

Ofatumumab: a novel monoclonal anti-CD20 antibody

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Abstract: Ofatumumab, a novel humanized monoclonal anti-CD20 antibody, was recently approved by the FDA for the treatment of fludarabine and alemtuzumab refractory chronic lymphocytic leukemia (CLL). Ofatumumab effectively induces complement-dependent cytotoxicity (CDC) *in vitro*, and recent studies demonstrated that ofatumumab also effectively mediates antibody-dependent cellular cytotoxicity (ADCC). Pharmacokinetic studies indicated that increased exposure to the antibody correlated with improved clinical outcome in CLL. Thus, pharmacogenomics may be important in identifying which patients are more likely to respond to ofatumumab therapy, although such studies have not yet been performed. Patients with the high-affinity FCGR3a 158 V/V polymorphism may be more likely to respond to therapy, if ADCC is the primary *in vivo* mechanism of action of ofatumumab. Patients with increased expression of the complement defense proteins CD55 and CD59 may be less likely to respond if ofatumumab works *in vivo* primarily via CDC. Patients with increased metabolism and clearance of ofatumumab may have lower exposure and be less likely to respond clinically. Thus, pharmacogenomics may determine the responsiveness of patients to ofatumumab therapy.

Keywords: monoclonal antibody, CD20, CLL, NHL, lymphoma

Introduction

The introduction of monoclonal antibodies such as the chimeric anti-CD20 antibody rituximab (Rituxan®, Genentech; MabThera®, Roche) and the chimeric anti-CD52 antibody alemtuzumab (Campath-1H®, Genzyme) has revolutionized the treatment of B-cell lymphoproliferative disorders such as B-cell non-Hodgkin's lymphoma (B-NHL) and chronic lymphocytic leukemia (CLL).¹⁻⁴ Unlike the idiotype vaccine which underwent clinical trials in follicular lymphoma (FL), monoclonal antibodies are not specific for an individual patient's tumor. Instead, antibodies recognize antigens which are expressed on the tumor cells of most or all patients with a certain type of cancer. However, these antigens are not truly tumor-specific; for example, CD20 is expressed on normal B lymphocytes, and CD52 is expressed on a variety of hematopoietic cells. Consequently, the toxicity profile of a monoclonal antibody depends largely on its antigen's expression on normal tissues. Monoclonal antibodies kill tumor cells through several mechanisms: antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct apoptosis.⁵⁻⁷ Monoclonal antibodies can be classified as type I or II, depending on their biological activity and mechanism of action in *in vitro* assays. Type I antibodies translocate CD20 into lipid rafts and are therefore more potent inducers of CDC, but are weak inducers of apoptosis. Lipid rafts are sphingolipid and cholesterol enriched-enriched microdomains of the

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plasma membrane which function as organizing platforms for cell signaling and receptor trafficking. The ability of anti-CD20 antibodies to induce CDC correlated with their ability to translocate CD20 into lipid rafts, whereas induction of apoptosis by anti-CD20 antibodies was independent of CD20 segregation into lipid rafts. In contrast, type II antibodies do not induce lipid rafts and are thus poor inducers of CDC, but more effectively induce direct apoptosis. Both type I and II antibodies effectively induce ADCC. However, the clinical activity of a monoclonal antibody may depend upon the affinity of an individual patient's Fc receptor for the Fc fragment of the antibody.⁵ Thus, the activity of an antibody in an individual patient may depend on the patient's genetic or biological profile.

The monoclonal antibody which has made the greatest clinical impact to date in hematologic malignancies is rituximab, which was initially approved for the treatment of FL more than a decade ago.¹ Rituximab and the majority of second-generation anti-CD20 antibodies in clinical study are type I antibodies and redistribute CD20 into lipid rafts, although several type II anti-CD20 antibodies are in preclinical and clinical development. While rituximab was initially indicated as single-agent therapy for relapsed FL, subsequent studies demonstrated its activity in combination with cytotoxic chemotherapeutic agents such as alkylating agents (cyclophosphamide, bendamustine) and nucleoside analogs (fludarabine, pentostatin) in FL, diffuse large B-NHL (DLBCL) and CLL.^{2,8-13} In fact, the primary clinical use of rituximab is in combination regimens, particularly in DLBCL and CLL. In CLL single-agent rituximab has had limited clinical impact, but rituximab has significantly changed the treatment of CLL, particularly in the upfront setting, due to combinations with fludarabine-based cytotoxic chemotherapy such as FCR.^{2,11,12} In contrast to FL, in which studies have demonstrated that rituximab works primarily via ADCC,⁵ rituximab's primary mechanism of action in CLL is undefined.⁶

