EXPERT OPINION

Update on obinutuzumab in the treatment of B-cell malignancies

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Introduction: The anti-CD20 mAb rituximab has revolutionized the treatment of B-cell malignancies, improving outcome for patients. Despite these improvements, the majority of patients still relapse and become refractory to rituximab. Further efforts to improve anti-CD20 mAb efficacy have recently focused on obinutuzumab/GA101, a novel anti-CD20 mAb glycoengineered to display enhanced Fc-mediated effector mechanisms and induce direct cell death.

Areas covered: We provide an overview of the current insights into the mechanisms of action of obinutuzumab focusing on how structural modifications and differences to rituximab led to designation of obinutuzumab as a type II antibody. We summarize data from preclinical studies and recent clinical trials including the Phase III trial in chronic lymphocytic leukemia (CLL), which led to FDA approval in November 2013.

Expert opinion: Clinical data are now emerging confirming the promise of the initial preclinical data that demonstrated superior efficacy of obinutuzumab over rituximab at similar dosing. The emerging randomized Phase III data from older comorbid patients with previously untreated CLL demonstrated significant improvements in molecular remission rates and median progression-free survival of obinutuzumab plus chlorambucil versus rituximab plus chlorambucil. This emerging data provide reasons to be optimistic that outcomes for patients with B-cell malignancies can be further improved with obinutuzumab.

Keywords: antibody, CD20, chronic lymphocytic leukemia, glycoengineering, non-Hodgkin lymphoma, obinutuzumab

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1. Introduction

An estimated 156,420 people in the US are expected to be diagnosed with a hematological malignancy in 2014 (9.4% of all new cancer cases) [1]. The majority of these is of B-cell origin and expresses the B-cell restricted antigen CD20 on the cell surface. The anti-CD20 mAb rituximab has been approved for use in the treatment of the B-cell malignancy follicular lymphoma (FL) since 1997. Subsequently, the addition of rituximab to chemotherapy demonstrated improvements in overall survival as part of the initial treatment of non-Hodgkin lymphoma (NHL) such as diffuse large B-cell lymphoma (DLBCL) and FL and more recently chronic lymphocytic leukemia (CLL) [2-8]. Although rituximab/chemotherapy combinations are now the standard of care in these diseases, a significant number of patients with DLBCL and most patients with FL or CLL eventually relapse and die of treatment refractory disease [9,10]. Given the success of rituximab as well as the urgent need to improve outcomes further, a number of different anti-CD20 mAb with novel or enhanced antibody effector mechanisms have been developed and are emerging into the market as potential competition for rituximab in first-line therapies and

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Box 1. Drug summary.

Drug name	Obinutuzumab
Phase	III/Licensed
Indication	Treatment of $CD20^+$ (B-cell) chronic lymphocytic leukemia
Pharmacology description/mechanism of action	mAb to CD20 eliciting antibody-dependent cell-mediated cytotoxicity (ADCC) and direct programmed cell death
Route of administration	Intravenous
Pivotal trial(s)	CLL11 Phase III trial in previously untreated elderly CLL pts with comorbidities in combination with chlorambucil showed ORR 26.7 versus 11.1 months chlorambucil alone and led to FDA approval in CLL.

CLL: Chronic lymphocytic leukemia; ORR: Overall response rates.

for use in rituximab refractory disease. To date, all of these anti-CD20 mAbs such as ofatumumab (US FDA licensed in CLL 2009) are type I anti-CD20 mAb. Obinutuzumab (**Box 1**) is the only glycoengineered type II mAb to enter the clinic and is the focus of this drug evaluation.

2. Preclinical studies

2.1 Type I/II antibody classification

Anti-CD20 mAb engage multiple Fab and Fc-mediated mechanisms of cellular cytotoxicity, including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and direct programmed cell death (PCD) [11]. The primary mode of action employed, together with the antigen-binding characteristics of the antibody, allows the discrimination of anti-CD20 mAbs into two distinct classes (**Table 1**). Those which induce high levels of CDC, but low levels of PCD, are designated type I [12-16] and include rituximab, ofatumumab, ublituximab, veltuzumab and ocaratuzumab [17-23]. Conversely, type II anti-CD20 mAb such as tositumomab and obinutuzumab only weakly induce CDC but potently elicit PCD [15,24,25].

