EXPERT REVIEWS

Obinutuzumab for chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is a frequent hematological malignancy that is incurable using standard approaches. Two anti-CD20 monoclonal antibodies (mAb), rituximab and ofatumumab, have been approved for CLL treatment. A new glycoengineered type II humanized anti-CD20 mAb, obinutuzumab (GA101), has been developed and demonstrates increased activity against B-cell malignancies by inducing direct cell death and better antibody-dependent cellular cytotoxicity. In a recent randomized Phase III study in patients with newly diagnosed CLL and coexisting conditions, obinutuzumab plus chlorambucil demonstrated significant improvement in progression-free survival and several other outcome parameters, in contrast to rituximab plus chlorambucil. Grade 3–4 infusion-related reactions and neutropenia occurred more frequently in patients who received obinutuzumab compared with those who received rituximab; however, the rate of serious infections was similar. Obinutuzumab represents a promising new option for patients with CLL and must be investigated with other chemotherapy regimens or with new targeted agents.

Keywords: antibody-dependent cellular cytotoxicity • anti-CD20 monoclonal antibody • chlorambucil • chronic lymphocytic leukemia • direct cell death • infusion-related reaction • obinutuzumab • rituximab

Chronic lymphocytic leukemia (CLL) is the most common chronic leukemia in Western countries and comprises 30% of all adult leukemias [1]. The median age of patients at the time of diagnosis is approximately 72 years; with prolonged life expectancy achieved in the last 50 years, an anticipated increased population of patients will be diagnosed with this disease. The frequency of CLL appears to be lower in Asia and the Far East than in Europe or North America. In addition to evaluating several familial cases of CLL, a genetic background associated with an increased susceptibility for developing CLL has been recently identified [2]. Classical CLL is of B-cell origin and results from the recirculation of mature B cells in the blood, bone marrow, spleen and lymph nodes. A slow proliferation of these B cells in lymphoid territories seems to depend on the activation of the B-cell receptor pathways and on signals provided by the microenvironment [3]. The chromosomal abnormalities of leukemia cells are found in more than 80% of the cases, the most common being deletion 13q14 (in ~50% of the cases), followed by deletion 11q, trisomy 12 and deletion 17p. Similar to other B-cell malignancies, advances in molecular biology and genetics have led to improved accuracy in the prognostication of CLL patient outcome. In addition to the cytogenetic abnormalities (del11q and del17p), the mutation pattern of the immunoglobulin heavy gene, the mutation of *P53* and the expression of protein markers, such as CD38 or ZAP70, have improved the likelihood of stratifying patients with different outcomes [4]. Recent advances in understanding the biology of malignant B cells have resulted in the development of new targeted therapeutic agents, such as kinase inhibitors [5].

More than two-thirds of newly diagnosed patients are initially asymptomatic and require no treatment until they develop symptoms and the disease progresses. The majority of these patients will remain asymptomatic for years and eventually die of unrelated diseases. However, at the time of their diagnosis or during follow-up, when patients present with significant lymph node or spleen enlargement, clinical symptoms, anemia, thrombocytopenia or other CLL-related complications (particularly autoimmune manifestations), a specific anti-tumor treatment is indicated. Previously, chemotherapy was used to treat CLL without substantial progress regarding outcome and survival [4]. Alkylating agents (such as chlorambucil), purine analogs

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(such as fludarabine, cladribine or pentostatin), bendamustine or eventually a combination of these medications constituted the primary armamentarium for controlling the disease. Recently, randomized trials have demonstrated that the addition of anti-CD20 mAb rituximab to fludarabine plus cyclophosphamide combination significantly improved the overall survival (OS) rate in patients with CLL [6]. However, this strategy was essentially developed for patients who were either young or fit enough to tolerate this regimen, which could potentially result in significant hematological and infectious toxicities [4]. Therefore, until recently, although the majority of patients diagnosed with CLL are older than 70 years of age and frequently present with co-morbidities or functional impairments, no treatment had proven to be superior to chlorambucil alone to improve the OS rate of this population [7,8].

From rituximab to third-generation anti-CD20 mAbs

Combined with conventional chemotherapy, the anti-CD20 mAb rituximab has significantly increased response rates, progression-free survival (PFS) and OS in patients with B-cell malignancies, leading to its wide use in non-Hodgkin B-cell lymphoma (NHL) and CLL [9–11]. Despite these significant advances, several indolent B-cell malignancies, such as CLL or follicular lymphoma, are incurable; patients treated with rituximab may also relapse or become resistant to rituximab-related regimens. Consequently, the development of new agents became a high priority to improve patient outcomes and to delay the onset of relapse and to achieve a higher cure rate.

Given the success of rituximab, CD20 remains an attractive therapeutic target, especially because its expression is restricted to B-cell precursors and mature B cells: the antigen is not expressed on hematopoietic stem cells (preventing severe hematological toxicities) or on plasma cells (contributing to preserve acceptable immunoglobulin serum concentrations) [12]. Thus, novel anti-CD20 antibodies with different functional activities have been developed to potentially ensure efficacy.