In order to improve upon the biological and clinical activity of rituximab, especially in the single-agent setting, several humanized monoclonal anti-CD20 antibodies have been engineered to improve ADCC or CDC. These agents are in various stages of preclinical and clinical investigation in B-NHL and CLL. To improve ADCC, particularly in patients whose Fc receptors bind rituximab with low affinity, the Fc portions of several of these antibodies have been modified to improve binding to the Fc receptor. Individuals who are homozygous for valine at amino acid 158 of Fc gamma receptor (FCGR)3a (V/V) respond significantly

better to rituximab than patients who are homozygous for phenylalanine (F/F) or who are heterozygous at amino acid 158 (V/F).⁵ Thus, one potential pathway to improve upon the activity of rituximab is to modify the Fc portion, in order to enhance binding to the Fc receptor of patients with low-affinity FCGR3a polymorphisms.

Ofatumumab

This review will focus on the second-generation anti-CD20 antibody which has undergone the furthest clinical development, ofatumumab (HuMax-CD20™, Genmab; Arzerra™, GSK).^{7,14-16} Ofatumumab was recently approved by the Food and Drug Administration (FDA) for the treatment of fludarabine and alemtuzumab refractory CLL, and the drug has been submitted for approval for the treatment of CALL in Europe. In addition, ongoing clinical studies are examining the safety and efficacy of ofatumumab as single-agent therapy for FL and in combination therapy for CLL, FL and DLBCL. Ofatumumab is a fully humanized, high-affinity monoclonal antibody whose epitope on CD20 is distinct from rituximab's target.¹⁴ The membrane proximal epitope recognized by ofatumumab encompasses both the small and large loops of CD20. In contrast, rituximab's binding site on CD20 is distal to ofatumumab's epitope and involves only the large loop. Preclinical studies indicated that ofatumumab has higher affinity for CD20 and activates CDC more effectively than rituximab.⁷

The initial form of ofatumumab, 2F2, was generated by immunization of human immunoglobulin (Ig) transgenic mice with NS/0 cells transfected with human CD20. 2F2 induced redistribution of CD20 into detergent insoluble plasma membrane fractions, consistent with translocation into lipid rafts.⁷ Thus, like rituximab, ofatumumab is a type I antibody. Cytotoxicity assays using the ARH-77 lymphoma cell line indicated that rituximab was unable to induce cytotoxicity with unfractionated blood but mediated efficient cell killing when incubated with a purified mononuclear cell fraction containing natural killer (NK) cells, confirming that rituximab induced killing via ADCC. In contrast, 2F2 was able to induce cell killing of ARH-77 cells when incubated with unfractionated blood. Furthermore, 2F2 was able to mediate cytotoxicity in the presence of plasma, and heat inactivation of plasma abolished this cell killing, suggesting that 2F2 induced cytotoxicity via complement fixation. Studies in SU-DHL-4 and Daudi lymphoma cells, which express high levels of CD20 and are sensitive to CDC, indicated that both rituximab and 2F2 were able to induce lysis of these cell lines. However, 2F2 was more effective than rituximab at

inducing CDC at low antibody concentrations. Furthermore, only 2F2 was able to induce CDC of Raji lymphoma cells, which have moderate CD20 expression but high levels of the complement defense molecules CD55 and CD59. Thus, 2F2 induced CDC more effectively than rituximab. Studies with primary CLL cells suggested that this difference may be clinically significant in CLL. 2F2, but not rituximab, induced killing of CLL cells in the presence of unfractionated blood, whereas both antibodies induced only low levels of ADCC in the presence of mononuclear cells.⁷

Subsequent studies using C1q-depleted serum supplemented with low concentrations of C1q, which initiates the classical pathway of complement, further illustrated the enhanced ability of ofatumumab, or 2F2, to induce CDC.¹⁷ These studies, which were conducted in Raji and Daudi lymphoma cell lines and primary CLL tumor cells, demonstrated that C1q bound more avidly to ofatumumab-opsonized cells than to rituximab-opsonized cells, resulting in high levels of complement fixation and CDC. Even when similar C1q binding was obtained with higher levels of C1q, rituximab-opsonized cells induced less complement fixation and CDC than ofatumumab-opsonized cells. Thus, the superior ability of ofatumumab to induce CDC resulted from both increased C1q binding and enhanced ability to activate complement after initial binding of C1q.¹⁷

