Expert Opinion

Obinutuzumab for the treatment of lymphoproliferative disorders

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Introduction: Targeted therapy against CD20 with the mAb rituximab has led to significant improvements in survival for patients with B-cell non-Hodgkin's lymphoma (NHL). Despite these improvements, many patients relapse and/or become refractory after rituximab-containing therapies and thus better therapies are required for NHL.

Areas covered: Obinutuzumab is a novel, humanized, anti-CD20 mAb currently being investigated in Phase III studies in comparison to rituximab. An overview of obinutuzumab, its mechanisms of action and the results of pre-clinical and Phase I/II studies are presented.

Expert opinion: Pre-clinical studies suggest that obinutuzumab is a more potent anti-CD20 mAb than Rituximab at inducing antibody-dependent cellular cytotoxicity (ADCC) and direct cell death (DCD). Obinutuzumab is safe and effective in CD20 + NHL and further study is warranted. Results of ongoing Phase III clinical trials comparing Obinutuzumab to Rituximab in different disease settings and with different chemotherapy regimens are eagerly awaited.

Keywords: antibody, CD20, chronic lymphocytic leukemia, lymphoma

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1. Introduction

Non-Hodgkin's lymphoma (NHL) comprises a variety of clinical entities of which the vast majority (80 – 85%) are derived from B cells. Over 90% of these B-cell lymphomas express CD20 and are thus amenable to targeted therapy directed against CD20. CD20 is an ideal target for directed therapy, being highly expressed on most B cells [1] and not expressed on stem cells or precursor cells nor on the majority of plasma cells, such that B cell development and mature antibody production are not impaired by anti-CD20 therapy [2].

The development of the anti-CD20 mAb rituximab provided one of the first examples of successful targeted therapy for cancer and has revolutionized the treatment of NHL. Immuno-chemotherapy, comprising chemotherapeutics and rituximab is now the standard treatment for most NHL and has resulted in progression-free (PFS) and overall survival (OS) advantages in diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL), the three most frequent lymphoproliferative disorders. The curability of DLBCL has increased by more than 15% since the addition of rituximab [3] and the introduction of rituximab to chemotherapy in CLL presented the first ever demonstration of an OS advantage in this disease [4]. Similarly, survival outcomes are improved in patients with FL treated with rituximab and chemotherapy [5,6]. However, despite the notable impact of rituximab in the treatment of NHL, its single-agent efficacy is only modest, especially in CLL [7], and many patients either fail to respond or relapse after rituximab-containing therapies.

Clearly, CD20 mAbs are of paramount importance in the treatment of NHL; therefore great efforts are underway to develop novel mAbs that can provide greater efficacy than rituximab. Several such mAbs have been developed and are currently being investigated. The majority of these novel anti-CD20 mAbs (including rituximab) are

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Box 1. Drug summary.

Drug name	Obinutuzumab
Phase	Phase II/III
Indication	Treatment of $CD20^+$ (B-cell) non-Hodgkin's lymphoma
Pharmacology description/mechanism of action	Anti-CD20 monoclonal antibody resulting in high levels of antibody-dependent cellular cytoxicity (ADCC) and direct cell death induction
Route of administration	Intravenous
Pivotal trials (s)	Ongoing