Development of next-generation mAbs has focused on structural modifications, which augment specific cytotoxicity mechanisms according to the type I/II bias. Thus, the type I ofatumumab that binds with higher affinity and slower off rate to an epitope of CD20 distinct from that recognized by rituximab, consequently displays greatly enhanced CDC activity [19,26]. Similarly, other mAbs such as ocaratuzumab (AME-133v) have been designed with enhanced affinity for both CD20 and CD16, leading to greater activation of NK cells [27,28].

Obinutuzumab (GA101/Gazyva, Roche Glycart AG) is a humanized, glycoengineered anti-CD20 mAb classified as a type II antibody on the basis of minimal capacity to elicit CDC (being 10 – 1000 fold less potent than rituximab/ofatumumab) [29], but displaying enhanced ability to activate ADCC and evoke PCD [25]. In part, these type II attributes are a consequence of a modified elbow-hinge sequence in the variable region, with substitution of a valine residue for a leucine at Kabat position 11, arising during the process of humanization. Indeed, mutation of the valine back to the original leucine leads to increased CDC, decreased homotypic adhesion and reduces PCD levels back to those seen with rituximab [25]. This molecular modification results in an altered binding orientation and wider elbow angle of nearly 30° compared to type I mAb [30] with recognition of the core epitopes 172 – 178 compared to 168 – 175 for rituximab. Consequently, obinutuzumab induces differential redistribution and compartmentalization of CD20 antigen compared to rituximab, resulting in distinct cellular responses according to the type I/II dichotomy (**Table 1**). Rituximab and other type I mAb bind between CD20 tetramers (inter-tetramer) whereas type II mAb may bind within one tetramer (intra-tetramer) resulting in a 2:1 binding ratio with two type I CD20 mAb per CD20 tetramer compared to one type II mAb [30,31]. Binding of obinutuzumab causes stable accumulation of CD20 at sites of homotypic adhesion during cellular aggregation, whereas ligation by rituximab in an inter-tetramer fashion sequesters CD20 molecules within dynamic assemblies of lipid raft membrane microdomains, providing a spatial distribution that favors enhanced complement deposition [25,30].

2.2 Glycoengineering of obinutuzumab

Although the principal effector mechanisms that mediate therapeutic efficacy achieved with rituximab are unclear, Fc-mediated effector mechanisms are thought to be important with Fc-γ receptor (FcγR) polymorphisms at residue 158 predicting response to rituximab monotherapy in FL patients [32]. The significance of this polymorphism is less clear for rituximab given in combination with chemotherapy in NHL with prognostic significance in some studies [33-36] but not others [37-41]. No prognostic significance has so far been observed in CLL patients, perhaps as a result of overall impaired function of effector cells in CLL [42-44]. Therefore, obinutuzumab was glycoengineered to enhance Fc effector functions. Core fucose residues were removed from carbohydrate moieties in the Fc region by defucosylation [25]. The glycosylation status of IgG, as determined by the oligosaccharide composition of the antibody heavy chain, is known to impact on effector function by altering the affinity for the cognate Fc receptor [45]. Defucosylation increases the affinity of the Fc for FcγRIIIa without impacting on binding to other FcγRs or FcRn (neonatal receptor), resulting in enhanced recruitment and activation of immune effector cells [45-49]. Consequently, obinutuzumab displays a 50-fold higher affinity for FcγRIIIa compared to nonengineered antibody [47,50] and in comparison to the type I mAbs rituximab and ofatumumab has 100-fold greater ADCC activity [29,51]. *In vitro*, whole blood

Table 1. Comparison of type I and type II anti-CD20 mAb.

	Type I mAb	Type II mAb
Associated mAb	Rituximab, ofatumumab, ublituximab, veltuzumab, ocaratuzumab	Obinutuzumab, tositumumab
Binding characteristics	Inter-tetramer binding	Inter-tetramer binding, resulting in half-maximal binding compared to type I mAb (i.e., 2:1 ratio type I:type II mAb)
CDC	Induce re-localization of CD20 to lipid rafts and potent CDC	Do not induce CD20 redistribution to lipid rafts; low levels of CDC
ADCC	Induce FcγR-mediated cell killing	Induce FcγR-mediated cell killing. Defucosylation increases affinity for FcγRIIIa and enhances ADCC, for example, obinutuzumab
ADCP	Induce phagocytosis of target cells	Induce phagocytosis of target cells. Enhanced by glycoengineering, for example, obinutuzumab
PCD	Low/negligible levels of direct PCD, apoptotic in nature	High levels of caspase-independent PCD, mediated by homotypic adhesion, lysosome permeabilization and ROS
Release of immunogenic factors	Complement-dependent release of HMGB1 (demonstrated for rituximab)	PCD-dependent release of HMGB1, HSP, ATP
Modulation of CD20 antigen	mAb binding results in internalization of CD20 antigen or shaving/trogocytosis	No internalization following mAb binding