While these studies demonstrated that ofatumumab effectively induces CDC, a recent abstract presented at the 2009 Annual Meeting of the American Society of Hematology (ASH) indicated that ofatumumab is also a potent inducer of ADCC. Craigen et al compared the ability of ofatumumab and rituximab to induce ADCC by purified NK cells from healthy donors expressing V/V (n = 10) or F/F (n = 10) at amino acid 158 of FCGR3a.¹⁸ Fc-mediated antibody binding to NK cells and ADCC were examined in a blinded study which incubated ARH-77 lymphoma cells with different concentrations of ofatumumab or rituximab in the presence of purified NK cells at an effector-to-target ratio of 5:1. Antibody binding was measured by flow cytometry in competition binding assays using ofatumumab or rituximab. Monomeric ofatumumab bound 4.4 times more strongly to NK cells expressing FCGR3a 158 V/V than to cells expressing 158 F/F, with EC₅₀ 900 and 3970 mg/mL, respectively ($P < 0.0001$). A similar 4.2-fold difference in NK cell binding between FCGR3a 158 V/V and 158 F/F was found for rituximab (EC₅₀ 1370 and 5720 mg/mL, respectively, $P < 0.0001$). Of note, ofatumumab bound 1.5-fold more tightly to both FCGR3a allotypes than rituximab ($P < 0.0001$). Ofatumumab induced potent NK-mediated

ADCC with cells from donors with FCGR3a 158 V/V and 158 F/F, with EC₅₀ 6.4 and 17.6 ng/mL, respectively ($P < 0.0001$). Higher concentrations of rituximab were required to induce ADCC by NK cells in this assay, with EC₅₀ 12 and 31 ng/mL, respectively, for donors with FCGR3a 158 V/V and 158 F/F. This 1.8-fold increase in ADCC potency between ofatumumab and rituximab ($P < 0.0001$) translated into increased killing of cells by ofatumumab, compared to rituximab, in the ADCC assay using NK cells from donors with FCGR3a 158 V/V (39.7% vs 27.6%) and 158 F/F (40.1% vs 35.4%).¹⁸ These studies indicated that ofatumumab effectively induces both ADCC and CDC.^{7,18}

Binding studies using Fab' fragments indicated that 2F2 bound more tightly to CD20 than rituximab, and additional studies in DOHH cells demonstrated a slower off-rate for 2F2 compared to rituximab.⁷ After 3 hours, more than 70% of 2F2 remained bound to cells, compared to 30% for rituximab. The faster off-rate of rituximab resulted in antibody dissociation and a concomitant loss in CDC assays, compared to 2F2. Cross-linking with sheep anti-human κ antibody prevented dissociation of rituximab from the cell surface and improved the CDC activity of rituximab, confirming that the faster off-rate of rituximab resulted in inferior CDC.⁷ Epitope mapping, using both mutagenesis studies and a novel epitope analysis method using overlapping 15-mer peptides of the extracellular loops of CD20 and a Pepscan-based ELISA assay, demonstrated that rituximab bound to the large extracellular loop of CD20. In contrast, 2F2 recognized a distinct epitope N-terminal of rituximab's binding site; furthermore, the epitope of 2F2 included both the small and large loops of the extracellular domain of CD20.¹⁴

Thus, ofatumumab's preclinical profile suggests several potential mechanisms of advantage over rituximab (Table 1). In *in vitro* assays ofatumumab induced both CDC and ADCC more effectively than did rituximab.^{7,18} While a higher concentration of ofatumumab was needed to bind NK cells from donors with the low affinity FCGR3a polymorphism 158 F/F than from donors with the high affinity polymorphism 158 V/V, resulting in a higher concentration for induction of ADCC in

Table 1 Preclinical characteristics of ofatumumab

Ofatumumab is a type I antibody (lipid rafts)
Epitope on CD20 is distinct from rituximab's epitope
Epitope encompasses both the small and large loops of CD20
Ofatumumab binds CD20 more tightly with slower off-rate
Ofatumumab induces complement-dependent cytotoxicity (CDC)
Ofatumumab induces antibody-dependent cytotoxicity (ADCC)
Ofatumumab caused prolonged B-cell depletion in animal studies

the low affinity patients, ofatumumab was more effective at binding NK cells and inducing ADCC than rituximab in both patient groups.¹⁸ In addition, ofatumumab recognized a distinct epitope on CD20 which is N-terminal to rituximab's epitope and encompasses both the small and large loops.¹⁴ Finally, ofatumumab bound more tightly, with a slower off-rate, than rituximab.⁷ Therefore, phase I/II clinical studies were initiated to determine whether these biological differences would translate into clinical activity in lymphoid malignancies.