termed type 1 antibodies. These antibodies function via stabilization of CD20 on lipid rafts, resulting *in vitro* in strong complement (C1q) binding and potent induction of complement-dependent cytotoxicity (CDCC) [8]. The exact mechanisms of *in vivo* activity of rituximab and other type 1 molecules remain controversial with some studies suggesting primarily CDCC activity [9-15] and others demonstrating more antibody-dependent cellular cytotoxicity (ADCC) [16,17]. A second class of mAbs, the type 2 antibodies, do not require lipid rafts and thus leave CD20 distributed across the surface of the B cell and have much lower *in vitro* complement binding and CDCC, but result in significantly greater ADCC and direct cell death (DCD) compared with type 1 mAbs [18]. The first well-studied type 2 mAb, iodine 131-tositumomab (B1, Bexxar, iodine-131 anti-B1) is a murine mAb conjugated to iodine 131, recognizing the B1 (CD20) antigen. Iodine-131-tositumomab has documented potent efficacy in indolent CD20 + NHL [19,20] but has not been widely adopted by hematologists/oncologists, probably due to a lack of comfort with radioactive compounds. A novel type 2 mAb, obinutuzumab (**Box 1**) (GA101, RO5072759) has shown efficacy in *in vitro* studies, animal models and in early-phase clinical trials and is currently being investigated in Phase III trials.

The exact mechanism(s) of resistance to rituximab are not well understood however, studies have suggested several mechanisms [21] including: CD20 'shaving' in which rituximab–CD20 complexes are removed from the B cell surface by monocytes through an endocytic reaction called trogocytosis [22-24], aberrant lipid raft composition of some malignant B cells [25,26], complement depletion [1,27], polymorphisms in the FcγRIIIa receptor reducing the affinity of the Fc receptor for rituximab [28,29], downregulation of pro-apoptotic proteins [30] and reduction in CD20 antigen expression levels after treatment with rituximab [31,32]. It remains unclear whether novel mAbs, such as the type 2 mAb obinutuzumab will be able to overcome these resistance mechanisms.

Obinutuzumab has demonstrated activity in pre-clinical studies and is currently being investigated in comparison with rituximab in several Phase III trials. Obinutuzumab is a type 2 humanized anti-CD20 mAb that has a glyco-engineered Fc portion, selected to increase its affinity for FcγRIIIa receptors on immune effector cells, intended to elicit enhanced ADCC. Obinutuzumab also contains a modified elbow hinge region to provide superior antigen binding [18,33]. The elbow hinge modification is reported to increase DCD but at the expense of reduced CDCC activity (**Figure 1**). Both antibody modifications were designed to induce much greater cell killing by obinutuzumab compared with rituximab [34].

2. Pre-clinical studies

Several *in vitro* studies of obinutuzumab have been performed and demonstrate superior efficacy over rituximab. Initial studies attempted to mimic *in vivo* conditions by examining the activity of obinutuzumab in whole-blood assays [33]. The assays incorporated immune effector cells as well as complement so that ADCC, CDCC and DCD could be measured. Effective B cell depletion was demonstrated in whole blood from 10 healthy donors, with obinutuzumab exhibiting significantly greater efficacy in B cell depletion than rituximab. Similar findings were noted with depletion of malignant B cells in the whole blood of a patient with CLL [33,35]. Several assays, including: binding to NHL cells lines, assays of cell death, ADCC and CDCC and B cell depletion measures in peripheral blood from healthy donors were also examined. Obinutuzumab exhibited increased DCD compared with type 1 mAbs in a panel of NHL cells lines, including DLBCL and mantle cell lymphoma (MCL) and exhibited up to 100 times higher ADCC activity but significantly less CDCC compared with the type 1 antibodies [33,36].

Several *in vitro* studies have also been performed to clarify the mechanism of cell death induced by obinutuzumab. Alduaij and colleagues determined that the DCD induced by obinutuzumab occurs by a non-apoptotic process involving actin reorganization and lysosomes. DCD by obinutuzumab was compared with that of rituximab in cells lines derived from Burkitt lymphoma, DLBCL, MCL and several patient-derived (primary) B cell malignancy samples. Significantly higher cell death was noted in response to obinutuzumab compared with rituximab in all experiments [34]. The importance of lysosomes in the induction of cell death was highlighted in these experiments and is a novel mechanism that appears to be unique to type 2 antibodies [37]. As this mechanism of cell killing is independent of classic apoptosis pathways, it is postulated that obinutuzumab may be able to overcome resistance to chemotherapy-induced apoptosis, an important resistance mechanism in multiply-treated NHL [34,38].

