaired by Drs Hagenbeek and Czuzman and sponsored by Genmab is ongoing (NCT00394836) and will randomize 112 patients with rituximab-refractory FL to an initial 300-mg infusion followed by seven weekly infusions of either 500 or 1000 mg of ofatumumab. The primary and secondary objectives are efficacy and safety, respectively. Disease status will be assessed every 3 months to complete 24 months of follow-up.

4.2 Chronic lymphocytic leukemia

4.2.1 *Phase I/II*

In a Phase I/II, open-label, multicenter, dose-escalating clinical trial (NCT00093314), 33 patients with relapsed or refractory

CLL were treated with once-weekly infusions of ofatumumab for 4 weeks: three patients received an initial dose of 100 mg, followed by three doses of 500 mg; three received 300 mg and three doses of 1000 mg; and 27 received 500 mg and three doses of 2000 mg [32-34]. Patients were previously treated with fludarabine (n = 20), rituximab (n = 7) and alemtuzumab (n = 6) and 67% of the patients were stage B according to the Binet clinical staging scale. Patients received oral acetaminophen, intravenous antihistamine and glucocorticosteroid before ofatumumab infusion and were followed up for 12 weeks. The overall objective response (ORR) was 44%; in the high-dose group (approximately 50% of evaluable patients; 1 withdrew from hepatic cytolysis), there was one nodular PR and 12 PRs and there was one PR in the low-dose group. In the high-dose group, 62% (16 out of 26) responded to treatment on physical examination and peripheral blood examination 4 weeks after treatment; three did not respond until week 7 or 11. The duration of response varied; at week 19, nine patients still had a response. Of those, seven had received previous therapy; two maintained response until week 27 and the remaining patients had PD with a median progression-free survival (PFS) of approximately 15 weeks and time to the next antileukemic therapy was 1 year. Of seven anemic patients, six improved at the two higher doses and eight of nine thrombocytopenic patients improved across all doses. In eight high-dose patients who responded, the median percentage of bone marrow lymphocytes was 78% and 50% before and after treatment, respectively, with three who had less than 30% posttreatment but had a nodular growth pattern. There was a median 55% reduction in $CD5^+/CD19^+$ B-cells in the blood in patients receiving the high dose after the first infusion, which increased to a median of 97% after the fourth infusion; similar reductions with $CD5^+/CD20^+$ B-cells and normal B-cells were observed; these reductions were mostly sustained until week 24.

In an ongoing Phase II, open-label, randomized, parallel-group, combination clinical trial in patients with B-cell CLL (NCT00410163), 56 patients will receive a 300-mg infusion of ofatumumab, followed by five infusions of 500 or 1000 mg with fludarabine and cyclophosphamide every 4 weeks until six infusions have been administered. Patients will be assessed every 4 weeks until week 24, every 12 weeks thereafter until disease progression or by 2 years. Patients that had not progressed at this time would be monitored at 24-week intervals to complete 4 years. The primary end point is CR rate from the initiation of treatment to 12 weeks after therapy; secondary end points are duration of response, time to next anti-CLL therapy, reduction in tumor size and AEs. At the time of this publication, the study was ongoing but recruitment had stopped; the estimated completion date is 2012.

In an international, multicenter study (NCT00349349), patients with double refractory (DR) and bulky fludarabine refractory (BFR) CLL, received eight weekly infusions of ofatumumab followed by four monthly infusions (dose 1: 300 mg; doses 2 – 12: 2000 mg) [35]. DR CLL was defined by failure to therapy with fludarabine and alemtuzumab. The primary end point was ORR over a 24-week period. Overall survival (OS) and safety were also evaluated. The interim analysis included all 138 treated patients (59 DR and 79 BFR patients); 54% received all 12 infusions and 90% received 8 infusions. The ORR, based on independent review committee assessment, was 51% for the DR group and 44% for the BFR group; one patient had CR. Additionally, 39 of 51 DR patients and 43 of 44 BFR patients had SD. Median time to next CLL therapy was 9 months for the DR group and 8 months for the BFR group. Clinical progression was usually demonstrated by worsening lymphadenopathy. The median OS was about 14 months for the DR group and 15 months for the BFR group. Based upon analysis at 12 weeks, response was significantly correlated with longer survival for both groups.

