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REVIEW

A Review of Obinutuzumab (GA101), a Novel Type II Anti-CD20 Monoclonal Antibody, for the Treatment of Patients with B-Cell Malignancies

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ABSTRACT

Obinutuzumab (GA101) is a novel, type II, glycoengineered, humanized anti-CD20 monoclonal antibody that has been developed to address the need for new therapeutics with improved efficacy in patients with lymphocytic leukemia and lymphoma of B-cell origin. Obinutuzumab has a distinct mode of action relative to type I anti-CD20 antibodies, such as rituximab, working primarily by inducing direct cell death and antibody-dependent cell-mediated cytotoxicity. Obinutuzumab is under investigation in a wide-ranging program of clinical trials in patients with B-cell malignancies. Efficacy as monotherapy has been reported in patients with relapsed/refractory indolent and aggressive non-Hodgkin lymphoma (NHL) and in chronic lymphocytic leukemia (CLL) of B-cell origin. Improved outcomes have also been noted when obinutuzumab is added to chemotherapy in patients with B-cell NHL, and superiority over rituximab has been reported with combination therapy in patients with CLL. Ongoing research is focusing on developing options for chemotherapy-free treatment and on new combinations of obinutuzumab with novel targeted agents.

Keywords: Antibody-dependent cell-mediated cytotoxicity; B-cell lymphoma; CD20; Chronic lymphocytic leukemia; Glycoengineering; Monoclonal antibody; Non-Hodgkin lymphoma; Obinutuzumab; Oncology; Rituximab

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INTRODUCTION

Lymphocytic leukemia and lymphomas are malignant neoplasms that disrupt normal lymphocyte development and function. Lymphocytic leukemia originates in bone marrow and is characterized by high numbers of abnormal lymphocytes in the blood [1]. Chronic lymphocytic leukemia (CLL) is a slowly progressing disease that affects B lymphocytes (B-cells), mainly in older adults, and is the most common form of leukemia in the Western world, with an annual incidence rate of 4.2 per 100,000 [2]. Lymphomas, on the other hand, encompass a group of hematologic malignancies that arise mainly from mature T lymphocytes (T cells) or B-cells in secondary lymphoid tissue, particularly the lymph nodes [3, 4]. They are subdivided based on the presence of Reed-Sternberg (or Hodgkin) cells into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) [5], of which NHL is by far the most common (accounting for around 90% of cases) [6].

There are many types of NHL, differing in terms of microscopic appearance, growth patterns, clinical impact, and treatment [3, 4]. However, NHL can be broadly divided into two major groups, B-cell and T-cell NHLs, of which the B-cell type accounts for approximately 85% of all cases [5]. Indolent NHL (iNHL) is a slow-growing form of B-cell NHL that includes follicular lymphoma (FL) and marginal zone lymphoma, whereas more aggressive and faster-growing presentations of NHL include diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma, and mantle cell lymphoma (MCL) [6].

FL is the most common type of iNHL, with an annual incidence that has risen from 2 to 3 per 100,000 in the 1950s to 5 to 7 per 100,000 more recently in Western Europe [7]. Median age at diagnosis is in the 6th decade of life, but up to 25% of patients with FL are aged 40 years or younger [8]. Approximately 55–70% of patients have bone marrow involvement at presentation, which is indicative of advanced disease [9–11]. Among aggressive subtypes of NHL, DLBCL is the most prevalent [12], having a crude annual incidence in Europe of 3.8 per 100,000 and accounting for between 30% and 58% of all cases of NHL [13]. Incidence of DLBCL increases with age, and risk factors include a family history of lymphoma, autoimmune disease, human immunodeficiency virus or hepatitis C virus infection, high body mass in young adulthood, and some types of occupational hazard exposure [13].

Management of NHL and CLL depends on stage, tumor burden, and other patient factors. For example, recent guidelines state that patients with early (stage I–II) iNHL should be offered radiotherapy [8, 14, 15], which can result in 10-year overall survival (OS) rates of up to 80% [14, 15]. However, most patients with iNHL are incurable [15], and successive relapses and increasing resistance to treatment characterize the individual course of the disease. Until the late 1990s (i.e., the period preceding the introduction of antibody therapy), these patients were usually treated with increasingly intensive combination chemotherapy regimens to reduce tumor burden and palliate symptoms. Response rates to conventional chemotherapy generally exceeded 50% [12], and maximal tumor reduction could be achieved with high-dose chemotherapy and autologous stem cell support, but there were no apparent improvements in failure-free survival [16]. Treatment of symptomatic CLL and the more aggressive forms of NHL have also traditionally relied heavily on intensive combination chemotherapy.