ADCC: Antibody-dependent cell-mediated cytotoxicity; ADCP: Antibody-dependent cellular phagocytosis CDC: Complement-dependent cytotoxicity; FcγR: Fc-γ receptor; HMGB1: High-mobility group box 1; HSP: Heat shock proteins; PCD: Programmed cell death; ROS: Reactive oxygen species.

B-cell depletion assays are a useful tool to measure the combined effects of mAb effector mechanisms and have demonstrated the superiority of obinutuzumab to rituximab and ofatumumab to deplete B cells (healthy volunteers) and leukemic B cells (CLL patients) [25,29,52-54]. Obinutuzumab was more effective than a glycoengineered version of rituximab, suggesting that this activity may be due in part to recognition of a distinct epitope and PCD [25]. Although rituximab activity was highly complement-dependent, obinutuzumab activity was less so [29] and could be reduced by blocking CD16 [52]. Obinutuzumab also induced greater NK-cell activation [53], neutrophil activation, cytokine production and phagocytosis than rituximab [54].

More recently, glycoengineering has been shown to result in enhanced ADCP. Although initial studies showed obinutuzumab did not induce greater ADCP than rituximab [29], later studies in situations more closely reflecting the natural physiological state where levels of competing nonspecific endogenous IgGs were high demonstrated enhanced ADCP in monocytes/macrophages with obinutuzumab through FcRIIIa binding [55]. Obinutuzumab has also been shown to have increased affinity for FcγRIIIb on neutrophils [54], leading to increased neutrophil activation and phagocytosis as a direct result of glycoengineering.

2.3 Direct cell death

In keeping with its classification as a type II anti-CD20 mAb, obinutuzumab has been shown to induce direct PCD in a variety of B-lymphoma cell lines and primary human malignancies, including FL, DLBCL and mantle cell lymphoma (MCL), as well as CLL [25,56,57]. Ligation of CD20 by type II mAb, including obinutuzumab, but not type I mAb such as rituximab, results in homotypic adhesion and caspase-independent, nonapoptotic cell death that is contingent upon lysosome permeabilization and cathepsin release (**Figure 1**) [24,56-59]. The terminal phase of the cell death pathway is also dependent on the extra-mitochondrial generation of reactive oxygen species, arising from the activation of an NADPH oxidase [60]. Both reactive oxygen species production and cell death are independent of Bcl2 expression or pharmacological inhibition of caspase activity, suggesting that obinutuzumab and other type II mAb are able to overcome apoptosis-resistance mechanisms and may enable effective deletion of malignant clones that are refractory to conventional chemotherapy or immunotherapy. Moreover, the Fc region of the mAb is dispensable for this mechanism, meaning that ligation of FcγR is not required for the induction of direct cell death. Therefore, this pathway potentially remains active in patients with impaired Fc-dependent responses, such as those with low-affinity FcγRIIIa polymorphisms, or in which immune effector cell saturation, exhaustion or depletion has occurred [32,61,62]. The exact contribution of PCD-induction to patient outcome remains to be determined but preclinical evidence supports the notion that type II mAbs such as obinutuzumab engage effector mechanisms, which may remain operational in clinical scenarios where type I mAb activity is diminished.