Finally, exploring the concept of maintenance therapy with ofatumumab, a Phase II trial (NCT00802737) will include patients who, after receiving eight weekly doses of ofatumumab (first dose 300 mg/m^2, then 2000 mg/m^2), have achieved at least SD. Patients will continue receiving ofatumumab at 2000 mg/m^2 once monthly to complete 2 years. Primary outcome is proportion of objective responders. Duration of response, PFS, time to next CLL therapy, OS, reduction in tumor size, AEs, major infections, human anti-human antibodies (HAHA) and pharmacokinetic parameters are secondary outcomes.

4.2.2 *Phase III*

A Phase III trial (NCT00748189) has started recruiting newly diagnosed CLL patients. Patients will be randomized to chlorambucil alone at 10 mg/m^2 p.o. for 7 days every 28 days or the combination of chlorambucil and ofatumumab at 300 mg/m^2 i.v. on day 1 followed by 1000 mg/m^2 i.v. on day 8 and every 28 days thereafter. Primary outcome is PFS and secondary outcomes are ORR and OS.

4.3 Rheumatoid arthritis

4.3.1 *Phase I/II*

In 2005, a Phase I/II, randomized, double-blind, placebo-controlled study (NCT00291928) was conducted to evaluate the safety and efficacy in patients with active RA who have failed one treatment with one or more disease-modifying antirheumatic drugs [36]. The study included 39 patients and 33 received either two infusions of 300, 700 and 1000 mg of ofatumumab or placebo, given 2 weeks apart. Efficacy was assessed by the ACR score at week 24. In the 300-mg dose group, 75% patients who received both doses obtained ACR20. In both the 700- and 1000-mg dose groups, 78% of patients who received both doses obtained ACR20. Results showed a 77% ACR20 response rate in patients who received two doses of ofatumumab. Patients who only received one dose showed a 66% ACR20 response. None of the patients receiving placebo achieved ACR20.

The previous study was expanded into a Phase II trial that included 200 additional patients randomized into four treatment groups. In each group, 50 patients received two infusions of 300, 700, or 1000 mg of ofatumumab or placebo, given 2 weeks apart. Patients were followed for 24 weeks to evaluate safety and efficacy and then every 12 weeks until B-cell counts returned to baseline levels. In the intention-to-treat analysis ACR20, ACR50 and ACR70 responses were achieved by 46,24 and 6% of all patients receiving ofatumumab compared with 15,5 and 0% in the placebo group. Evaluated by dose groups, an ACR20 response was obtained by 41,49 and 46% of patients receiving 300, 700 and 1000 mg of ofatumumab, respectively. An ACR50 response was obtained by 19,26 and 26% of patients receiving the varying doses of ofatumumab, with 9,4 and 6% obtaining an ACR70 response. In the subgroup of 178 patients receiving concomitant stable doses of MTX, results across the three dose levels of ofatumumab studied showed that an ACR20 response was obtained by 42,56 and 50% of patients in the 300-, 700- and 1000-mg dose groups, respectively, compared with 16% in the placebo group. An ACR50 response was obtained by 21,26 and 26% of patients receiving the varying doses of ofatumumab, with 8,2 and 5% obtaining an ACR70 response. The corresponding responses for the placebo group were 7% and 0%, respectively. Overall, 72% of patients treated with each ofatumumab dose experienced at least a moderate EULAR response compared with 40% of patients receiving placebo at week 24. Patients in this study will be part of another Phase II, nonrandomized, open-label, active-control study that started in January 2008 set to evaluate the long-term effectiveness of repeated courses of ofatumumab (NCT00655824).

Following a protocol amendment, 203 patients from the total patient population were subject to efficacy measurements for a 48-week follow-up period [37]. Continuation of ongoing therapy with MTX and low-dose prednisolone was permitted. Patients on 700-mg and 1000-mg doses of ofatumumab maintained numerically higher ACR20 response rates than those on placebo at the end of follow-up. The percentage of patients with a good or moderate EULAR response was statistically significantly higher in the 700-mg group compared with placebo at week 48 (70% vs 49%, respectively). Only two possibly/probably related AEs, cellulitis and Clostridium colitis, were recorded from weeks 24 to 48 across all active arms.