The therapeutic landscape for all forms of B-cell NHL changed markedly in the late 1990s with the approval of a CD20-directed monoclonal antibody (mAb), rituximab, after demonstration of significant activity as a single agent in patients with iNHL [17, 18]. The antigen CD20 is a membrane protein found on the surface of all mature B-cells that typically has a constitutive and constant expression and therefore provides an excellent therapeutic target [19]. Moreover, it is found in 95% of B-cell malignancies [20]. Rituximab acts by engaging Fc receptors on immune effector cells, such as natural killer (NK) cells and macrophages, and mediating complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity/phagocytosis (ADCC/ADCP), in addition to exerting direct antiproliferative and pro-apoptotic effects [12, 21].

The development of rituximab initiated the introduction of targeted immunotherapy for the treatment of indolent and aggressive forms of B-cell NHL and CLL and improved the general prognosis for patients with these diseases [9]. For instance, using a series of patients (with previously untreated stage I–II FL) from Stanford University, Tan et al. described four eras of FL treatment from 1960 to 2003: pre-anthracycline (1960–1975, 180 patients), anthracycline (1976–1986, 426 patients), aggressive chemotherapy/purine analogs (1987–1996; 471 patients), and rituximab (1997–2003, 257 patients) [22]. Median OS improved from 11 years in eras 1 and 2 to 18.4 years in era 3; at the time of reporting in 2013, median OS for era 4 had not been reached [22]. The development of rituximab in combination with chemotherapy represented a major step forward, significantly improving survival outcomes in not only patients with FL [23] but also in other CD20-positive B-cell malignancies, including DLBCL [24, 25] and CLL [26]. These advances led to the adoption of rituximab as standard of care in patients with CLL, FL, and DLBCL who require systemic therapy [2, 8, 13, 14].

Despite the major therapeutic advances brought about by rituximab [15, 27], relapse and development of resistance to treatment are eventually seen in the majority of B-cell NHL patients [12, 15, 28]. The disease course is characterized thereafter by an ongoing decrease in the quality and duration of response with each subsequent course of therapy [15]. Mechanisms of resistance that have been suggested include increased mAb metabolism, reduced tumor penetration, reduced mAb binding (via FcγRIII polymorphisms), resistance to mAb effector mechanisms, complement depletion, abnormal lipid raft composition of some malignant B-cells, downregulation of pro-apoptotic proteins, and impairments in immune effector cell recruitment or function [29, 30].

CD20 ‘shaving’ is another potential resistance mechanism to type I anti-CD20 mAbs, whereby mAb-CD20 complexes are rapidly removed from the surface of B-cells via monocytes/macrophages through a mechanism known as trogocytosis [31–33]. This resistance mechanism can lead to fewer cell-surface CD20 antigens (with a consequent reduction in anti-CD20 mAb binding) and a decrease in Fc-mediated effector functions as well as a reduced mAb half-life. Additionally, there is evidence to indicate that FcγRIIb-mediated internalization and degradation of the complex formed between the mAb and CD20 from the surface of some B-cell malignancies may cause resistance to type I anti-CD20 mAbs, such as rituximab [34–36], although this process of CD20 downregulation appears to be slower

than trogocytosis [32]. This mechanism of mAb-CD20 internalization limits the engagement of natural effectors, reduces mAb half-life, and increases mAb turnover [37]. mAb-CD20 internalization correlates strongly with *cis* expression of the inhibitory Fc receptor FcγRIIb on target B-cells and has been shown to predict less durable responses to rituximab therapy in patients with MCL [37].

Management of relapse and resistance in rituximab-treated patients presents a significant challenge [6], and there is a need for treatments with improved activity across B-cell NHL subtypes and CLL. Better understanding of antibody biology and modes of action, together with increased ability to design highly efficient therapeutics, has led to the development of novel mAbs with improved activity. As a review article, the following paper does not contain any new studies with human or animal subjects performed by any of the authors.