The underlying mechanisms associated with acquired rituximab resistance are currently poorly defined. In one study, a quarter of relapsed NHL patients were shown to have lost CD20 protein expression [63] and CD20 gene mutations have been reported [64,65] but other mechanisms such as

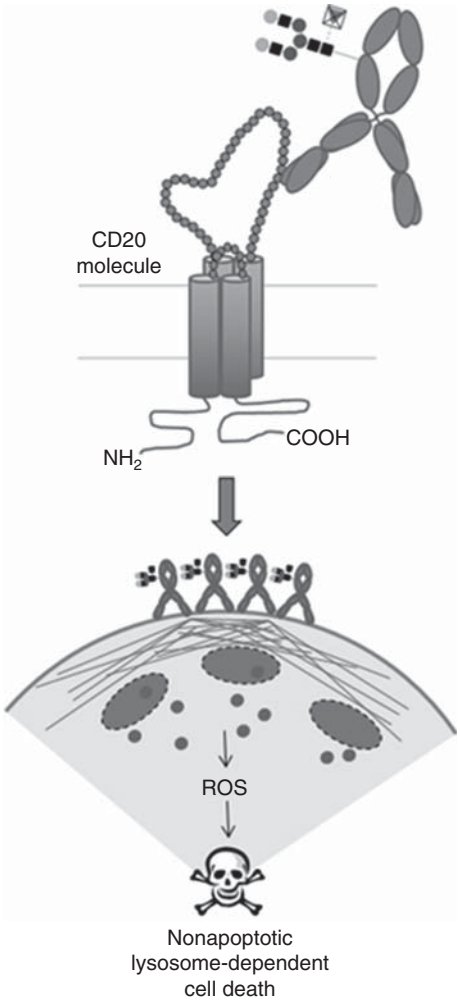

Figure 1. The Type II anti-CD20 mAb obinutuzumab has been glycoengineered by defucosylation of the glycan chain. Binding of obinutuzumab to CD20 leads to intra-tetramer binding, actin reorganization, homotypic adhesion, lysosomal permeabilization and ROS-dependent cell death.
ROS: Reactive oxygen species.

effector cell exhaustion [62] and tumor/immunological microenvironment must also have a role to play. Type I mAbs such as rituximab have been shown to downregulate CD20 on the cell surface through internalization of the CD20-mAb complex [66] or trogocytosis (shaving of rituximab-CD20 complexes from the cell surface in an Fc-dependent manner) [67], leading to reduced mAb half-life. Type II mAbs on the contrary do not induce CD20 internalization and were shown to be 5 times more potent in B-cell depletion in a human CD20 transgenic mouse model [66]. Tumor cell resistance to apoptosis can predict impaired response to chemoimmunotherapy with CLL patients having high expression of the antiapoptotic protein mcl-1 having reduced responses to pentostatin, cyclophosphamide and rituximab [68]. Similarly, expression of the BCL6 marker of germinal center origin, a protein involved in the suppression of p-53-induced apoptosis did not benefit from R-CHOP compared to CHOP [69]. Thus, the induction of nonapoptotic direct cell death by obinutuzumab may offer benefits for those patients whose tumors are resistant to induction of classical apoptosis.

2.4 Immunogenic cell death

A further consequence of anti-CD20 mAb-mediated cell death, which has recently been identified, is the release of intracellular constituents that can function as immune danger signals. PCD evoked by type II mAb such as obinutuzumab results in the loss of cellular plasma membrane integrity and liberation of high-mobility group box 1 (HMGB1), ATP and heat shock proteins (HSP) 60 and 90; type I mAbs such as rituximab appear able to mediate release of HMGB1 and HSP90 via a mechanistically distinct pathway that is dependent upon CDC [70]. When present in the extracellular milieu, these molecular determinants can function as damage-associated molecular patterns (DAMP) and potentially enhance immunogenicity and priming of tumor-specific CD8 T-cells [71]. In keeping with this concept, DAMP released following treatment of human lymphoma cells with obinutuzumab are able to stimulate maturation and activation of primary human DC and induce T-cell proliferation, at least during *in vitro* co-culture assays [70].

Current data on the induction of immunogenic cell death (ICD) following anti-CD20 mAb therapy remain limited, and require further characterization and validation. However, these initial observations raise the possibility that ICD may contribute, at least in part, to the induction of tumor immunity occasionally observed following treatment with anti-CD20 mAb. Emerging data suggest that anti-CD20 mAb can induce a 'vaccination effect' with levels of FL idiotype-specific T cells increased relative to baseline post-rituximab therapy in five FL patients [72,73]. Furthermore, both CD4 and CD8 T cells have been implicated in therapy and protection from further disease in immunocompetent mice [74] with the Fc component critical for long-lasting tumor protection. Induction of a vaccination effect post-mAb treatment is likely mediated by antigen-presenting cells (APCs) that have phagacytosed dead or dying cells, which subsequently process and present tumor antigens to T-lymphocytes to induce long-term immune responses. Thus, it can be predicted that increased direct cell death and enhanced binding of obinutuzumab to APC through enhanced affinity for FcγR through glycoengineering [75,76] could optimize APC-related functions and subsequently obinutuzumab may induce more potent long-term antitumor T-cell responses.