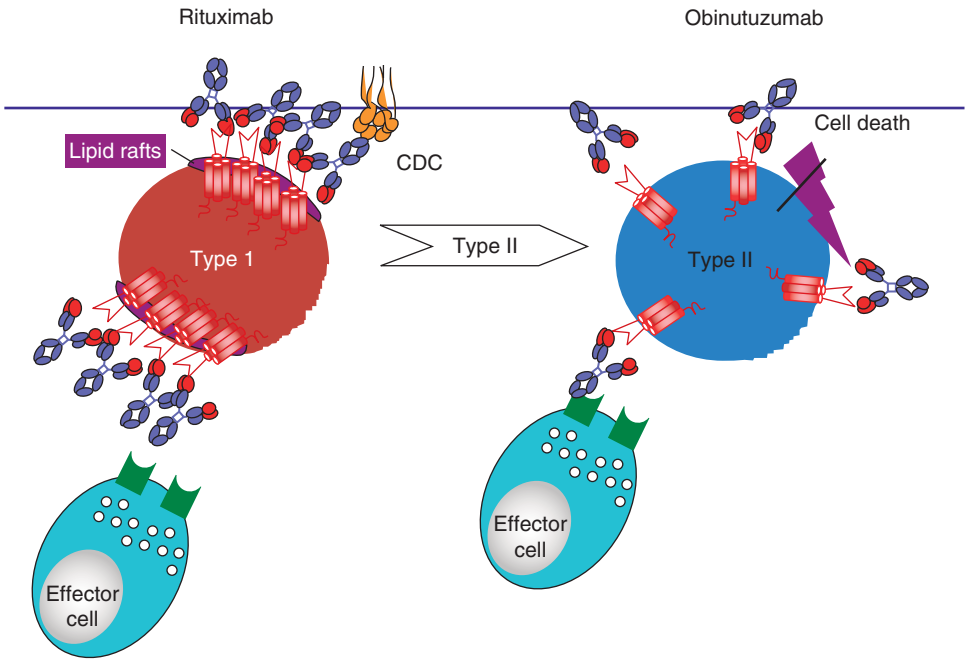

Figure 1. The differing mechanisms of action of type 1 (rituximab) and type 2 (obinutuzumab) antibodies. Type 1 antibodies function via stabilization of CD20 on lipid rafts, resulting in vitro in strong complement-dependent cytotoxicity (CDCC), while type 2 antibodies leave CD20 distributed across the surface of the B cell and have much lower in vitro CDCC, but greater antibody-dependent cellular cytotoxicity (ADCC) and direct cell death (DCD). Obinutuzumab has a glyco-engineered Fc portion, selected to increase its affinity for FcγRIIIa receptors on immune effector cells, and a modified elbow hinge region to provide superior antigen binding.

Source: GA101 overview presentation at B021005 and B021223 study investigator meetings, July 2011, San Fransisco, CA. Permission to use and modify from Michael Wenger, Global Clinical Lead GA101, Hoffmann-La Roche.

The efficacy of obinutuzumab has also been demonstrated in animal models, showing effective B cell depletion by the drug in Cynomolgus monkeys. Obinutuzumab showed a particular improvement in B cell depletion in the spleen and lymph nodes of the monkeys compared with rituximab [33]. Xenograft models including SCID beige mouse models of DLBCL using a human DLBCL cell line, SUDHL-4, were also investigated, with obinutuzumab demonstrating improved tumor killing compared with rituximab. SUDHL-4 cells were injected into the mice allowing measurable growth of tumors, then treatment was initiated with either rituximab or obinutuzumab. Obinutuzumab resulted in complete tumor eradication in all animals at the highest dose with durable tumor control in nine out of ten mice treated at the highest dose level. In comparison, tumor growth was inhibited by rituximab but no tumor regression was observed at any dose [33].