Antibodies against CD20 utilize complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and direct induction of cell death to various extents according to their intrinsic mechanism of action and their classification in type I or type II antibody molecules [12,13]. This classification translates their ability to induce the reorganization of CD20 molecules into lipid rafts in the cell membrane after antibody binding. Type I and II mAbs differ in how they bind to CD20, which influences the activation of cell death pathways. The majority of new anti-CD20 antibodies have been characteristic of the type I variety, including veltuzumab [14], ocrelizumab [15], ofatumumab [16], ocaratuzumab [17] or ublituximab [18].

Type I antibodies (rituximab-related) induce the translocation and thus the stabilization of CD20 molecules into lipid rafts, which leads to stronger C1q recruitment and efficiently activates complement-mediated cell death (CDC) but only low levels of direct cell death [13,19]. The occurrence of resistance to type I mAbs appears to be associated with the loss of CD20 by 'shaving' (also designated as trogocytosis, which is an endocytic process for removing CD20 molecules from the cell surface) or antibody internalization [20].

Because type II mAbs do not generate the translocation of CD20 molecules into lipid rafts, C1q binding is reduced and complement is consequently poorly activated [13,21]. As observed for type I mAbs, ADCC and phagocytosis are induced. However, in contrast to type I antibodies, type II mAbs are able to induce significantly increased direct cell death [21]. Less resistance to therapy can be expected because of significantly less antigenic internalization [20], which may prolong the presence of the antibody on the cell surface and, therefore, likely increase ADCC. The prototype of type II antibody is the murine antibody tositumomab, which was developed for therapeutic use as a radio conjugate but not as a humanized antibody.

Advances in mAbs-based therapy were allowed with the generation of humanized or fully human second-generation mAbs. Early examples include veltuzumab (with enhanced CDC compared with rituximab) [14], ocrelizumab (with increased binding affinity for the low-affinity variant of the FcγRIIIa receptor) [15] and ofatumumab. The latter is a human mAb type I anti-CD20 antibody that binds to a novel epitope of the CD20 antigen. Several preclinical data indicated that ofatumumab demonstrated a slower off-rate from the target and an increased CDC, compared with rituximab [22]. Ofatumumab demonstrated single-agent activity in patients with CLL or NHL in Phase I/II clinical trials. In the initial Phase II study conducted in 33 patients with relapse or refractory CLL [23], the response rate was 50%; however, the median time to disease progression was only 3.5 months. A pivotal study was conducted in 138 CLL patients who were refractory to both fludarabine and alemtuzumab or had bulky disease refractory to fludarabine [24]. The proposed therapeutic regimen consisted of 300 mg on day 1 followed by 2000 mg weekly for 12 doses. The response rates of 47–58% and the median PFS of 5.9–5.7 months were achieved for patients with bulky or double refractory disease [24]. These results were used for the initial registration of the medication (in the USA and Europe) in this restricted population of patients with refractory CLL. More recently, the combination of ofatumumab and chlorambucil was approved in the USA as the first-line therapy of CLL based on the results of a randomized study evaluating this combination against chlorambucil alone [25].

Obinutuzumab

Chemistry & formulation

Obinutuzumab (formerly designated as GA101 and marketed under the trade name Gaziva® by F. Hoffmann-La Roche) is a unique glycoengineered type II humanized anti-CD20 monoclonal antibody of the IgG1 subclass. It recognizes a specific epitope of the CD20 molecule found on B cells [26]. The molecular mass of the antibody is approximately 150 kDa [27]. The obinutuzumab drug substance is manufactured by fermenting a recombinant mammalian cell suspension culture and subsequently purifying the antibody. An antibody-producing cell line was established in a Chinese hamster ovary K1 cell line.

Obinutuzumab is intravenously administered as a sterile, clear, colorless to slightly brown and preservative-free liquid concentrate. The agent is supplied at a concentration of 25 mg/ml in 1000 mg single-use vials. In addition to the antibody, the liquid contains histidine, trehalose, poloxamer 188 and highly purified water. The pH is 6.0 [28].

Improving activity by antibody engineering

GA101 was derived by humanization of the parental murine IgG1-K antibody B-ly1 and glycoengineering [21]. cDNAs encoding variable chain regions were cloned from the B-ly1 hybridoma, and their complementarity-determining regions were grafted onto variable acceptor frameworks. The variants with complete identity to the human germline and with high-binding affinity to human CD20 were selected. Furthermore, the variants were screened for their ability to induce direct cell death in human B-cell lymphoma cells *in vitro*. A sequence alteration in the immunoglobulin heavy chain elbow-hinge region, an area that is known to affect the flexibility of the Fab domain, was demonstrated. The most active variants, including GA101, harbored a human germline variable heavy framework 1 with a valine residue instead of leucine residue present in the murine Ab [21].

During the stage of elaboration of the agent, this substitution led to a different membrane compartmentalization of obinutuzumab–CD20 complexes compared with type I mAbs. Thus, obinutuzumab/A101 induces homotypic cell aggregation and direct cell death by forming stable complexes with CD20 at the sites of cell–cell contact. These differences with type I mAbs, which are known to stabilize a different and more dynamic population of CD20 within lipid rafts, appear to be associated with superior direct cell killing properties [21,29].