2.5 *In vivo* studies

Obinutuzumab has demonstrated superiority to rituximab and ofatumumab in a variety of lymphoma xenograft models, even when saturating high doses of 30 mg/kg rituximab are used [25,29,56]. Crucially, efficacy was seen with obinutuzumab as a second-line therapy after relapse post-rituximab treatment

in DLBCL models when tumors failed to respond to further treatment with rituximab or ofatumumab [25,29]. In contrast to rituximab, therapy was not dependent on complement [56]. Combination studies with cyclophosphamide [56], bendamustine, fludarabine, chlorambucil or cyclophosphamide/vincristine [77] all showed that obinutuzumab/chemotherapy combinations were superior to rituximab/chemotherapy combination with obinutuzumab monotherapy as effective as rituximab/chemotherapy combinations [77]. In a cynomolgus monkey model, although rituximab and obinutuzumab induced near equivalent levels of B-cell depletion in peripheral blood, obinutuzumab was superior at B-cell depletion in lymphoid and splenic tissue [25].

Preclinical studies suggest that obinutuzumab is able to induce marked tumor cell killing and B-cell depletion through mechanisms of action that are in part due to its glycoengineered Fc arm and in part due to its properties as a type II mAb which recognizes a distinct epitope of CD20 through a differing spatial arrangement [31,78]. The combination of these two properties suggests that obinutuzumab may offer increased clinical efficacy over rituximab and other novel anti-CD20 mAbs such as the type I glycoengineered ublituximab and may be of particular benefit in rituximab-refractory patients.

3. Clinical studies

3.1 Early-phase clinical trials

Obinutuzumab underwent testing as monotherapy in Phase I trials in both CLL and NHL. Twenty patients with relapsed/refractory NHL received eight cycles of obinutuzumab monotherapy every 3 weeks at doses ranging from 50/100 to 1200/2000 mg with five complete responses (CR) and four partial responses (PR) [79]. In the second Phase I study, 22 patients including 5 with CLL received 200 – 2000 mg of obinutuzumab every 4 weeks for eight cycles with 5 PR and 13 stable disease (1 of whom later improved to PR) [80]. Importantly, obinutuzumab induced responses in rituximab refractory patients, mirroring xenograft studies, and no significant activation of the complement cascade was observed, emulating *in vitro* mechanism of action data [25]. Similarly, 62% (8/13) of heavily pretreated relapsed/refractory CLL patients given a single dose of obinutuzumab (400 – 2000 mg) every 3 weeks for eight cycles achieved a partial response with a significant reduction in B-cell counts [81]. However, the Phase II arm reported a much lower end-of-treatment response rate with 25% (4/16) of patients achieving PR [82], potentially as a result of fewer patients with low tumor burden. Baseline tumor burden below 2400 mm^2 was predictive of response in both arms of the study, as previously shown for type I mAbs where serum concentrations can be lower in patients with high tumor burden and consequently associated with poorer prognosis [83-86].

Reported adverse events (AEs) post-obinutuzumab infusion were similar to those observed with other anti-CD20 mAbs. Although infusion-related reactions (IRRs) were common at first infusion, there were few grade 3/4 events with the exception of several episodes of grade 3/4 neutropenia in CLL patients, which were resolved with or without G-CSF administration [79,80].