OBINUTUZUMAB, A NOVEL HUMANIZED TYPE II MAB

Obinutuzumab (GA101) is a novel, type II, glycoengineered, humanized anti-CD20 mAb that has been developed to address the need for novel therapeutics with higher activity than rituximab. The post-translational glycoengineering process used in the development of this agent [resulting in the absence of a fucose sugar residue from immunoglobulin G (IgG) oligosaccharides in the Fc region of the mAb molecule] was developed to increase activity by enhancing binding affinity to the FcγRIII receptor on immune effector cells (Fig. 1) [38, 39]. Additionally, obinutuzumab has a modified

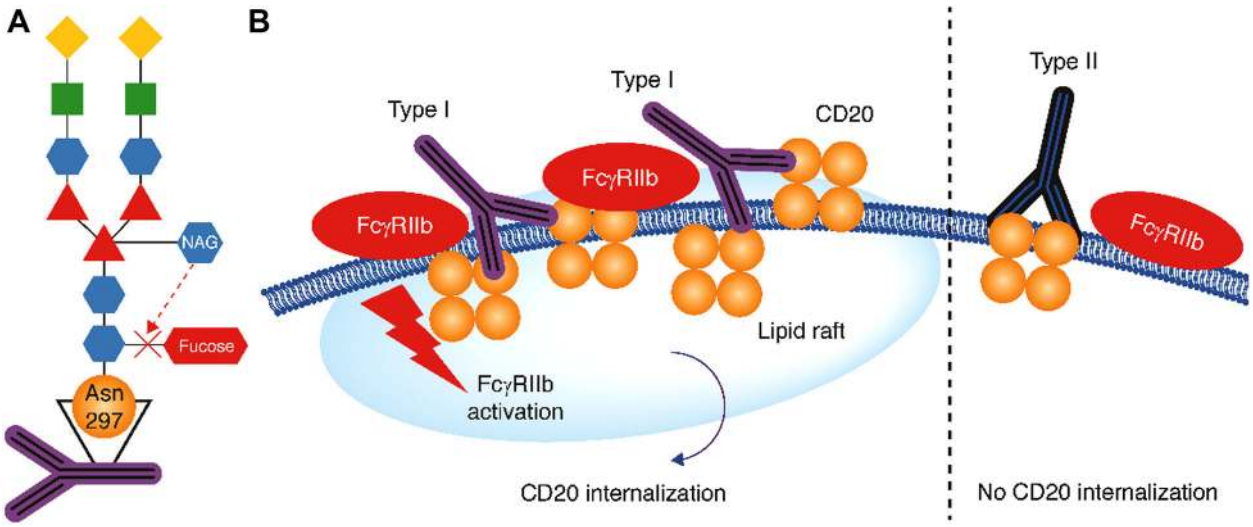


Fig. 1 Structure and binding behavior of obinutuzumab. Glycoengineered structure and type II binding properties of obinutuzumab. **a** Glycoengineering by defucosylation of immunoglobulin G oligosaccharides in the Fc region of obinutuzumab. In Chinese hamster ovary producer cells, N-acetylglucosamine (NAG) is assembled into oligosaccharides, which sterically prevents the addition of fucose to the carbohydrate attached to asparagine (Asn) 297. **b** Hypothetical model of CD20 binding properties of type I and II antibodies. In contrast to inter-tetrameric CD20 binding of type I antibodies, intra-tetrameric binding of type II antibodies to CD20 does not lead to FcγRIIb-mediated internalization of CD20 in lipid rafts (reproduced from Goede et al. [38] with permission; copyright © 2015 Karger Publishers, Basel, Switzerland)

elbow-hinge amino acid sequence compared to type I agents, which together with the unique epitope recognized by obinutuzumab results in spatial alterations of the CD20-mAb complex on B-cells [39, 40]; this is believed to be the molecular basis for the type II biology of obinutuzumab [40] as both type II character and cell death induction (as described below) can be switched on and off by mutating this elbow-hinge region [39].

The type II mechanism of action of obinutuzumab together with glycoengineering acts to enhance direct cell death and ADCC/ADCP, while decreasing CDC (Fig. 2) [41], and differentiates the drug from classical type I anti-CD20 mAbs, such as rituximab and ofatumumab [39, 40, 42–44]. Rituximab, by comparison, works primarily via CDC (by clustering CD20 within lipid rafts) and by ADCC/ADCP, with direct cell death contributing much less to the overall antitumor activity [45]. Ofatumumab also acts primarily via CDC after binding both loop domains of CD20 at a different epitope compared to rituximab [46].