Moreover, ADCC mechanisms, mediated by effector cells expressing FcγRI, FcγRII and primarily FcγRIII (displayed on NK cells), might contribute to the clinical effect of anti-CD20 mAbs [12]. Enhanced ADCC activity can be obtained by engineering the Fc portion of the antibody (amino acid substitution or glycosylation modification) to improve its affinity for FcγRIII receptor and subsequently to increase the activation of effector cells. In addition to modifications of its variable region, obinutuzumab harbored a glycoengineered afucosylated Fc segment to improve the binding affinity for FcγRIII. Thus, obinutuzumab induces increased antibody-dependent cytotoxicity that is 5- to 100-fold greater than that observed with rituximab [30,29].

In summary, the glycoengineered obinutuzumab mediates B-cell lysis through the following characteristics: high ADCC, high-affinity binding to the CD20 antigen, low CDC activity and high levels of direct cell death induction (Figure 1).

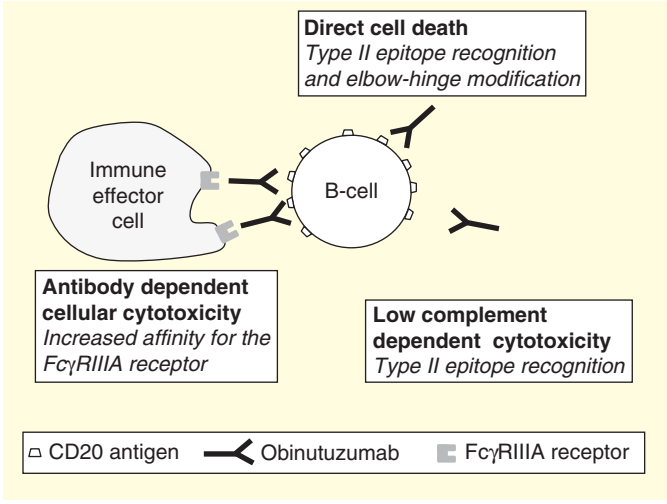

Figure 1. Schematic representation of the activity of the obinutuzumab monoclonal antibody on B-cells.

Pharmacodynamics, pharmacokinetics & metabolism of obinutuzumab

Pharmacodynamics

In Phase I of the Phase I/II GAUGUIN trial (BO20999) of obinutuzumab monotherapy in patients with relapse or refractory CLL, rapid (within days) and sustained elimination of CLL cells was achieved; 12 of 13 patients exhibited B-cell depletion ($CD19^+$ cell counts $<0.07 \times 10^9$/l). The initial CD19 B-cell recovery was observed in several patients approximately 9 months after the last dose. At 18 months of follow-up, several patients remained B-cell depleted [31].

In Phase II of this trial, peripheral B-cell depletion occurred rapidly after the first infusion of obinutuzumab; all of the patients (n = 20) achieved levels below 4000 B cells/µl within 1 week of treatment [32].

Results from the Phase I study of 21 patients with relapse/refractory $CD20^+$ NHL showed no significant changes in the serum levels of complement fraction. For nine patients for whom data were available, no clinically significant changes in IgA, IgG and IgM levels were observed 4 or more months after treatment was completed compared with pretreatment [33].

Pharmacokinetics

A two-compartment pharmacokinetic model comprising both a linear clearance pathway and a non-linear time varying clearance pathway was fitted to the data from Phase I/II GAUGUIN (BO20999) [34].

Tumor burden is perceived to contribute significantly to the clearance of obinutuzumab, especially at the commencement of treatment. With decreasing tumor burden, the clearance was shown to reach an asymptote. Consequently, the available PK data indicate that patients with a higher disease burden may have faster clearance of obinutuzumab. The clearance decreased with time and for repeat treatment. This occurrence was perceived to be attributed to a decline in the number of $CD20^+$ tumor cells. These findings are consistent with previous assumptions for CD20 antibodies in which elimination is primarily a function of the target antigen [34].

In the dose ranges explored, obinutuzumab serum concentrations increased with each escalated dose. In Phase I of the GAUGUIN study of 21 patients with heavily pretreated,

Table 1. Response rates observed during Phase I and Phase II studies in patients with chronic lymphocytic leukemia.

Study	Obinutuzumab dose	Patients (n)	Overall response rate (%)[§]
Phase I	400/800 mg[†]	3	33
	800/1200 mg or 1000/1000 mg[†]	6	50
	1200/2000 mg[†]	4	100
Phase II	1000 mg[‡]	20	25

[†]Patients in Phase I were scheduled to receive the lower flat dose on day 1 of cycle 1, the escalated flat dose on day 8 of cycle 1 and thereafter on day 1 of cycles 2–8 (total = 9 doses).
[‡]Patients in Phase II were scheduled to receive the same dose (1000 mg flat) on day 1, day 8 and day 15 of cycle 1, then on day 1 of cycles 2–8 (total = 10 doses).
[§]All responses were PR except one patient with CR at the dose level of 800/1200 mg.
Data taken from [31,32].

relapse or refractory CD20$^+$ indolent NHL, the patients received GA101 in a dose-escalating fashion (3 per cohort, range 50/100–1200/2000 mg) for 8 × 21-day cycles. For the three lower dose cohorts (50/100, 100/200 and 200/400 mg), the serum concentrations remained persistently below 200 μg/ml and the increases in concentration were small. Following doses of 400/800 mg and higher, there were substantial increases in serum obinutuzumab concentrations compared with the lower doses and a tendency of serum concentrations to increase during the treatment course [33,35,34].