Other xenograft models included Z138, a MCL line [39] and RL, a FL cell line, also assessed in SCID mice [13]. In the MCL model, obinutuzumab and rituximab were examined in combination with fludarabine or bendamustine. Obinutuzumab in combination with fludarabine or bendamustine exhibited significantly better tumor inhibition than the same chemotherapy with rituximab. Obinutuzumab as a single agent was observed to be equally efficacious compared with the combination of rituximab with bendamustine or fludarabine [39]. Similarly, obinutuzumab induced stronger inhibition of tumor growth than rituximab in the FL cell line, both as a single agent and in combination with cyclophosphamide [13].

An additional study attempted to investigate the efficacy of obinutuzumab in the setting of rituximab resistance. SUDHL-4 DLBCL mice were treated with weekly rituximab and followed until tumor development reached an advanced stage. The mice were then randomized to receive more rituximab, a placebo injection or obinutuzumab. The tumors continued to grow rapidly in the rituximab and placebo arms but tumor growth was successfully arrested in the obinutuzumab-treated animals, suggesting efficacy of obinutuzumab in this model of rituximab-resistance [33].

3. Clinical studies

3.1 Phase I studies

Table 1 summarizes the published clinical studies of obinutuzumab in NHL. Two Phase I studies of obinutuzumab have been performed in patients with relapsed/refractory CD20 + NHL. The B020999 study assessed dose escalation from 50 to 2000 mg starting with once weekly infusions on days 1 and 8 and then continued at three-weekly intervals for six

Table 1. Summary of clinical trials examining the efficacy of obinutuzumab in lymphoma.

Trial	Study design	Number of subjects		Patient population	Obinutuzumab dose/ combination
B020999	Phase I	34		Relapsed/refractory $CD20^+$ NHL (indolent NHL, aggressive NHL and CLL)	Single-agent, dose-escalation (400 – 2000 mg)
	Phase II	100	40	Relapsed/refractory indolent NHL	Low dose (LD) cohorts: 400 mg days 1 and 8 of cycle 1, 400 mg day1 cycles 2 – 6
			40	Relapsed/refractory aggressive NHL	High dose (HD) cohorts: 1600 mg days 1 and 8 of cycle 1, 800 mg day 1 of cycles 2 – 6
			20	Relapsed/refractory CLL	
B021003	Phase I	22		Relapsed/refractory NHL	200 – 2000 mg days 8 and 22 and repeated every four weeks for six cycles (ay 1 50% dose). Responding patients receive maintenance therapy every 3 months for 24 months
	Phase II	149		Relapsed/refractory indolent NHL	Obinutuzumab 1000 mg weekly for 4 weeks followed by maintenance every 2 months for 24 months
B021000	Phase Ib	56	28	Relapsed/refractory FL	LD cohort : 400 mg days 1 and 8 of cycle 1, 400 mg day 1 cycles 2 – 6 + CHOP or FC
			28	Relapsed/refractory FL	HD cohort : 1600 mg days 1 and 8 of cycle 1, 800 mg D1 of cycles 2 – 6 + CHOP or FC
B021004	Phase III	Not yet reported		Previously untreated CLL with comorbidities	Obinutuzumab 1000 mg days 1, 8 and 15 for cycle 1 then 1000 mg day1 every 28 days for cycles 2 – 6 + chlorambucil
B021005	Phase III	Not yet reported		Previously untreated advanced DLBCL	Obinutuzumab 1000 mg days 1, 8 and 15 for cycle 1 then day1 every 21 days for cycles 2 – 6 + CHOP
B021223	Phase III	Not yet reported		Previously untreated patients with advanced FL or. MZL	Obinutuzumab 1000 mg days 1, 8, 15 for cycle 1 then day 1 for cycles 2 – 6 + chemotherapy