A two-part Phase I/II study to evaluate a subcutaneous route of administration of ofatumumab in RA patients, stable on MTX, is underway (NCT00686868). Part A will characterize the safety and tolerability of ofatumumab when administered subcutaneously. The primary end point is safety and tolerability. Part B will characterize the pharmacokinetics/pharmacodynamics of subcutaneous dosing. Patients in both parts are allowed to continue a stable dose of MTX therapy. Part A will be a randomized, single-blind, placebo-controlled, dose-range finding study of approximately 40 patients; administration of ofatumumab will occur in a hospital-based unit. Part B will be a blinded, randomized, placebo-controlled study. On the basis of findings in part A of the study, selected doses will be taken forward for administration in part B. Administration of ofatumumab for part B is planned to be conducted in an outpatient setting.

4.3.2 *Phase III*

At present, two Genmab-sponsored, Phase III, randomized, double-blind, placebo-controlled, parallel assignment clinical trials comparing ofatumumab with placebo in RA patients are ongoing. The primary end point for both would be reduced clinical signs and symptoms of RA after a single course of ofatumumab, measured by an ACR20 response at 24 weeks; the secondary outcome of both trials would be safety and efficacy after repeated doses of ofatumumab in a 120-week, open-label period. In one trial, 248 patients with an inadequate response to MTX will be randomized to a single course of two 700-mg doses of ofatumumab 2 weeks apart or placebo, in addition to background MTX (NCT00611455). In the second trial, 236 patients refractory or with inadequate response to anti-TNFα therapy will be randomized again to a single course of ofatumumab 700 mg or placebo (NCT00603525).

4.4 Multiple sclerosis

4.4.1 *Phase I/II*

In December 2007, a double-blind, randomized, placebo-controlled, multicenter, dose-finding trial of ofatumumab in RRMS patients was announced (NCT00640328). The first patient in the study was treated in June 2008. The purpose of the trial is to investigate the safety and the dose response of three doses of ofatumumab compared with placebo. It is a two-part study. In Part A, 36 patients will be treated in cohorts of increasing doses of ofatumumab of 100, 300 or 700 mg. In Part B, 288 patients will be randomized to one of three ofatumumab dose groups or placebo and followed for a 48-week treatment period. After week 24, patients on an active dose will receive re-treatment with the same dose of ofatumumab or placebo. Patients in the placebo group will receive ofatumumab at the highest tolerated dose from Part A. The dose response will be determined on disease activity as measured by MRI scans of the brain at 8 and 24 weeks.

4.4.2 *Phase III*

No Phase III data were available at the time of this publication.

5. Adverse events and contraindications

In a Phase I/II trial evaluating safety and efficacy of ofatumumab in relapsed or refractory FL, no safety concerns or maximum tolerated dose were identified [31]. A total of 274 AEs were reported; 190 were judged related to ofatumumab, most occurring on the first infusion day with Common

Terminology Criteria (CTC) grade 1 or 2. Eight related events were grade 3. Treatment caused immediate and profound B-cell depletion.

In the NCT00349349 CLL trial, ofatumumab infusion-related adverse events on the first infusion day occurred in 46% of patients in the DR group and 38% in the BFR group, which were grade 3 in 7% and 3%, respectively. The subsequent infusions did not demonstrate the potency of the initial infusion reactions. The most common CTC grade 3 or 4 toxicities were infections (25% in DR; 27% in BFR group) and hematologic events including neutropenia (12% in DR; 10% in BFR group) and anemia (8% in DR; 4% in BFR group). Early death, defined as within 8 weeks from start of treatment, occurred in two patients (3%) in the DR group (sepsis, n = 1; fungal pneumonia, n = 1) and three patients (4%) in the BFR group (PD, n = 1; sepsis, n = 1; myocardial infarction, n = 1). None of the patients tested developed human antibodies against ofatumumab.