Obinutuzumab appears to be distributed primarily intravascularly. Based on a population pharmacokinetic analysis, the geometric mean (CV%) volume of the distribution of obinutuzumab at steady state is approximately 3.8 l. The mean terminal clearance and the elimination half-life are approximately 0.09 l/day and 28.4 days, respectively [34].

The pharmacokinetics of obinutuzumab was studied in specific populations. Neither the age nor renal impairment with baseline creatinine clearance (CLcr) >30 ml/min affected the pharmacokinetic parameters of the drug. Obinutuzumab has not been studied, neither in patients with a baseline CLcr <30 ml/min nor in patients with hepatic impairment [27].

Metabolism

Metabolic studies were not conducted for obinutuzumab. Protein agents are degraded into amino acids that are subsequently recycled, eventually for synthesis of other proteins. An assessment of protein binding is not applicable to biologics.

Efficacy

Phase I & II studies

Early Phase I and Phase II results using obinutuzumab monotherapy in patients with relapse or refractory CLL were only presented in abstract form at the time of this review. During the Phase I study [31] with escalating doses of GA101 from 400 to 2000 mg, the investigators reported an optimal overall response rate of 62% in 13 patients, according to the International Workshop on Chronic Lymphocytic Leukemia; 1 patient achieved an incomplete complete response (CR) and 7 patients a partial response (PR). Several responses appeared to be sustained, with a median response duration of 10.5 months. B-cell depletion (assessed using CD19 staining) was nearly complete for all 13 patients. In the Phase II study [32], 20 patients with relapse/refractory CLL received a flat dose of 1000 mg GA101 administered on days 1, 8, 15 and 22 and q21 (total of 10 infusions). Only 16 patients were evaluated for hematology results and tumor volume assessment using CT scans; the response rate was 25%; 4 patients had PR, 5 patients had stable disease and 7 patients had disease progression. Why these preliminary results appeared discordant between the Phase I and the Phase II studies (Table 1) has not been established; however, the differences in patient selection, tumor volume at the time of inclusion, higher GA101 doses in the last phase cohort of Phase I (1200 and 2000 mg flat doses) might all be considered relevant to these observations. Based on these preliminary data, the development of obinutuzumab in patients with CLL was essentially conducted using combinations of mAb and other agents, either cytotoxic chemotherapy agents or targeted therapies. Notably, a single agent study comparing the activity of obinutuzumab at doses of 1000 or 2000 mg in untreated patients with CLL was recently conducted [36].

Phase III pivotal study

The recently published, randomized, pivotal Phase III study conducted in previously untreated elderly patients with CLL aimed at comparing three treatment arms: one arm comprised chlorambucil alone, one arm comprised chlorambucil plus rituximab and one arm comprised chlorambucil plus obinutuzumab [37]. This trial [38], called CLL11, was conducted in collaboration with the German CLL Study Group and F. Hoffmann-La Roche in 26 countries. The eligibility criteria included patients with confirmed CD20$^+$ CLL who were diagnosed according to standard international criteria [39] and required treatment (i.e., patients with Binet stage B or C or symptomatic disease). Additionally, the patients had to present a cumulative illness rating scale score higher than 6, which reflects the presence of clinically meaningful coexisting conditions, or an alteration of the CLcr (between 30 and 69 ml/min using the Cockcroft-Gault formula). All of the patients received chlorambucil at 0.5 mg/kg on day 1 and on day 15 of six 28-day cycles. In the rituximab–chlorambucil treatment arm, rituximab was added on day 1 to each of the 6 cycles at the dose of 375 mg/m^2 on cycle 1 and at 500 mg/m^2 on subsequent cycles. In the obinutuzumab–chlorambucil treatment arm, obinutuzumab was delivered at 1000 mg flat doses on days 1, 8 and 15 of cycle 1 and then on day 1 of each

Table 2. Summary of the results of CLL11, comparing chlorambucil with obinutuzumab–chlorambucil and rituximab–chlorambucil.

	Study arm	Patients (n)	Overall response rate	Complete response rate	Negative MRD testing (PB/BM)	Median PFS (months)	Hazard ratio (95% CI)
Stage 1a analysis	Ob-Clb	238	75.5%	22.2%	31.1%/ 17%	23.0	0.14 (0.09–0.21)
	Clb	118	30%	0%	0%/0%	10.9	
Stage 2 analysis	Ob-Clb	333	78.4	20.7	37.7%/ 19.5%	26.7	0.39 (0.31–0.49)
	R-Clb	330	65.1	7.0	3.3%/ 2.6%	15.2	

The study design allowed for comparison of the stage 1 analysis of paired obinutuzumab–chlorambucil versus chlorambucil alone (stage 1a, first 2 lines) as well as rituximab–chlorambucil versus chlorambucil (not shown). The stage 2 comparison (lines 3 and 4) was performed after additional follow-up and included a higher number of patients treated using one of the antibodies in combination with chlorambucil.
BM: Bone marrow; Clb: Chlorambucil; MRD: Minimal residual disease; Ob-Clb: Obinutuzumab plus chlorambucil; PB: Peripheral blood; PFS: Progression-free survival; R-Clb: Rituximab–chlorambucil.
Data taken from [37].

subsequent cycle. The primary end point of the study was PFS assessed by the site investigators. Secondary end points included centrally assessed PFS (by an independent review committee), response rates and the rate of negative testing for minimal residual disease (MRD) at the end of treatment, the OS rate and adverse events (AEs). The study was designed to provide a first-stage comparison of chlorambucil alone versus rituximab–chlorambucil and versus obinutuzumab–chlorambucil as well as a second-stage comparison of rituximab–chlorambucil versus obinutuzumab–chlorambucil. Hence, the CLL11 aimed to address the following interrogations: whether the addition of each of the anti-CD20 antibodies to chlorambucil was superior to chlorambucil alone and whether obinutuzumab–chlorambucil was superior to rituximab–chlorambucil.