CHOP: Cyclophosphamide, doxorubicin, vincristine, prednisone, CLL: Chronic lymphocytic leukemia, DLBCL: Diffuse large B cell lymphoma, FC : Fludarabine, cyclophosphamide, FL: Follicular lymphoma, MZL: Marginal zone lymphoma, NHL: Non-Hodgkin's lymphoma.

cycles (nine doses of obinutuzumab in total). The drug was safe with no dose-limiting toxicities and responses were noted at all dose levels. Grade 1 – 2 infusion-related reactions (IRR), most frequently with the first dose, were very common as were minor (Grade 1 – 2) infections. Rare cases of tumor lysis syndrome (TLS) were observed [40]. In patients with CLL, Grade 3 – 4 neutropenia was also frequent [41].

A second Phase I study (B021003) examined obinutuzumab monotherapy followed by maintenance therapy in responding patients. Obinutuzumab was administered as a flat dose (200 – 2000 mg) on days 1, 8 and 22 and repeated every four weeks for six cycles but with the first infusion administered at 50% dose. Responding patients received maintenance obinutuzumab every three months for two years. Again, the most common adverse events were Grade 1 – 2 IRRs; however five Grade 3 – 4 reactions were recorded with four Grade 3 IRRs, one associated with TLS and 1 Grade 4 IRR with hypoxia, for which the patient was discontinued from further obinutuzumab therapy [42].

The results of a Phase Ib combination chemo-immunotherapy study (B021000) including obinutuzumab have also been reported. A total of 56 patients with relapsed/refractory FL were treated with four to six cycles of obinutuzumab with fludarabine + cyclophosphamide (FC) (n = 28) or obinutuzumab with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) (n = 28). Patients were randomized to either a low dose or a high dose of obinutuzumab. The low-dose cohort was treated with 400 mg of obinutuzumab for all infusions while the high-dose cohort received 1600 mg on days 1 and 8 and 800 mg for all subsequent infusions. Responding patients were then offered maintenance at their randomized dose level every 3 months for 2 years or until progression. Again, IRRs were the most common adverse events with up to 80% of patients experiencing IRRs, mostly with the first infusion. Grade 3 – 4 IRRs occurred in 7% of patients. Grade 3 – 4 neutropenia was frequent, occurring in 36 and 50% of patients in the obinutuzumab–CHOP and obinutuzumab–FC arms, respectively. Infections occurred in 16% of patients with no deaths reported. The end of treatment response (EOR) was 94% in the obinutuzumab-CHOP arm and 93% in the obinutuzumab–FC arm. While the results are too immature for assessment of duration of response, these early results suggest safety and potent activity of obinutuzumab in combination with chemotherapy [43].

3.2 Phase II studies

3.2.1 Indolent lymphoma

The results of a Phase II component of the B020999 study, examining monotherapy with obinutuzumab without maintenance therapy, were recently presented for 40 patients with indolent NHL. The patients were randomized to receive obinutuzumab in the low-dose (n = 18) or high-dose (n = 22) cohorts as previously described, with nine total doses received. The patients were heavily pre-treated with a median of three prior regimens and 55% were rituximab-refractory. The EOR

was 17% in the low-dose cohort and 55% in the high-dose cohort. Of the rituximab-refractory patients in the high-dose cohort 50% had objective responses and responses were recorded in patients with the lower affinity FcγRIIIa receptor. The median PFS was 6 months for the low-dose cohort and 11.3 months for the hgh-dose cohort and ten patients had ongoing responses at the time of presentation. Toxicities were similar to those described in the Phase I study, with the addition of three Grade 3 – 4 neutropenia events, one febrile neutropenia and one Grade 3 – 4 thrombocytopenia, all in the high-dose cohort [44].