During the early stages of the initial RA trial [36], two patients experienced infusion-related serious AEs–one anaphylactoid reaction and one urticaria–and one patient had a CTC grade 3 bronchospasm. These were observed when using the 300-mg dose, so premedication with corticosteroids and a slower infusion rate were implemented before the use of higher doses. After premedication in all active groups, three CTC grade 2 events–one fatigue and two bronchospasms–were observed in the 700- and 1000-mg groups, but no serious AEs were reported. At the conclusion of the study, the incidence of CTC remained the same but one serious AE was reported. Most nonserious AEs were observed on the first infusion day, were grade 1 and 2 CTC events and were reported in 87% of patients in the 300 mg group, 75% in the 700 mg group and 58% in the 1000 mg group, compared with 20,33 and 0%, respectively, during the second infusion. In a recent study to evaluate the long-term effects of two i.v. doses of 300, 700 and 1000 mg of ofatumumab given 2 weeks apart in patients with RA, two serious AEs (cellulitis and clostridium colitis) were reported. Of note, only serious AEs were reported at 24 and 48 weeks [37].

6. Conclusions

Ofatumumab is a second-generation anti-CD20 MAb that has been demonstrated to be safe and efficacious in treating patients with NHL, CLL, RA and MS. Ofatumumab is a fully human MAb, attaches to a newly described epitope and shows lower off-rates and improved CDC effect than rituximab. Initial clinical data present ofatumumab as an attractive agent with lower rates on infusion-related events than rituximab. Ongoing Phase III trials in patients with FL, CLL and RA are ongoing and Phase II trials in patients with DLBCL and MS are also under development. Thus far, ofatumumab has shown safety and efficacy as a single agent as well as in combination with chemotherapy. Future research should be directed towards using ofatumumab in rituximab-resistant patients and comparing ofatumumab with rituximab in treatment-naive patients.

7. Expert opinion

The advent of anti-CD20 MAbs has changed the treatment paradigm of LPDs and AIDs. Rituximab-containing regimens have improved response and survival rates in low-grade and aggressive NHL and in CLL [10,13,38,39]. Patients with RA and MS have also responded to anti-CD20 MAb therapy and current practice is moving away from other harder-to-manage immunosuppressants [36]. Despite this fact, rituximab therapy is far from perfect; the infusion-related reactions are common, some of them are life-threatening, and patients will ultimately develop resistance following repetitive exposure to rituximab [10,20,21].

The CD20 antigen continues to be a great target for MAb therapy. CD20 is not shed in the bloodstream and is very specific to B-lymphocytes [40]. Although CD20 function has not been definitively elucidated, its modulation by MAb induces ADCC, CDC and apoptosis in the malignant or autoimmune cell [19]. Current research has shown that not all anti-CD20 MAbs are created equal. Ofatumumab has specific characteristics that differentiate it from rituximab, starting from a different binding site and binding properties to longer half-life and higher intensity of CDC [24,25]. Ofatumumab is also a fully humanized MAb with a low immunogenic potential and, so far, formation of human anti-human antibodies has not been observed in patients exposed to ofatumumab. All these features allow shorter infusions with fewer infusion-related reactions than rituximab and make ofatumumab an attractive product to be used in repetitive doses in the treatment of LPDs and AIDs. Much like rituximab, infusion-related AEs have been reported more commonly during the first infusion. Administration of corticosteroids should be routinely used before ofatumumab infusions and the rate of infusion should be decreased according to a protocol similar to that used with rituximab.

Ofatumumab has shown preclinical and clinical efficacy in different rituximab-resistant settings [41]. Ofatumumab induced lysis in cell lines with low expression of CD20 and also has shown efficacy in cases of NHL that are primarily resistant or refractory to rituximab and in cases of CLL, which are characterized by inherently lower expression of CD20 [25]. There are ongoing Phase III trials using ofatumumab in low-grade NHL, CLL and RA. Of note, none of these trials is a head-to-head comparison against rituximab. There is a general agreement that rituximab therapy has potentially serious complications, but the clinical experience has been positive so far; as more years of experience accumulate in favor of rituximab it could potentially be harder to introduce a new anti-CD20 MAb, even if it proves to be noninferior.