Increased Direct Cell Death Induction

Obinutuzumab has been shown to be faster than and superior to both rituximab and ofatumumab in inducing direct cell death in malignant B-cells. This was demonstrated by phosphatidylserine exposure and propidium iodide staining [with analysis by

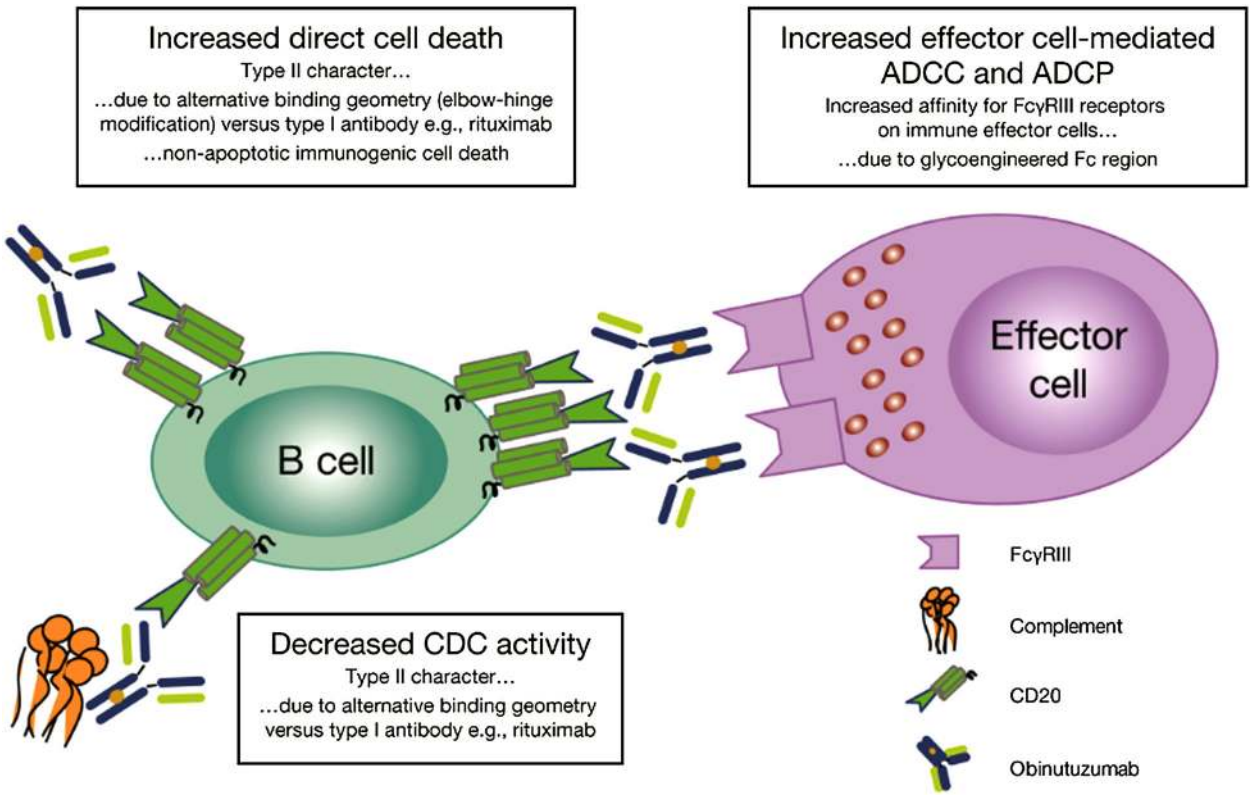


Fig. 2 Putative mechanisms of action of obinutuzumab. Please refer to the text for further information and supporting references. *ADCC* antibody-dependent cell-mediated cytotoxicity, *ADCP* antibody-dependent cellular phagocytosis, *CDC* complement-dependent cytotoxicity (adapted from Goede et al. [41] with permission)

fluorescence-activated cell sorting (FACS) and time-lapse microscopy] in a panel of CD20-expressing tumor cell lines [39, 43]. While some researchers have questioned the validity of using FACS to assess mAb-induced direct cell death (because of potential mechanical interference with mAb-mediated homotypic adhesion) [47], multiple studies, using a variety of methods (including FACS), have confirmed that, overall, obinutuzumab induces greater direct cell death than type I mAbs [48–53] and occurs without disruption of homotypic aggregates [43, 54].