Clinical development in CLL

A phase I/II study of ofatumumab was conducted in CLL, based on these *in vitro* studies as well as animal studies demonstrating prolonged B-cell depletion in cynomolgus monkeys exposed to ofatumumab, compared to rituximab. Ofatumumab was given by intravenous (IV) infusion weekly for 4 doses to 33 patients with relapsed CLL.¹⁶ Median age of patients was 61 years (range 27–82), and 58% were male. Four patients had Rai stage III/IV disease at screening, whereas 28 patients were stage I/II. Four patients had bulky lymphadenopathy greater than 5 cm in diameter. Patients were a median of 6.3 years (range 1.2–14) from diagnosis and had received a median of 3 prior therapies (range 1–9). Cohort 1 administered ofatumumab 100 mg followed by 3 doses of 500 mg to 3 patients, whereas 3 patients in cohort 2 received 300 mg followed by 3 doses of 1000 mg. Cohort 3 administered 500 mg followed by 3 doses of 2000 mg to 27 patients. Twenty-seven patients experienced 246 adverse events (AEs), of which 150 were judged to be related to ofatumumab and 19 were grade 3 or 4. Fifty-six percent of AEs were infusion-related and decreased in incidence and severity with subsequent infusions of ofatumumab. Two patients developed grade 4 neutropenia, and 2 patients experienced grade 3 thrombocytopenia judged to be related to therapy. Seventeen patients (51%) experienced a total of 25 infections, most

of which were grade 1–2. Three patients developed grade 3 infections (herpes zoster, nasopharyngitis, pneumonia), and one patient died of infectious interstitial pneumonitis. In total, 10 serious adverse events (SAEs) were observed in 9 patients, and the maximum tolerated dose (MTD) was not reached. One patient in cohort 3 was withdrawn from therapy due to transient grade 3 hepatitis after the first 500 mg infusion of ofatumumab, but the other 32 patients were evaluable for response. Thirteen patients in cohort 3 responded (48%), including one nodular partial response (nPR) and 12 PR (Table 2). Pharmacokinetic (PK) analysis demonstrated that clinical response to ofatumumab correlated with the drug's minimum or trough serum concentration (C_{min} , $P = 0.009$), maximum or peak concentration (C_{max} , $P = 0.003$), area under the curve (AUC, $P = 0.006$), and half-life ($t_{1/2}$, $P = 0.009$).

Based on these promising results, a pivotal phase II licensing trial was initiated in patients with relapsed or refractory CLL.¹⁹ Patients were eligible for this study if they were refractory to both fludarabine and alemtuzumab (FA-REF) or were refractory to fludarabine and had bulky nodal disease defined as at least one lymph node of 5 cm or greater in dimension (bulky fludarabine refractory, BFR). The primary endpoint was overall response (OR) rate, and a planned interim analysis was performed when 66 patients were enrolled in the FA-REF group. At the time of the interim analysis, 154 patients had been enrolled, including 59 patients in the FA-REF group, 79 patients in the BFR group, and 16 patients who did not meet the eligibility criteria of either treatment cohort (Table 2). Median age of the 59 FA-REF patients was 64 years (range 41–86), 75% were male, and 54% had Rai stage III/IV disease. Median number of prior therapies was 5 (range 1–14), 93% of patients had received alkylator therapy, and 59% had received rituximab therapy. Median age of the 79 BFR patients was 62 years (range 43–84), 72% were male, and 70% had Rai stage III/IV disease. Median number

Table 2 Clinical studies of weekly single-agent ofatumumab

Phase	N	Disease	Dosing schedule	Response rate	Median PFS
I/II	27 ^a	CLL (relapsed)	500 mg week 1 2000 mg weeks 2–4	48%	Not reported
II	59	CLL (FA-REF)	300 mg week 1	58%	5.7 months
	79	CLL (BFR)	2000 mg weeks 2–8 2000 mg monthly × 4	47%	5.9 months
I/II	38 ^b	FL (relapsed)	300–1000 mg weeks 1–4	43%	8.8 months
II	116	FL (refractory)	300 mg week 1	11%	6.0 months
			500–1000 mg weeks 2–8		

^aPatients in cohort 3; ^bPatients who were evaluable for response.