The final study population included 781 patients with a median age of 73 years, a median cumulative illness rating scale score of 8 and a median CLcr of 62 ml/min [37]. Approximately 78% of the patients had Binet stage B or C disease. There were no significant differences in the baseline characteristics between groups in the three pairwise comparisons.

The analysis of the first stage of the study was reported in May 2013 during the 49th meeting of the American Society of Clinical Oncology [40]. The analysis demonstrated a superior efficacy of both rituximab–chlorambucil and obinutuzumab–chlorambucil for the primary end point, that is, PFS: the median PFS was 10.8 months in the chlorambucil arm versus 15.7 months in the rituximab–chlorambucil arm, with a hazard ratio (HR) of 0.32 (95% CI: 0.24–0.44; $p < 0.0001$) and 23.0 months in the obinutuzumab–chlorambucil arm (HR: 0.14; 95% CI: 0.09–0.21; $p < 0.0001$). These first-stage results were confirmed in the final analysis [37] (Table 2), with a significant superiority of obinutuzumab–chlorambucil (HR: 0.18; 95% CI: 0.13–0.24; $p < 0.0001$) over chlorambucil alone. The overall response rate with obinutuzumab–chlorambucil was 77% (including 55% of the patients with PR and 22% with CR, respectively) versus 31% with chlorambucil alone (all of these patients were in the PR category). Furthermore, there was a significant OS benefit (HR: 0.41; 95% CI: 0.23–0.74; $p = 0.002$) for the patients who received obinutuzumab–chlorambucil compared with the patients who received chlorambucil only.

The comparison of the efficacy of the obinutuzumab versus rituximab combinations was examined in the second stage of the analysis (Table 2) [37]. A significant improvement of PFS was observed in patients who received the type II antibody compared with patients who received the type I antibody (median PFS 27 vs 15 months, respectively; HR: 0.39; 95% CI: 0.31–0.49; $p < 0.0001$). Similarly, the clinical response rate was significantly improved ($p < 0.0001$) in patients who received obinutuzumab (58% of PR and 21% of CR) versus those who received rituximab (58% of PR and 7% of CR). This difference in the quality of response was exemplified with the results of the MRD data; 19.5% of the patients were noted to be MRD negative in bone marrow and 38% in peripheral blood in the obinutuzumab-containing arm versus 3 and 3%, respectively, for those treated in the rituximab-containing arm ($p < 0.001$ for both comparisons). Despite a lower rate of deaths observed in the obinutuzumab arm (8%) than in the rituximab arm (12%), no significant OS difference was observed between the two [37].

In conclusion (Table 2), this large Phase III study established that the addition of obinutuzumab to chlorambucil is clearly superior to chlorambucil alone in this population of previously untreated patients with CLL in terms of PFS, response rate and OS. Furthermore, obinutuzumab combined with chlorambucil significantly improved the quality of response (with more patients in CR and more patients with a negative MRD test) and significantly delayed the risk of disease progression compared with the rituximab–chlorambucil combination.

Table 3. Adverse drug reactions of any grade with an incidence of at least 3% higher in patients with obinutuzumab–chlorambucil compared with patients with chlorambucil alone in the CLL11 study.

	Ob-Clb	Clb
	n = 241	*n = 116*
General disorders and administration site conditions (n, %)		
Infusion-related reaction	166 (69)	–
Pyrexia	25 (10)	8 (7)
Cough	23 (10)	8 (7)
Blood and lymphatic system disorders (n, %)		
Neutropenia	98 (41)	21 (18)
Thrombocytopenia	37 (15)	9 (8)
Leukopenia	17 (7)	–
Metabolism and nutrition disorders (n, %)		
Tumor lysis syndrome	10 (4)	1 (<1)
Hyperuricemia	8 (3)	–

Clb: Chlorambucil alone; Ob-Clb: Obinutuzumab plus chlorambucil.
Data taken from [37].

Obinutuzumab safety

Emerging safety signals during Phase I/II studies

In the Phase I and II studies [31,32], with a limited number of patients (n = 13 in Phase I and n = 20 in Phase II), a few AEs of grade 3 or 4 emerged in several patients consisting essentially of transient neutropenia (9/13 patients in Phase I but only 4/20 patients in Phase II), thrombocytopenia (2 patients in Phase I and 2 patients in Phase II), infusion-related reactions (2 patients in Phase I and 6 patients in Phase II) and tumor lysis syndrome (1 patient in Phase I), as well as a few cases of infections (3 patients in Phase I and 3 patients in Phase II) with or without neutropenia. These data were consistent with those observed in other B-cell malignancies in the North American and European trials [41,33], in which grade 3–4 infusion-related reactions (18 and 14%, respectively) and neutropenia (23 and 10%, respectively) were also reported.