The mechanisms that may underlie the ability of type II anti-CD20 mAbs to directly evoke programmed cell death (PCD) are still poorly understood, but have been investigated in several studies [39, 48, 55]. Honeychurch et al. demonstrated actin-dependent, lysosome-mediated induction of PCD by type II mAbs, such as obinutuzumab or tositumomab, which was directly correlated with the production of reactive oxygen species (ROS) [48]. In contrast, type I mAbs, such as rituximab, induced only minimal levels of ROS and PCD. Generation of ROS mediated by nicotinamide adenine dinucleotide phosphate oxidase, independently of mitochondria, was unaffected by B-cell lymphoma 2 (BCL-2) overexpression and took place downstream of mAb-induced actin cytoskeletal reorganization and lysosome membrane permeabilization. The results thus indicated a newly characterized cell death pathway that is independent of the classical hallmarks of apoptosis and has the potential to bypass mechanisms of apoptotic resistance, thereby eliminating malignant cells that are refractory to conventional chemo- or immunotherapy. A similar mechanism of cell death has also been described for other antibodies targeting B-cell surface antigens, such as HLA-DR or CD37 [56, 57]. In a separate study, Cheadle and colleagues found that PCD induced by type II agents such as obinutuzumab is a form of immunogenic cell death that is characterized by the release of damage-associated pattern molecules, including heat shock protein 90 and adenosine triphosphate. It is believed that this mechanism of PCD could enhance the immune response by inducing dendritic cell mutation and subsequent T-cell proliferation [49].

In contrast to these data, which are largely based on NHL cell lines, ex vivo studies utilizing CLL primary samples suggest that direct cell death induction is not the major mechanism of obinutuzumab-mediated B-cell depletion in CLL [50, 55, 58–61]. This observation may be a consequence of the lower proliferative state of non-stimulated CLL cells, as compared to NHL cell lines. Thus, in CLL, immune effector-mediated mechanisms (as described below) may play a more important role than direct cell death induction in mediating the antiproliferative effects of obinutuzumab. In line with this assumption, a study has demonstrated that CD40 stimulation can sensitize CLL cells to lysosomal cell death induction by obinutuzumab, providing evidence that drug sensitivity in CLL cells can be modulated by microenvironmental stimuli [55].

Complement-Dependent Cytotoxicity

Antibody-mediated CDC is initiated by fixing of C1q (the initiating component of the classical complement pathway) to the Fc portion of target-bound antibodies. This triggers a cascade that in turn leads to the formation of C3 and C5 convertase and ultimately to the membrane attack complex (MAC). The MAC then causes cell lysis by disrupting the plasma membrane of the target cell [62].

The ability of CD20 antibodies to mediate CDC appears to be determined by the effect of mAb binding and cross-linking on the redistribution of the target antigen into lipid rafts on the surface of the target cell. The density and positioning of the antigen-antibody complexes influence C1q binding and, in turn, the complement cascade [62]. Complement recruitment as such is a key characteristic distinction between type I CD20 mAbs, such as rituximab and ofatumumab, which mediate strong CDC, and type II CDC antibodies like obinutuzumab, which only mediate weak CDC activity in cellular assays [39, 42, 43]. CDC is therefore not thought to contribute meaningfully to the overall activity of obinutuzumab [38, 62]. Interestingly, the limited capacity of obinutuzumab to fix complement via its Fc portion may further enhance its capability to bind to FcγRIII and mediate ADCC [63].

ADCC/ADCP

Glycoengineering as well as subsequent enhancement of affinity for FcγRIIIa (an activating Fc receptor expressed primarily on NK cells) was confirmed as the predominant determinant of the superior ADCC activity of obinutuzumab over type I anti-CD20 mAbs in a series of experiments using NHL cell lines and human PBMCs expressing the V158/V158 or F158/F158 FcγRIIIa receptor [43]. While obinutuzumab was superior to rituximab and ofatumumab in terms of potency and overall cell killing, the ADCC activity of a non-glycoengineered version of obinutuzumab was similar to that of rituximab and ofatumumab, confirming the additional benefit conferred by glycoengineering [43]. Notably, the induction of ADCC was found to be particularly more potent with obinutuzumab than rituximab in the presence of nonspecific human IgG at physiological concentrations, as found in human blood [39].

In an assessment of ADCP, Herter et al. used FACS analysis to show that obinutuzumab, rituximab, and ofatumumab have comparable overall phagocytic activity in NHL cell lines and primary human monocyte-derived macrophages (MDMs) [44]. Rafiq et al. have demonstrated the phagocytic activity of obinutuzumab against membrane-dyed CLL cells undergoing flow cytometry, although ofatumumab and rituximab produced greater phagocytosis than obinutuzumab in these experiments [50]. In a recent report, intravital imaging revealed improved Kupffer cell-mediated phagocytosis of B-cells as an important in vivo mode of action of glycoengineered anti-CD20 mAbs, such as obinutuzumab, which underlies their improved activity compared with non-Fc-engineered antibodies [64].