Other second-generation anti-CD20 MAbs are under development. Veltuzumab (Immunomedics, Inc.), a humanized

anti-CD20 MAb with high CDC effect and slow off-rates, is undergoing Phase II studies in NHL, CLL, RA and idiopathic thrombocytopenic purpura. Veltuzumab has been shown to be safe and efficacious at low doses using intravenous and, more recently, subcutaneous formulations [42-44]. Ocrelizumab (Biogen Idec, Inc., Genentech, Inc., Roche Holding AG and Chugai Pharmaceuticals Co. Ltd.), another humanized anti-CD20 MAb, is undergoing Phase III trials in combination with MTX for the treatment of patients with RA and lupus nephritis, and Phase II trials for systemic lupus erythematosus and hematological malignancies [45,46].

The success of ofatumumab in front-line settings will be based on showing noninferiority to rituximab in achieving and sustaining clinical responses and improving survival. The Phase III NHL trial is comparing two different doses of ofatumumab and the Phase III CLL trial is comparing ofatumumab versus the combination of ofatumumab and chlorambucil. These trials will be unlikely to change the current management of these disorders, although they can prove the principle of ofatumumab benefit in these settings. Future Phase III trials in the development of ofatumumab should evaluate combination with chemotherapy and/or other targeted therapies in comparison with what is considered standard of care, namely R-CHOP in DLBCL, R-CVP (rituximab, cyclophosphamide, vincristine and prednisone) in FL and FCR (fludarabine, cyclophosphamide and rituximab) in CLL patients. A Phase III trial comparing FC against FC in combination with ofatumumab in CLL patients will start recruiting patients this year (NCT00824265). The latter trial is of great interest since recent Phase III trials evaluating the addition of rituximab to FC in CLL patients have shown higher response rates and prolonged PFS in front-line [38] and relapsed settings [39].

In relapsed and refractory settings, ofatumumab has a great opportunity to show superiority to rituximab since most of the patients will already be rituximab failures. In DLBCL patients, the combination of ofatumumab and ICE (ifosfamide, carboplatin and etoposide) and DHAP (dexamethasone, cytarabine and cisplatin) regimens should be studied. In FL and CLL patients, combinations with bendamustine, immunomodulators such as thalidomide and lenalidomide, and other monoclonal antibodies such as galiximab (Biogen Idec, Inc.), lumiliximab (Biogen Idec, Inc.) and epratuzumab (Immunomedics, Inc.)–anti-CD80, anti-CD23 and anti-CD22 mAbs, respectively–should be attempted. Ofatumumab alone or in combination with chemotherapy and/or bortezomib could also be of value in Waldenstrom's macroglobulinemia (WM) and mantle cell lymphoma. A Phase II trial of ofatumumab in patients with WM will start in 2009 (NCT00811733). The concept of maintenance ofatumumab should also be studied in patients with indolent NHL and CLL even after maintenance rituximab failure. It would be of interest to see if an effective B-cell suppression can be achieved with fewer infusions of ofatumumab in this setting. Given that both antibodies bind different epitopes in the CD20 antigen, maybe a combination of both CD-20 MAbs will induce more potent and longer responses, although the implications of such profound B-cell depletion are unknown. Finally, ofatumumab-based radioimmunotherapy could prove to be useful given the characteristics of the binding site and longer binding affinity.

Ofatumumab as a novel anti-CD20 MAb has shown to be safe and effective, alone or in combination, in treating patients with indolent and aggressive NHL, CLL, RA and MS, but its role is not clearly defined. Probably, ofatumumab will have an important role in treating patients who cannot tolerate rituximab or have developed rituximab resistance; but it is unclear how ofatumumab will compare to rituximab as a single agent or in combination with chemotherapy in front-line settings. The best way to answer these questions is through the careful design, planning, execution and analysis of significant randomized controlled trials. Since many of these patients will have an insidious clinical course, it is also necessary to improve our present understanding of the malignant and autoimmune cell biology to personalize and tailor therapies so that better response and longer survival rates may be obtained without affecting significantly patients' quality of life.

Declaration of interest

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