Consolidated safety data from the pivotal Phase III study

However, solid safety outcomes in patients with CLL were identified in the randomized, controlled, open-label Phase III trial CLL11 conducted in treatment-naïve CLL patients [37].

As expected, AEs occurred more frequently in the antibody groups than in the chlorambucil-alone group, including patients treated with obinutuzumab–chlorambucil. Several AEs were reported to have an incidence of 3% or higher in patients with obinutuzumab–chlorambucil compared with patients with chlorambucil alone (Table 3), thus reflecting the additional toxicity related to obinutuzumab. According to the Medical Dictionary for Drug Regulatory Activities, the AEs concerned general disorders, such as infusion-related reactions and hematological events, such as leukopenia, neutropenia, thrombocytopenia and tumor lysis syndrome [37].

When focusing on the toxicities of grade 3 and higher, the same AEs were most frequently reported in patients with obinutuzumab–chlorambucil compared with patients with chlorambucil alone, with infusion-related reactions (21 vs 0%), leukopenia (5 vs 0%), neutropenia (35 vs 16%) and thrombocytopenia (11 vs 4%) [37]. Notably, although the incidence of hemorrhagic events was not different between the three study arms, four cases of fatal hemorrhagic events occurred in the obinutuzumab–chlorambucil arm after cycle 1 (vs none in the rituximab–chlorambucil arm and one in the chlorambucil-alone arm).

Despite an increased incidence of neutropenia observed with both obinutuzumab and rituximab in the CLL11 trial, this finding did not result in an increased incidence of infection of any grade (Table 4). Grade 3–5 infection rates ranged from 11 to 14% and did not differ significantly among the treatment groups. The majority of reported infections were of bacterial origin [37]. Similarly, although several neoplasms were diagnosed in patients with obinutuzumab during the study, the frequencies were similar among the treatment groups. Finally, the AEs-related death rate was lower in the obinutuzumab-containing arm (4%) compared with the rituximab–chlorambucil (6%) or chlorambucil-alone (9%) arm.

Particular attention was drawn to the infusion-related reactions occurring in obinutuzumab recipients and led to recommend premedication prior to administration. Moreover, grade 3 and higher infusion-related AEs occurred in 20% of patients in the obinutuzumab plus chlorambucil group versus 4% of those in the rituximab plus chlorambucil group [37]. Although prophylactic measures were implemented during the trial (e.g., premedication using glucocorticoids and administration of the first dose of obinutuzumab over a 2-day period), infusion-related reactions were reported, including severe reactions leading to the withdrawal of therapy, and were identified as a particular risk of obinutuzumab–chlorambucil treatment. A tumor lysis syndrome occurred in 14 patients in the obinutuzumab plus chlorambucil group versus zero in those in the rituximab plus chlorambucil group. All grade 3 or 4 infusion-related reactions occurred during the first infusion of obinutuzumab but rarely during subsequent infusions, which indicated that this event was linked to the rapid and profound depletion of B cells following the first dose of obinutuzumab infused. Neither the lymphocyte count nor the tumor burden at baseline was a strong predictor of obinutuzumab-related infusion reactions [37].

Other safety & prescribing information

As with other anti-CD20 antibodies, rare but potentially serious reactions that may occur with obinutuzumab include hepatitis B virus reactivation and progressive multifocal leukoencephalopathy [28].

According to the manufacturer and the US FDA recommendations [27,28], patients are advised to seek immediate attention

Table 4. Infections of any grade reported in the Phase III trial of CLL11 study.

	Infections of any grade in the different study arms n (%)					
	Ob-Clb vs Clb		R-Clb vs Clb		Ob-Clb vs R-Clb	
	Ob-Clb	*Clb*	*R-Clb*	*Clb*	*Ob-Clb*	*R-Clb*
	n = 241	n = 116	n = 225	n = 116	n = 336	n = 321
Febrile neutropenia	6 (2)	5 (4)	4 (2)	5 (4)	10 (3)	4 (1)
Urinary tract infection	15 (6)	3 (3)	2 (<1)	3 (3)	18 (5)	5 (2)
Oral herpes	9 (4)	1 (<1)	3 (1)	1 (<1)	11 (3)	5 (2)
Nasopharyngitis	17 (7)	8 (7)	7 (3)	8 (7)	19 (6)	10(3)
Bronchitis	11 (5)	8 (7)	10 (4)	8 (7)	12(4)	16 (5)
Pneumonia	12 (5)	4 (3)	12 (5)	4 (3)	17 (5)	20 (6)
Upper respiratory tract infection	5 (2)	5 (4)	10 (4)	5 (4)	8 (2)	15 (5)
Respiratory tract infection	8 (3)	4 (3)	6 (3)	4 (3)	9 (3)	7 (2)

The study design allowed for comparison of the stage 1 analysis of paired obinutuzumab–chlorambucil versus chlorambucil alone (stage 1a, first 2 columns), rituximab–chlorambucil versus chlorambucil (stage 1b, columns 3 and 4), where the chlorambucil arm as reference is similar in columns 2 and 4. The stage 2 comparison was performed after a longer follow-up and included a higher number of patients treated with one of the antibody in combination with chlorambucil.
Clb: Chlorambucil alone; Ob-Clb: Obinutuzumab plus chlorambucil; R-Clb: Rituximab plus chlorambucil.
Data taken from [37].

if they experience any of the following symptoms of infusion reactions (i.e., dizziness, nausea, chills, fever, digestive troubles, breathing problems or chest pain), symptoms of tumor lysis syndrome (i.e., nausea, vomiting, diarrhea and lethargy), signs of infection (i.e., fever and cough) and new or changes in neurological symptoms (i.e., confusion, dizziness or loss of balance, difficulty talking or walking or vision problems). Other recommendations include periodic monitoring of blood counts and the avoidance of vaccinations using live viral vaccines. Patients with a history of hepatitis B should be monitored and should eventually receive prophylactic treatment against hepatitis B reactivation. The potential effects of obinutuzumab on the QTc interval have not been studied.