ADCC is carried out mainly by NK cells, which carry inhibitory killer cell immunoglobulin-like receptors (KIRs) that interact with exposed epitopes on the class I human leukocyte antigen (HLA). Experiments using peripheral blood mononuclear cells (PBMCs) from healthy donors, primary CLL cells from patients, and a variety of cell lines have shown that, because of its Fc modification, obinutuzumab can compensate for inhibitory KIR/HLA interactions [65]. This results in the recruitment of additional NK cells for ADCC, and target cell depletion is not negatively impacted by KIR/HLA interactions [65]. Genotyping of patients participating in the CLL11 study (comparing chlorambucil, rituximab-chlorambucil, and obinutuzumab-chlorambucil in previously untreated patients with CLL) has shown that the prognosis for patients with a low number of KIR/HLA interactions is better than that for

Fig. 3 Percentage tumor growth inhibition (TGI) in combination studies of mouse Z138 MCL xenografts [69]. *Statistically significant ($p < 0.001$; Tukey-Kramer test) vs. single-agent treatments.**Statistically significant vs. G monotherapy and RIT + FLU. ***Statistically significant vs. RIT monotherapy. TGI was calculated from tumor volume (TV) [(length × width^2)/2], calculated from staging until study termination, as follows: $\text{TGI}\ (\%) = 100 - \frac{\text{Median}\ [\text{TV(treated)}_{\text{day}z} - \text{TV(treated)}_{\text{day}x}]}{\text{Median}\ [\text{TV(respective control)}_{\text{day}z} - \text{TV(respective control)}_{\text{day}x}]} \times 100$.

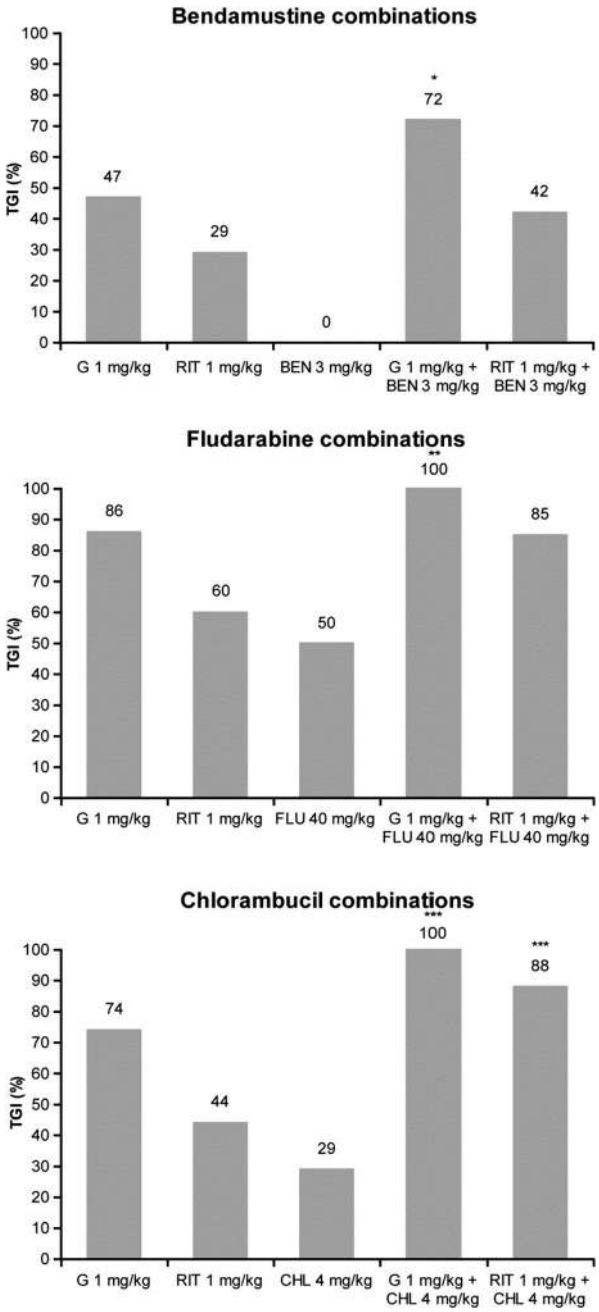
Each treatment group was compared with its respective vehicle control. *TVday z* represented TV for an individual animal at a defined study day (day *z*), and *TVday x* represented TV of an individual animal at the staging day (day *x*). Animals in control groups received 0.9% sodium chloride vehicle; randomization to treatments took place 9–27 days after tumor cell injection. Animals were killed at various time points from day 30 to day 66. *BEN* bendamustine, *CHL* chlorambucil, *FLU* fludarabine, *G* obinutuzumab (GA101), *MCL* mantle cell lymphoma, *RIT* rituximab, *TGI* tumor growth inhibition