Abbreviations: N, number of patients; PFS, progression-free survival; CLL, chronic lymphocytic leukemia; FA-REF, fludarabine and alemtuzumab refractory; BFR, bulky fludarabine refractory; FL, follicular lymphoma.

of prior therapies was 4 (range 1–16), 92% of patients had received alkylator therapy, and 54% had received rituximab therapy. Ninety-three percent of FA-REF patients and 100% of BFR patients had at least one lymph node of 5 cm or greater in dimension by physical examination and/or radiographic imaging.

Ofatumumab was given weekly for 8 doses, followed by 4 maintenance monthly doses.¹⁹ All patients received 300 mg for the first infusion, and a dose of 2000 mg was administered for each of the remaining 11 infusions; thus, patients received a total of 22,300 mg. Patients received acetaminophen 1000 mg and cetirizine 10 mg or equivalent prior to infusions. Patients also received prednisolone 100 mg or equivalent prior to infusions 1, 2 and 9; the glucocorticoid dose could be reduced for other infusions if the first two infusions were well-tolerated. Antibiotic prophylaxis was not mandated.

Infusion-related reactions, nearly all of which were grade 1–2 and occurred primarily during the first two infusions, were seen in 64% of FA-REF patients and 61% of BFR patients.¹⁹ The most common AEs were infections (67%), cough (18%), diarrhea (16%), anemia (16%), fatigue (15%), fever (15%) and neutropenia (15%). AEs judged to be related to ofatumumab treatment included 2 cases each of grade 3–4 thrombocytopenia and grade 3–4 hemolytic anemia and 1 case of grade 3 febrile neutropenia. Ninety-two patients experienced a total of 189 infections, of which 139 (74%) were grade 1–2. Among 37 grade 3–4 infections, pneumonia ($n = 14$) and other respiratory tract infections ($n = 6$) were most common. Thirteen infections resulted in death, including sepsis ($n = 6$), pneumonia ($n = 5$), fusarium infection ($n = 1$) and progressive multifocal leukoencephalopathy (PML, $n = 1$).

Response was assessed by an Independent Review Committee (IRC) using the 1996 NCI-WG response criteria. The OR rate was 58% and 47% for FA-REF and BFR patients, respectively, with stable disease in 31% and 41% of FA-REF and BFR patients, respectively. All responders achieved PR except for one patient who attained a complete response (CR). The OR rate among patients who previously received a rituximab-containing regimen was 54% and 44% in the FA-REF and BFR groups, respectively.¹⁹ Ofatumumab was rapidly effective in responding patients, with approximately 80% of responses occurring within 2 months of treatment initiation. The median duration of response was 7.1 months in FA-REF patients and 5.6 months in BFR patients. Median progression-free survival (PFS) was 5.7 months and 5.9 months for FA-REF and BFR patients, respectively. Median overall survival (OS) was 13.7 months

and 15.4 months for FA-REF and BFR patients, respectively. An exploratory landmark analysis was performed at week 12 to determine if there was a correlation between response and OS. Median OS was significantly longer among responding patients; the median OS had not yet been reached for responders in either the FA-REF or BFR groups, compared with 9.8 months and 10.2 months, respectively, for non-responders. Based on the results of the planned interim analysis of this pivotal phase II study, ofatumumab was recently approved by the FDA for the treatment of fludarabine and alemtuzumab refractory CLL.

Osterborg et al examined the relationship among serum ofatumumab concentrations, baseline patient characteristics and clinical outcomes in this study, which he presented at the 2009 ASH meeting.²⁰ Multivariate analysis demonstrated that a higher C_{max} following the first dose of ofatumumab was associated with a lower percentage of bone marrow infiltration by CLL, lower Rai stage, lower lymphocyte count, small body surface area and lower total bilirubin. Notably, the first three associations were with parameters of lower tumor burden and, thus, slower antibody clearance. The likelihood of response correlated with three parameters of antibody exposure at dose 8, which was the final dose of weekly treatment. Responders demonstrated a 37% higher AUC, a 23% higher C_{max} and a 91% higher C_{min} at dose 8 than non-responders. Interestingly, no association was found between PK parameters at dose 12 and clinical response. In addition, higher AUC, C_{max} and C_{min} , as well as lower clearance, correlated with improved PFS at both dose 8 and dose 12. However, exploratory multivariate analysis did not indicate that PK parameters independently predicted either ORR or PFS. Nonetheless, these studies indicated that increased exposure to ofatumumab may result in improved clinical outcome, although more detailed studies will need to be performed to define this relationship.