Given the recent approval (1 November 2013) of obinutuzumab in the USA, no public post-marketing surveillance report has been released.

Phase Ib/II ongoing studies with obinutuzumab

Given the results achieved with chlorambucil and obinutuzumab combinations, several other combinations are currently being evaluated. These include combination with classical cytotoxic agents such as the fludarabine–cyclophosphamide (FC) association or bendamustine [42–44]. Preliminary results of the non-randomized study GALTON evaluating the safety and efficacy of these combinations for 6 cycles in previously untreated patients with CLL were recently presented [45]. A total of 41 patients were enrolled and the preliminary results were reported with a median follow-up of about 1 year. The overall and CR rates were respectively of 90 and 20% in the bendamustine plus obinutuzumab combination arm (20 patients), and 62 and 10% in the FC plus obinutuzumab arm (21 patients). Grade 3–4 AEs observed in more than 10% of the patients included neutropenia (50%), febrile neutropenia (10%) and thrombocytopenia (10%) in the bendamustine–obinutuzumab arm; and neutropenia (29%), infections and febrile neutropenia (10% each), anemia (14%) and elevation of transaminases (19%) in the FC–obinutuzumab arm. Infusion-related reactions were observed in 91% of the patients in the FC–obinutuzumab (29% grade 3–4) arm and 90% (10% grade 3–4) in the bendamustine–obinutuzumab arm. With a short follow-up (11.9 months), no patient experienced disease progression during the study.

Also of interest are combinations of obinutuzumab with targeted therapies. Presently active studies include combinations with another biological agent, otlertuzumab (TRU-016), a novel anti-CD37 therapeutic protein [46], with a PI3 kinase delta-specific inhibitor (TRG-1202) [47] and with the specific Bcl2 inhibitor GDC/ABT-0199 [48]. This latter combination is of great interest given the high efficacy of GDC/ABT-0199 in previously treated CLL patients [49] and the potential synergy of both drugs to induce cell death in CLL.

Regulatory affairs

Obinutuzumab (Gazyva) was approved by the FDA for '*for use in combination with chlorambucil for the treatment of patients with previously untreated* CLL' on 1 November 2013 in the USA based on the results of the CLL11 study [50]. This agent received the first breakthrough therapy designation delivered by the FDA. This designation, requested by the sponsor, can be recognized '*if preliminary clinical evidence indicates the drug offers a substantial improvement over available therapies for patients with serious or life-threatening diseases*'. The medication

was also granted a priority review by the FDA because of the significant improvement of a serious condition and an orphan product designation given the rarity of CLL [50]. The posology of obinutuzumab approved follows the dose and schedule that were used in the CLL11 study, with the recommendation to fractionate the first dose between day 1 (100 mg) and day 2 (900 mg). A boxed warning underscores the risks of hepatitis B reactivation and progressive multifocal leukoencephalopathy. Other warnings include the risk of infusion-related reactions (and the need for premedication using glucocorticoid, acetaminophen and anti-histamine) and tumor lysis syndrome, neutropenia, thrombocytopenia and the contraindication for immunization using live virus vaccines [27].

In Europe [51], obinutuzumab was granted by the European Commission to Roche Registration Limited, UK, an orphan designation (EU/3/12/1054) for the treatment of CLL. After a positive opinion of the Committee for Medicinal Products for Human Use [52], the European Commission has approved obinutuzumab (as Gazyvaro®) as the first-line treatment for patients with CLL in July 2014 [53].

Conclusion

In conclusion, obinutuzumab represents the first humanized glycoengineered type II antibody developed in humans; this anti-CD20 mAb presents an original mode of action that includes direct B-cell kill activity and enhanced ADCC. The safety data are consistent with those of other anti-CD20 antibodies and focus on infusion reactions and tumor lysis syndrome during the first infusion in patients with CLL. Presently, the efficacy data for a single agent in relapsed/refractory patients with CLL are incomplete. In the setting of previously untreated patients with CLL who are unable to tolerate intensive treatments, obinutuzumab combined with chlorambucil dramatically improved the outcome of these patients. Furthermore, in the same population, the combination of obinutuzumab and chlorambucil was shown to be significantly superior to the rituximab and chlorambucil treatment in terms of response rates, depth of response and delay in disease progression. These data, together with a re-assuring safety profile in the Phase III study, led to the marketing approval of obinutuzumab in combination with chlorambucil in the USA and Europe. This anti-CD20 antibody appears to constitute a pivotal component for the treatment of CLL patients and will likely contribute to improved patient outcome.