patients with a higher number of such interactions [66]. Taken together, these results indicate that ADCC is a key mode of action for obinutuzumab and is influenced by the number of the inhibitory interactions.

Unlike type I anti-CD20 mAbs, type II agents are found to undergo reduced CD20 internalization (Fig. 1) [67]. Indeed, increased stability of surface-accessible CD20 was noted with obinutuzumab relative to both rituximab and ofatumumab using FACS in a human DLBCL cell line and in blood from patients with CLL [43]. Ultimately, this may further enhance the immune effector cell-mediated mechanisms of type II anti-CD20 mAbs, independently of, but in conjunction with, glycoengineering. Furthermore, type II anti-CD20 mAbs may be less susceptible to the development of resistance mechanisms affecting effector-cell-mediated cytotoxicity. The impact of trogocytosis on reducing the efficacy of type II mAbs through shaving of CD20 molecules from the cell surface requires further investigation.

Superior and/or Faster Whole Blood B-cell Depletion

Measurement of overall B-cell depletion in whole blood allows all mechanisms of action of therapeutic antibodies to be assessed in a single assay. Potent B-cell depleting activity in peripheral blood and lymphoid tissue superior to that of rituximab has been reported with obinutuzumab in non-human primate models [39]. In addition, Mössner et al. showed obinutuzumab to be significantly (10–25 times) more potent and 1.5–2.5 times more effective ($p < 0.001$) than rituximab in depleting B-cells in whole blood from healthy human donors [39]. These results were then confirmed using primary malignant B-cells from a patient with CLL [39]. Superior B-CLL cell depletion in whole blood with obinutuzumab was demonstrated independently by several other research groups [50, 58, 60, 61], whereas Bologna et al. showed that obinutuzumab treatment depleted B-cells in whole blood from CLL patients to a similar extent to rituximab, but at a much faster rate [59]. These findings may reflect the different experimental methods that were used but overall demonstrate the enhanced B-cell-depleting activity of obinutuzumab. Recently, Ysebaert et al. described the effects of rituximab and obinutuzumab on B-cell depletion in 96 CLL patient samples with different prognostic factors [68]. Median proportions of B-cell depletion after whole-blood assay were 22% with rituximab and 63% with obinutuzumab ($p < 0.001$), independent of their prognostic factors [68].



Superior Antitumor Efficacy In Vivo

Finally, obinutuzumab has shown superiority over rituximab in vivo in various human lymphoma xenograft models [39]. Dose-dependent efficacy in the range of 1–30 mg/kg was noted in a staged aggressive SU-DHL4 DLBCL model, with complete tumor regression in all animals and lasting tumor eradication in 90% at the highest dose of 30 mg/kg. In contrast, tumor regression was not shown at any equivalent dose of rituximab [39] or with ofatumumab [43]. In this model, second-line obinutuzumab treatment was also effective in inhibiting the progression of tumors that had progressed under first-line treatment with rituximab and that were no longer responsive to rituximab [39] or ofatumumab [43]. Superior efficacy of obinutuzumab over rituximab or ofatumumab has also been shown in an aggressive disseminated MCL model [39, 43].

Herting et al. described the antitumor activity of obinutuzumab and rituximab alone and in combination with bendamustine, fludarabine, chlorambucil, doxorubicin, and cyclophosphamide/vincristine in subcutaneous murine xenograft models using Z138 MCL and WSU-DLCL2 DLBCL tumors [69]. As obinutuzumab had high single-agent activity in these models, suboptimal doses were used in order to observe combination effects. Superior tumor growth inhibition was achieved with obinutuzumab plus bendamustine over rituximab plus bendamustine, and statistically significant effects versus the respective single treatments were also observed (Fig. 3). In addition, obinutuzumab showed significantly greater activity than rituximab when combined with fludarabine, chlorambucil, or cyclophosphamide/vincristine. Obinutuzumab monotherapy was as effective as, or more effective than, rituximab plus chemotherapy in vivo [69].