Wierda et al recently presented preliminary results of a randomized phase II study examining two doses of ofatumumab, 500 mg and 1000 mg, in combination with standard dose fludarabine and cyclophosphamide (FC) every 28 days in previously untreated CLL.²¹ The median age of patients was 56 years (range 38–73), 46% of patients had Rai stage III/IV disease, and the median β -2-microglobulin level was 4.0 mg/L. Patients received 300 mg of ofatumumab IV on day 1 of cycle 1, followed by fludarabine 25 mg/m² IV and cyclophosphamide 250 mg/m² IV on days 2 to 4 of cycle 1. The ofatumumab dose was increased to 500 mg ($n = 31$) or 1000 mg ($n = 30$) on day 1 of cycles 2 to 6, and FC was administered at the same doses on days 1 to 3 of cycles 2 to 6. OR

and CR rates were 75% and 41%, and there was a trend toward a higher CR rate in the 1000 mg arm (50% vs 31%). A higher CR rate was observed in patients with a β -2-microglobulin level <4.0 mg/L compared to those with β -2-microglobulin level ≥ 4.0 mg/L (53% vs 29%), and patients who completed all 6 planned cycles of therapy had higher OR (92% vs 48%) and CR (55% vs 17%) rates than patients who received fewer than 6 cycles. While 4 patients came off study due to failure to respond, most patients withdrew from therapy due to toxicity, most notably cytopenias. Only 1 of 8 patients with del(17p13), corresponding to loss of p53, achieved CR. Due to short median follow-up time, no PFS data are available. The small sample size, high β -2-microglobulin level in both cohorts, and high percentage of del(17p13) patients in the 1000 mg treatment arm make it difficult to compare these results with historical results of other chemoimmunotherapy regimens such as FCR.

In summary, ofatumumab demonstrated significant single-agent activity in fludarabine and alemtuzumab refractory CLL and recently was approved by the FDA for this indication.¹⁹ Preliminary PK studies indicated that increased antibody exposure may correlate with improved clinical outcome, although no pharmacokinetic parameter independently predicted for OR rate or PFS.²⁰ A small phase II study of ofatumumab in combination with FC demonstrated activity, although comparison to historical results with rituximab-based combination regimens was limited by the small size of the study, high β -2-microglobulin level, and high percentage of patients with the poor-risk del(17p13) cytogenetic abnormality.²¹

Ofatumumab in indolent B-NHL

Ofatumumab has also demonstrated clinical activity in relapsed FL. A multi-center phase I/II study administered ofatumumab to 40 patients with relapsed FL.¹⁵ Median age was 58.5 years (range 34–75), and 50% of patients were male. Median time from diagnosis was 4.5 years (range 0.7–17.1), and 31 patients were stage III/IV at screening. Patients had received a median of 2 prior therapies (range 1–8), and 15 patients had received prior rituximab either as monotherapy or in combination with cytotoxic chemotherapy. Ofatumumab was given at a dose of 300, 500, 700 or 1000 mg IV weekly to 10 patients per cohort. Forty patients developed a total of 274 AEs, of which 190 were judged to be related to therapy. Similar to the experience in CLL, infusion toxicity was seen with the first ofatumumab dose in virtually all patients but extinguished with subsequent infusions. Ninety-five percent of AEs were grade 1 or 2, but 7 patients