3.2 Phase II studies in NHL

The Phase II GAUGUIN study recently reported results in relapsed/refractory indolent NHL and aggressive DLBCL/MCL. Patients were randomized to receive 400 mg obinutuzumab on day 1 and 8 of cycle 1 and day 1 of cycles 2 – 8 (400/400) or 1600 mg on day 1 and 8 of cycle 1 and 800 mg on day 1 of cycles 2 – 8 (1600/800) every 3 weeks. Response rates were consistently higher in the 1600/800 mg arms with end of treatment overall response rates (ORR) of 55 and 32% (1600/800 mg) versus 17 and 24% (400/400 mg) for indolent and aggressive NHL, respectively [87,88]. The ORR in rituximab refractory patients was similar at 50% in indolent NHL and 33% in aggressive NHL with the higher dose of obinutuzumab. Few grade 3/4 AEs were reported (2/40 and 3/40) and encouragingly best ORR in heavily pretreated aggressive NHL (median of three prior treatments, many including rituximab) of 32% in DLBCL and 27% in MCL were comparable to those reported for rituximab (30%) [89,90] in a less heavily pretreated, rituximab naïve population. The median response duration was 17.2 months for indolent NHL and 9.8 months for aggressive NHL.

A Phase II randomized trial comparing 4 weekly infusions (days 1, 8, 15, 22) of obinutuzumab (1000 mg) or rituximab (375 mg/m^2) in 175 relapsed indolent NHL patients that had previously demonstrated a response to rituximab reported investigator assessed end of induction ORR of 43.2% for obinutuzumab versus 38.7% for rituximab (difference in response rates 4.6, 95% CI 12, 21.1) [91]. A higher number of CR were achieved with obinutuzumab (10.8 vs 6.7%) although no difference in progression-free survival (PFS) was observed. No new toxicities were reported although more patients in the obinutuzumab arm reported IRRs (72 vs 49, grade 3/4 11 vs 5) and 5 times more AEs occurred with obinutuzumab than rituximab – coughs (10 vs 1), back pain (7 vs 2), decreased appetite (7 vs 2), insomnia (5 vs 0) and fatigue (23 vs 17). However, more patients discontinued treatment during the induction phase with rituximab than obinutuzumab (7 vs 4) and a greater number of SAEs were reported (9 vs 5).

Obinutuzumab has also shown significant activity when used in combination with chemotherapy. A Phase Ib study (GAUDI) of 56 patients with relapsed/refractory FL showed induction end ORR of 96% for G-CHOP (obinutuzumab, cyclophosphamide, doxorubicin, vincristine and prednisone) and 93% for G-FC (obinutuzumab, fludarabine and cyclophosphamide) [92] with CR of 39 and 50%, respectively. Notably, of the rituximab refractory patients all 14 experienced at least a PR.

3.3 Phase III trial in CLL

To date, no new treatments/regimens have been shown to be superior to chlorambucil for the treatment of comorbid CLL or elderly patients with CLL [93]. In a trial designed to test whether addition of type I or type II anti-CD20 mAb can enhance the efficacy of chlorambucil 781 patients with previously untreated CLL (median age 73) and a score higher than six on the cumulative illness rating scale (median 8) were randomized to receive chlorambucil, rituximab plus chlorambucil or obinutuzumab plus chlorambucil [94]. Approximately 21% of patients were Binet stage A, 42% stage B and 37% stage C. Patients received treatment over six 28-day cycles with chlorambucil administered orally at 0.5 mg/kg on days 1 and 15 of each cycle, rituximab intravenously at 375 mg/m^2 on day 1 of cycle 1 and 500 mg/m^2 on day 1 of cycle 2 – 6 and obinutuzumab intravenously at 100 mg on day 1, 8 and 15 of cycle 1 and day 1 of cycles 2 – 6. Grade 3/4 IRRs were higher with obinutuzumab–chlorambucil than rituximab–chlorambucil (20 vs 4%) during the first infusion but no grade 3/4 IRRs were reported during subsequent infusions and no deaths were associated with IRRs. Higher levels of grade 3 or above AEs were also reported with obinutuzumab–chlorambucil than rituximab–chlorambucil such as thrombocytopenia (10 vs 3%) and neutropenia (33 vs 28%). Overall, a greater number of patients on the obinutuzumab–chlorambucil arm discontinued treatment than those on the rituximab–chlorambucil arm (8 vs 3), primarily as a result of IRRs although there were more deaths from AEs with rituximab–chlorambucil (6 vs 4%). Obinutuzumab–chlorambucil prolonged PFS (26.7 months) over both rituximab–chlorambucil (15.2 months, hazard ratio [HR] 0.39 [95% CI, 0.31 – 0.49]) and chlorambucil monotherapy (11.1 months) ($p < 0.001$, HR 0.18 [95% CI, 0.13 – 0.24]). ORR were 77.3% for obinutuzumab–chlorambucil versus 65.7% for rituximab–chlorambucil and 31.4% with chlorambucil alone. There was a higher complete response rate with obinutuzumab–chlorambucil than rituximab–chlorambucil (22.3 vs 7.3%, $p < 0.001$) and no CR were obtained with chlorambucil alone. Minimal residual disease (MRD) negativity was higher in the obinutuzumab–chlorambucil arm than rituximab–chlorambucil (bone marrow, 19.5 vs 2.6%; blood, 37.7 vs 3.3%, respectively, $p < 0.001$) with no patients obtaining MRD^{-ve} status with chlorambucil monotherapy. Overall survival analysis showed a significant benefit for obinutuzumab–chlorambucil over chlorambucil (rates of death 9 vs 20%, respectively). As a result of these data obinutuzumab was licensed by the FDA in November 2013 for use in combination with chlorambucil in previously untreated CLL patients.