A randomized Phase II study (B021003) has also been performed in relapsed indolent CD20 + NHL, comparing single agent obinutuzumab at 1000 mg weekly for four doses, followed by maintenance therapy every two months for two years, compared with single agent rituximab at 375 mg/m^2 weekly for four doses followed by the same maintenance schedule, in patients who were not rituximab-refractory. Assessment after completion of induction in 149 patients with FL showed ORR of 43% for obinutuzumab versus 28% for rituximab when assessed by central blinded radiology review. The difference was less marked when based on investigator assessment with ORR rates of 43 and 39% for obinutuzumab and rituximab, respectively. No unexpected toxicities were noted [45].

3.2.2 *Aggressive lymphoma*

A total of 40 patients with relapsed/refractory CD20 + aggressive lymphoma were also reported in the Phase II B020999 study, including 25 patients with DLBCL and 15 patients with MCL. The patients were randomized to receive obinutuzumab in the low-dose (n = 21) or high-dose (n = 19) cohorts as previously described. The EOR was 24% in the LD and 32% in the high-dose cohorts and was equivalent for DLBCL (28%) or MCL (27%) patients. IRRs were common including three severe IRRs with the first infusion and two episodes of TLS. Grade 3 – 4 neutropenia was uncommon, occurring in only one patient in the high-dose cohort. Thus the tolerability and efficacy of obinutuzumab was thought to be encouraging given the high risk nature of these heavily pre-treated patients. The median response duration was 8.6 months for the low-dose cohort and had not yet been reached for the high-dose cohort [46].

3.2.3 *Chronic lymphocytic leukemia*

The B020999 Phase II study in CLL patients included 20 patients with relapsed/refractory CLL who received obinutuzumab at 1000 mg on Days 1, 8, 15, 22 and then every three weeks for a total of ten infusions. The 1000 mg dose was selected based on the higher efficacy of the HD compared to the LD regimen and based on pharmacokinetic data and modeling and simulation [47]. There were six Grade 3 – 4 IRRs and four Grade 3 – 4 neutropenias. The EOR was 20% with four PR. A relationship was noted between the level of tumor burden and the response rate as the four PR patients had lower tumor burdens than the non-responding patients. The conclusion from this study was that obinutuzumab is safe in patients with advanced CLL but that the single-agent activity is modest and combination chemo-immunotherapy was likely to be necessary for most CLL patients, especially those with higher tumor burdens [48].

3.3 Current Phase III studies

Given the encouraging results of these early-phase clinical trials, there is much interest in the outcomes of several recently started Phase III clinical trials. Three such studies are underway in the frontline treatment setting including: B021005 investigating obinutuzumab and CHOP versus rituximab-CHOP in previously untreated DLBCL, B021223 investigating obinutuzumab with chemotherapy versus rituximab with chemotherapy in previously untreated indolent lymphoma and the B021004 study of obinutuzumab with chlorambucil (CLB) versus rituximab with CLB or CLB alone in previously untreated elderly or unfit patients with CLL. These studies are currently ongoing and results are eagerly anticipated.

The run-in phase of the B021004 study in CLL patients was recently reported addressing the safety and tolerability of obinutuzumab in this elderly or unfit CLL population. Six patients were enrolled to receive six cycles of obinutuzumab and CLB. None of the patients required withdrawal of treatment though treatment was delayed in three patients, mainly due to neutropenia. IRRs were very common, occurring in five patients but were mostly of Grade 1 – 2. Rapid clearance of lymphocytes was reported within days of the first dose of obinutuzumab and CLB, though no EORs have yet been reported [49].

Phase III studies are also being conducted in patients with relapsed/refractory CD20 + NHL including a study comparing obinutuzumab and bendamustine to monotherapy with bendamustine.

4. Expert opinion

The addition of rituximab to the treatment of NHL and CLL has had a significant and clinically important effect on survival. Since the introduction of rituximab, many pharmaceutical companies have been attempting to develop a better anti-CD20 mAb to out-perform rituximab. To date, none of these novel compounds has proven to be sufficiently 'better' than rituximab in the clinical setting though many have stimulated early interest with improved potency *in vitro*. Most of these novel mAbs are type 1 antibodies similar to rituximab, and this may explain why most have not resulted in a marked difference in efficacy *in vivo* but the lack of improvement with these other novel agents warns that the same may prove true for obinutuzumab.