developed a total of 8 grade 3 AEs judged to be related to therapy. Thirteen patients experienced a total of 20 infections; 18 infections were grade 1 or 2 and included 11 cases of upper respiratory tract infection. Two grade 3 infections (urinary tract infection, neutropenic sepsis) were reported as SAEs but were judged unrelated to ofatumumab. There was no relationship between dose and toxicity, and the MTD was not reached. Sixteen of 38 evaluable patients (43%) responded (Table 2); 5 patients achieved a CR, and 2 attained an unconfirmed CR (CRu). The OR rate varied between 20% and 63% across cohorts, and there was no dose-response relationship. Nine of 14 evaluable patients who previously received rituximab responded (64%), including 3 of 4 rituximab-refractory patients. Six of 10 patients in cohort 4 (1000 mg dose), 6 of 10 patients responded, including 4 of 5 patients who had failed prior rituximab therapy. Median time to progression (TTP) was 8.8 months for all subjects and 32.6 months for responding patients, and median duration of response was 29.9 months. Thus, ofatumumab demonstrated significant activity in FL patients who had relapsed after prior rituximab therapy. Ofatumumab caused immediate, profound B-cell depletion lasting 6 to 10 months after end of therapy, and 65% of evaluable patients converted from Bcl-2 positive to Bcl-2 negative peripheral blood. Clinical response did not correlate with C_{max} or AUC, but $t_{1/2}$ and clearance correlated with clinical response at week 26 but not week 19.

To determine if single agent ofatumumab has activity in patients with refractory FL, a multi-center study administered 8 weekly doses of ofatumumab to 116 patients with rituximab refractory FL.²² Patients were initially randomized to receive 500 mg or 1000 mg of ofatumumab. However, due to slow accrual, the study was amended to close the 500 mg treatment arm. Median age of all patients was 61 years (range 37–82), 86% of patients were Ann Arbor stage III/IV, and 75% of patients had intermediate or high-risk disease by the Follicular Lymphoma International Prognostic Index (FLIPI) including 47% with high-risk disease. Patients were refractory to rituximab monotherapy (23%), rituximab-based combination chemotherapy (38%), or rituximab maintenance therapy following cytotoxic chemotherapy (39%). Interestingly, 65% of patients were refractory to cytotoxic chemotherapy. Patients in the 500 mg and 1000 mg treatment cohorts had similar characteristics, with no significant differences between the two groups. All patients received 300 mg for the first infusion, followed by 500 mg ($n = 30$) or 1000 mg ($n = 86$) for their 7 remaining weekly infusions. Patients received acetaminophen 1000 mg and cetirizine 10 mg or equivalent prior to infusions, as well as prednisolone 100 mg

or equivalent prior to infusions 1 and 2; the glucocorticoid dose could be reduced for other infusions if the first two infusions were well-tolerated. Antibiotic prophylaxis was not mandated. Treatment was well tolerated, with primarily grade 1–2 infusion toxicity associated with the first ofatumumab dose which extinguished with subsequent infusions. There were only three cases of grade 3–4 non-hematologic, non-infectious toxicity (2 cough, 1 urticaria). Twenty-eight patients experienced infection, mostly grade 1–2 upper respiratory infections, with only 2 cases of grade 3 infection. Fifteen percent of patients experienced transient grade 3–4 neutropenia which resolved a few weeks after completion of therapy.

The primary endpoint of the study was OR rate (Table 2). The OR rate was 11%, including 10% of patients in the 1000 mg arm and 13% of patients who received 500 mg.²² Three patients attained CR or CRu, and 10 patients achieved PR. Since there was no difference in primary endpoint between the two treatment arms, patients in both cohorts were combined for all other analyses. Patients who were refractory to rituximab monotherapy appeared to be most sensitive to single-agent ofatumumab; 22% of patients refractory to rituximab monotherapy responded, compared to 7% of patients refractory to rituximab combination chemotherapy and 9% of patients refractory to rituximab maintenance therapy. Although the OR rate was 11%, nearly half of patients experienced some diminution in the sum of the products of the diameters (SPD) of their target lesions. Median PFS was 6.0 months. Thus, ofatumumab demonstrated limited single-agent activity in this heavily pretreated, refractory population. Thus, current efforts in FL are focused on the use of ofatumumab monotherapy in patients with previously untreated FL or relapsed, rituximab-sensitive FL, as well as combination regimens in both rituximab-sensitive and rituximab-refractory FL.