4. Conclusion

The routine use of rituximab has improved patient outcomes in CD20-positive B-cell malignancies. Despite the clinical success achieved with rituximab further improvements in anti-CD20 mAb efficacy are required to improve outcomes further. Obinutuzumab is the only type II glycoengineered anti-CD20 mAb to enter clinical testing. Obinutuzumab utilizes distinct mechanisms of action relative to type I antibodies such as rituximab, which include enhanced direct cell death, increased ADCC but reduced CDC. Obinutuzumab mediates direct cell death via a nonapoptotic, caspase-independent mechanism that is independent of Fcγ receptors, which may enable activity in patients with impaired Fc-dependent immune effector mechanisms or tumors resistance to the induction of chemotherapy-induced apoptosis. In preclinical models, obinutuzumab induced tumor remission, with high activity in rituximab-refractory tumors. Obinutuzumab has demonstrated encouraging efficacy as monotherapy in NHL and CLL and when combined with chemotherapy in relapsed/refractory NHL and treatment-naïve symptomatic CLL. In the first report of a recently completed randomized Phase III trial of patients with previously untreated comorbid CLL, overall response rate was significantly greater (78 vs 65%, $p <. 0001$) and median PFS was significantly prolonged (26.7 vs 15.2 months, $p < 0001$) for obinutuzumab plus chlorambucil versus rituximab plus chlorambucil. The encouraging early-phase trial data and these emerging data from Phase III trials in patients with CLL suggest that patient outcomes can be further improved by the introduction of obinutuzumab in B-cell malignancies. Further results from other Phase III clinical trials including obinutuzumab are eagerly awaited. Ongoing studies are comparing obinutuzumab plus CHOP versus rituximab plus CHOP in previously untreated patients with CD20-positive DLBCL (NCT01659099, NCT01287741), obinutuzumab or rituximab plus CHOP, CVP or bendamustine in previously untreated advanced indolent NHL (NCT01332968) in patients with previously untreated indolent NHL as well as an important study in rituximab-refractory indolent NHL comparing Bendamustine versus Bendamustine and obinutuzumab (NCT01059630). In CLL ongoing Phase III studies are evaluating obinutuzumab alone or in combination with chemotherapy (NCT0190 5943) and a number of early-phase trials are evaluating obinutuzumab in combination with ABT-199 (NCT01685892) or lenalidomide (NCT01995669). The results of these trials will potentially provide additional evidence of whether patient outcomes in other B-cell malignancies can be further improved by the introduction of obinutuzumab.

5. Expert opinion

Most anti-CD20 antibodies have been developed based on improved modes of action to enhance CDC or on structural modifications to the Fc region that enhance lymphoma cell killing via ADCC. Obinutuzumab is a unique, glycoengineered type II anti-CD20 antibody with different mechanisms of action compared with rituximab, which