Obinutuzumab, a type 2 mAb has demonstrated increased potency over rituximab in CD20 + NHL and CLL in several early-phase studies. Many investigators have criticized the current comparisons of rituximab and obinutuzumab due to the higher administration dose of obinutuzumab. Unfortunately,

no study has yet clearly examined the pharmacokinetics of rituximab, particularly in relation to rituximab-resistance and it is possible that larger doses of rituximab would be more effective. Some early studies of single-agent rituximab suggested improved results in patients with higher serum concentrations of the antibody [50] and a recent study in elderly DLBCL patients suggested that higher doses of rituximab resulted in improved PFS and OS in high-risk patients compared with results in similar patients treated previously with standard doses of rituximab [51]. Therefore, it may be that the current comparisons of rituximab and novel antibodies, like obinutuzumab are not fair comparisons and that higher doses of rituximab should be examined. This weakness of the Phase III studies that are currently accruing may prevent clinicians from switching from rituximab to obinutuzumab even if clinically significant differences in outcomes are observed. Similarly, some have suggested that the potency of rituximab might be increased by combining it with hypomethylating agents or histone deacetylase inhibitors in an attempt to prevent downregulation of CD20 on malignant cells [52]. While rituximab dose escalation studies are likely to be safe, studies involving combinations with novel agents will require more detailed safety analysis and would probably be associated with more adverse events than would the replacement of rituximab with a more potent antibody like obinutuzumab.

The preliminary obinutuzumab data indicates that this novel type 2 antibody is the most potent anti-CD20 antibody in current development and has justified the current Phase III studies, which are comparing obinutuzumab to rituximab in a number of CD20 + lymphoproliferative disorders. Despite encouraging early-phase clinical results and the wealth of *in vitro* studies showing improved efficacy over rituximab, the effect of obinutuzumab in the clinical setting still needs to be objectively measured. The results of the largest clinical study to date showed no ORR difference by investigator assessment but a clinically significiant 15% improvement in ORR by independent radiology review. Thus, good quality evidence of improved clinical outcomes with obinutuzumab is not yet available.

Thus, while it is feasible that obinutuzumab may one day replace rituximab in all areas of hematological oncology, it is also possible that the efficacy and/or superiority of obinutuzumab may vary in different subtypes of CD20 + NHL and/or in different individuals. It is probable that superiority of obinutuzumab will be easier to demonstrate in diseases with poor rituximab single-agent efficacy, such as CLL and MCL. However, the limited single-agent activity of obinutuzumab in the B020999 study in CLL patients, suggests that even this agent may not have sufficient potency to result in marked improvement in CLL. Additionally, given the excellent modern-day outcomes for DLBCL, a marked improvement in outcomes would be required to justify replacing rituximab in this disease.

We must also carefully assess possible early and late toxicities that may be associated with the potential higher potency of this novel mAb. This includes not only early IRRs and infections, but also less anticipated late effects of obinutuzumab. Unusual effects of rituximab were reported years after routine use, including delayed neutropenia, progressive multifocal leukoencephalopathy and possible lung toxicity [53,54]. Therefore, vigilance must be exercised while investigating for adverse effects of this more potent anti-CD20 mAb.

While it will probably be several years before we have a full understanding of the value of obinutuzumab in NHL and CLL, current evidence suggests that this novel agent may help improve outcomes for some patients with CD20 + lymphoproliferative disorders.

Declaration of interest

Both authors have received honoraria for Advisory Boards and institutional research support from Hoffmann-La Roche, Canada, and have participated in Obinutuzumab clinical trials sponsored by Hoffmann-La Roche.

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