Role of pharmacogenomics

What is the potential role of pharmacogenomics in ofatumumab therapy? The answer to this depends on the relative clinical importance of ADCC and CDC as potential mechanisms of action, as well as the clearance or metabolism of ofatumumab by patients' bodies (Table 3). The importance of polymorphisms at amino acid 158 of FCGR3a has been well demonstrated in FL patients treated with rituximab, which is thought to work primarily by ADCC in FL.⁵ Individuals with 158 V/V respond significantly better to single-agent rituximab than patients with 158 F/F or 158 V/F. However, a similar relationship between FCGR3a polymorphisms

Table 3 Potential role of pharmacogenomics in ofatumumab

Antibody-dependent cellular cytotoxicity (ADCC)

FCGR3a polymorphisms

158 V/V (high affinity)

158 F/F (low affinity)

158 V/F (intermediate affinity)

Complement-dependent cytotoxicity (CDC)

CD55 and CD59

High expression (resistant to CDC)

Low expression (sensitive to CDC)

Exposure to drug–drug clearance

Increased metabolism of antibody (lower exposure/activity)

Increased antibody clearance (lower exposure/activity)

and response to therapy has not been reported for combination regimens of rituximab and cytotoxic chemotherapy in FL. Furthermore, the importance of FCGR3a polymorphisms in determining response to therapy has not been described for more aggressive B-NHL histologies such as DLBCL, and a study in CLL demonstrated no difference in clinical response to rituximab monotherapy between patients with 158 V/V and those with 158 F/F or 158 V/F. It is unclear if ofatumumab also is more effective in FL patients with the high-affinity 158 V/V polymorphism, but recent data presented at the 2009 ASH meeting indicated that ofatumumab may work *in vivo* via ADCC.¹⁸ Therefore, studies to examine the effect of FCGR3a polymorphisms upon the response to ofatumumab, both as single-agent therapy and in combination regimens with chemotherapy, are ongoing. Similarly, pharmacogenomics may be important in determining patients' susceptibility to ofatumumab if CDC is the antibody's primary *in vivo* mechanism of action. *In vitro* preclinical studies demonstrated that ofatumumab, but not rituximab, was able to induce CDC of Raji lymphoma cells with high levels of the complement defense molecules CD55 and CD59.⁷ Thus, patients with high levels of CD55 and CD59 may potentially be more resistant to ofatumumab therapy than patients with lower levels of these complement inhibitory proteins. Interestingly, a recent report indicated that the complement regulatory proteins CD46 and CD59 were higher in 15 patients with B-NHL who responded to rituximab and chemotherapy than in 7 patients who failed to respond to chemotherapy.²³ While this finding appears counterintuitive, it should be noted that CDC is not thought to be an important mechanism of action of rituximab in FL and other B-NHL. Given the increased ability of ofatumumab to induce CDC *in vitro*, it would be intriguing to examine the effect of CD55 and CD59 expression upon the clinical response of FL patients to ofatumumab, in order to determine whether patients with increased expression of

these complement inhibitory proteins are less likely to respond to ofatumumab based therapy.

Finally, the presentation by Osterborg et al at the 2009 ASH meeting demonstrated that increased exposure to ofatumumab correlated with clinical outcome in refractory CLL patients treated with the antibody.²⁰ Lower tumor burden correlated with increased C_{max} , which along with increased AUC and C_{min} , correlated with an improved OR rate. Thus, pharmacogenomic differences which result in slower metabolism of ofatumumab, decreased antibody clearance, and increased exposure to ofatumumab may result in increased clinical activity of the antibody.

In summary, ofatumumab is a humanized monoclonal anti-CD20 antibody which was recently approved by the FDA for the treatment of fludarabine and alemtuzumab refractory CLL, and which is currently undergoing further clinical studies as monotherapy and in combination therapy in CLL and B-NHL such as FL. Ofatumumab effectively induced CDC *in vitro*, and recent studies demonstrated that ofatumumab is also an effective mediator of ADCC. PK studies indicated that increased exposure to the antibody correlated with improved clinical outcome including OR rate and PFS. Thus, pharmacogenomics may be important in identifying which patients are more likely to respond to ofatumumab therapy, although such studies have not yet been performed. Patients with the high-affinity FCGR3a 158 V/V polymorphism may be more likely to respond to therapy, if ADCC is an important *in vivo* mechanism of action of ofatumumab. Patients with increased expression of the complement defense proteins CD55 and CD59 may be less likely to respond to ofatumumab if the antibody works *in vivo* primarily via CDC. Finally, patients with increased metabolism and clearance of ofatumumab may experience lower *in vivo* exposure to the antibody and be less likely to respond clinically. Thus, several potential avenues of pharmacogenomic research may help to delineate patients who are more or less susceptible to ofatumumab therapy.

Disclosure

The author is an employee of GSK.

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