include increased induction of direct cell death and enhanced ADCC. Preclinical data have demonstrated superior efficacy of obinutuzumab over rituximab at the same dose of mAb. These preclinical observations of improved efficacy with the same mAb dosing are important given the reservations expressed in the clinical community that any enhanced treatment efficacy is simply related to the increased mAb dosing of obinutuzumab over rituximab [95]. Indeed the early-phase clinical trials in relapsed/refractory CLL and NHL subtypes confirmed this encouraging clinical efficacy of obinutuzumab as monotherapy using higher doses of obinutuzumab (1000 mg) than the standard dose of rituximab 375 mg/m^2. In these early-phase trials, more detailed pharmacokinetics and defining of the optimal dose were performed than with the pragmatic rituximab dosing adopted into routine clinical practice. This higher dose of 1000 mg of obinutuzumab was taken forward in the randomized trials with the comparator of standard therapy, including rituximab at registration dose of 375 mg/m^2. The emerging randomized Phase III data from older comorbid patients with previously untreated CLL demonstrated impressive improvements in molecular remission rates and median PFS of obinutuzumab plus chlorambucil versus rituximab plus chlorambucil. These emerging data provides reasons to be optimistic that outcomes for patients with B-cell malignancies can be further improved with obinutuzumab. While it is likely that the increased efficacy over rituximab may be at least partially related to the increased dosing of anti-CD20 mAb known to be important to increasing efficacy with rituximab in CLL, it appears most unlikely to be the only explanation for this increased efficacy. Instead, this increased efficacy is more likely to be related to the novel mechanisms of action of obinutuzumab of this type II anti-CD20 mAb and the clinical results are entirely in keeping with the preclinical studies.

The data using ofatumumab as a single agent and in combination with chlorambucil are also compelling and have led to recent US FDA approval in April 2014. In the randomized, open-label, pivotal Phase III COMPLEMENT 1 study, ofatumumab in combination with chlorambucil versus chlorambucil alone was evaluated in 447 patients with CLL who were previously untreated and for whom fludarabine-based therapy was considered inappropriate by study investigators. Among the 447 patients (median age 69 years) included in the study, the majority of patients (72 per cent) had two or more comorbidities. The results of ofatumumab and chlorambucil (n = 221) versus chlorambucil alone (n = 226) demonstrated statistically significant improvement in median PFS in patients randomized to ofatumumab and chlorambucil compared to patients randomized to chlorambucil alone (22.4 months versus 13.1 months, respectively) (HR = 0.57 [95% CI, 0.45, 0.72] $p < 0.001$) [96]. So which of these anti-CD20 mAb should be used to replace rituximab in CLL or other B-cell NHL? In the absence of data from a direct comparison of the type I anti-CD20 ofatumumab and the type II obinutuzumab, this question is difficult to answer definitely and the possibility of such a randomized study being performed appears remote. Certainly, the preclinical insights regarding CD20 expression and modulation imply that the type II anti-CD20 mAb obinutuzumab may be superior although it is possible that differential effects may be seen using obinutuzumab with the largest benefits being seen in B-cell malignancies such as B-CLL and MCL rather than FL. The opinion among many clinical trial groups is that obinutuzumab is the anti-CD20 mAb to take forward and forms the basis of a number of ongoing Phase III clinical trials in patients with rituximab-refractory indolent NHL. In particular, a direct comparison of obinutuzumab-CHOP versus rituximab-CHOP in previously untreated patients with CD20-positive DLBCL and obinutuzumab or rituximab plus CHOP, CVP or bendamustine in previously untreated advanced indolent NHL. The results of these trials will potentially provide further evidence of whether patient outcomes in other B-cell malignancies can be further improved by the introduction of obinutuzumab.

In CLL, the therapeutic alternatives and scheduling options are even more bewildering with the emerging data of high clinical activity of the many novel agents that target the signaling of the cell proliferation pathway such as the PI3K inhibitor idelalisib and the Bruton's tyrosine kinase inhibitor ibrutinib. The Bcl-2 antagonist ABT-199 also appears highly active and acts to induce cell death (apoptosis) via an unrelated mechanism. For CLL many trial groups are considering perhaps using 'mild' short-acting chemotherapy to debulk the tumor, then intervening using at least 2 – 3 of the best novel agents: for example, ABT-199 plus obinutuzumab, or ibrutinib plus obinutuzumab, or idelalisib/ibrutinib/ABT-199 [97]. All of these combinations will need rigorous testing in well-designed clinical trials assessing MRD status.

The results of these ongoing randomized clinical trials in other B-cell malignancies are eagerly awaited and are required to provide further evidence of whether results in other B-cell malignancies can be further improved by the introduction of obinutuzumab. The results emerging in CLL provide at least some reasons to be hopeful that outcomes can be further improved.

Declaration of interest

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