Expert commentary

The development of obinutuzumab in CLL was rapid, based on early positive signals obtained during the Phase I study. The results of the CLL11 study indicate that obinutuzumab is superior to rituximab in CLL patients, when obinutuzumab is combined with a weak cytotoxic agent, for example, chlorambucil. Based on preclinical data and pharmacokinetic information during the Phase I/II studies, the chosen dose and the schedule of administration are specific for this antibody and range between the doses approved for rituximab and those approved for ofatumumab. The safety of the antibody does not appear to be markedly different from that of other anti-CD20 antibodies; however, infusion reactions (and tumor lysis) are more frequent, which most likely reflects the potency of obinutuzumab, and its capability of rapidly depleting circulating B cells in the peripheral blood. Precautionary actions and monitoring during the first infusion could help to easily manage this AE. The combination of obinutuzumab plus chlorambucil appears to represent currently a standard therapeutic approach for patients with CLL who are ineligible for the classical FC–R (FC and rituximab) combination.

However, the type I anti-CD20 mAb ofatumumab has shown a superiority to chlorambucil when it is combined with chlorambucil in the same setting [25]. Although the patients in both studies are not strictly comparable, the CR rate and median PFS achieved in the ofatumumab–chlorambucil combination study (12% of CR and 22 months of median PFS) might be slightly inferior to those observed with the obinutuzumab combination (22% of CR and 27 months of PFS) in the CLL11 study [37]. A head-to-head clinical comparison of both antibodies, which elicits distinct mechanisms of action *in vitro* (predominantly complement-dependent cell cytotoxicity for ofatumumab versus direct cell kill and increased ADCC for obinutuzumab) will be interesting.

In the near future, several points must be addressed concerning the optimal use of the anti-CD20 antibody. The optimal dose, schedule of administration and the duration of treatment for patients with CLL can be explored in the following query: Will higher doses or a more prolonged treatment (than the 6 cycles used in the CLL11 study) result in an additional clinical benefit, without compromising the safety of its use?

Another crucial point to investigate is the efficacy and safety of obinutuzumab with other cytotoxic agents, such as bendamustine (which can be used in younger and older patients), purine analogs or the combination of fludarabine and cyclophosphamide. Such studies are underway (see section "Phase Ib/II ongoing studies with obinutuzumab") and their results are eagerly anticipated. Finally, given the rapid emergence of new agents with different modes of action [5], such as B-cell receptor inhibitors (ibrutinib), inhibitors of the PI3 kinase delta subunit (idelalisib) or agonists of the Bcl2 protein (ABT-199), other combinations will need to be assessed.

Five-year view

Obinutuzumab will likely replace rituximab in the daily use of anti-CD20 antibodies in patients with CLL, if the safety of its combination with other cytotoxic agents is confirmed. However, the field of CLL is rapidly changing with the emergence of new compounds targeting intracellular signaling pathways (ibrutinib, idelalisib, ABT-199 and others) or the tumor microenvironment (lenalidomide) [4,5]. The rapid development of these new compounds would lead to the use of chemotherapy-free approaches, especially in the elderly population or in the relapse setting. A multiplicity of combinations would need to be investigated. If the therapeutic goal is aimed at achieving the

best quality of response, hoping to ensure a prolonged PFS and an improved OS, then regimens that will achieve the higher rates of clinical, immunophenotypic and molecular CR will be preferable. Considering the data on the negative rates of MRD in the CLL11 study, obinutuzumab will most likely constitute one component in these regimens. However, prolonged therapy with oral agents, if the emergence of resistance to those remains low, may constitute another alternative for frail patients or for those who have failed previous therapies. The choice of medications and combinations will likely be driven not only by convenience, but also by cost. The future of CLL treatment is likely to be a field with multiple options, and therapeutic objectives, patient wishes and healthcare resources will likely be considered to optimize patient care.

Information resources

Relevant information regarding CLL management can be found in [4] and [5]. The most relevant articles on anti-CD20 antibodies are [13] and [14].

The preclinical development of obinutuzumab is presented in [21]. The CLL1 pivotal study is discussed in [37].

Additional information is available on the following sites:

www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm373263.htm

www.gazyva.com/patient?cid=gne_WE_00000083

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Key issues

- As in other B-cell malignancies, anti-CD20 monoclonal antibodies constitute a pivotal component in the treatment of patients with chronic lymphocytic leukemia (CLL). Rituximab and ofatumumab are type I anti-CD20 mAb that are already approved for the treatment of CLL.
- Obinutuzumab is a new and distinct anti-CD20 antibody, which recognizes a different epitope of the CD20 molecule and engages different modes of action *in vitro* and *in vivo* to deplete B cells. Specifically, this type II glycoengineered and humanized mAb exerts a high level of direct cell kill and enhanced antibody-dependent cell-mediated cytotoxicity.
- In a Phase III randomized study for the first-line treatment of CLL patients with advanced age and co-morbidities, the combination of obinutuzumab plus chlorambucil demonstrated a significant clinical benefit over chlorambucil alone (significant overall survival benefit) and a superior efficacy (CR rate and PFS) over the combination of rituximab and chlorambucil. The obinutuzumab combination showed a favorable safety profile, with a higher incidence of infusion-related reactions at the time of the first infusion that must be prevented and monitored.
- Obinutuzumab has been approved as the first-line treatment of patients with CLL in combination with chlorambucil and is likely to play a key role in the evolving management of this disease in the future.

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