CLINICAL TRIALS OF OBINUTUZUMAB IN B-CELL NHL

Obinutuzumab is now under evaluation in an extensive clinical trial program in patients with B-cell malignancies.

Obinutuzumab Monotherapy

Obinutuzumab given as monotherapy has been investigated in the GAUGUIN study (NCT00517530), a multicenter phase Ib/II clinical trial in patients with B-cell malignancies [70–73]. The primary objective of the phase Ib part of the study was to investigate the safety and tolerability of escalating intravenous doses of obinutuzumab in patients with CD20-positive lymphoid malignancies, including NHL and CLL. This was followed by a phase II part to study efficacy and safety. Patients were adults aged 18 years or older with an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2.

The first two reports from GAUGUIN (Table 1) communicated phase I [70] and II [71] results from patients with relapsed/refractory iNHL, most of whom had FL. In the phase I part, 21 patients (median of five prior therapies, 20/21 with prior rituximab) received escalating doses of obinutuzumab monotherapy over eight 21-day cycles. The majority of adverse events (AEs) were infusion-related reactions (IRRs), nearly all of which were of grade 1–2 in severity (Table 1).

IRRs with obinutuzumab, like those seen with rituximab, have been attributed to cytokine release. In previous studies of rituximab, patients with IRRs were found to release greater amounts of interleukin (IL)-8, IL-6, and tumor necrosis factor-alpha than those without IRRs [76–78]. Patients with a baseline absolute lymphocyte count of $\geq 50 \times 10^9$/l appear to have a particularly high risk of developing IRRs [76–78]. The incidence and severity of IRRs in patients treated with obinutuzumab is reportedly greater than with rituximab and has been linked to immediate and marked release of IL-6 and IL-8 that is limited to the first infusion and is accompanied by rapid destruction of circulating B-cells and disappearance of circulating NK cells from the peripheral blood [79].

In GAUGUIN, 18 grade 3–4 AEs occurred in 7 patients; all treatment-related grade 4 events were neutropenias, while most grade 3 AEs were hematologic events and IRRs. Notably, there were no dose-limiting toxicities (DLTs). Two of nine rituximab-refractory patients showed a tumor response, and the best overall response rate (ORR) was 43%, with five complete responses (CRs) and four partial responses indicating the promising activity of obinutuzumab.

Phase II data from relapsed/refractory iNHL patients ($n = 40$; Table 1) showed promising efficacy and acceptable tolerability, particularly in the higher dose group (1600/800 mg) over a median observation time of 33.7 months [71]. Of the phase II patients, 90% had stage III or IV disease and had received a median of two prior rituximab treatments (range 0–5); 45% and 55% were refractory to their last treatment or to rituximab, respectively. End-of-treatment ORRs in rituximab-refractory patients were 8% in the 400/400 mg group and 50% in the 1600/800 mg group. The two CRs were seen in the higher dosage group.

The phase II GAUGUIN study for patients with aggressive forms of B-cell NHL showed similar tolerability and promising efficacy (Table 1) [72]. These patients (25 with DLBCL and 15 with MCL) had received a median of three prior treatments (range 1–17), and 25 (63%) were rituximab-refractory. Of the 25 rituximab-refractory patients, 4 (16%) had objective responses to induction treatment and one responded during follow-up. Four of the five responses were achieved at the 1600/800 mg dose. The best ORR in all patients with aggressive B-cell NHL was 30%. Median response duration (all responders) was

Table 1 Monotherapy trials of obinutuzumab in patients with B-cell NHL

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses		AEs
				ETR	BOR	
GAUGUIN						
Salles et al. [70] r/r iNHL	Phase I; mc, dose escalation	21 (13 FL; 4 MCL; 1 DLBCL; 3 others)	G 50/100–1200/2000 mg; 3 patients per cohort	4 CR; 3 PR ETR = 33%	5 CR; 4 PR BOR = 43%	IRRS in 86% of patients (98% grade 1–2); no DLTs
Salles et al. [71] r/r iNHL	Phase II; mc, ol, r	18 (14 FL; 4 others)	G 400/400 mg	3 PR (17%) FL ETR = 21% (3/14)	2 CR/CRu (11%); 4 PR (22%) Median PFS = 6.0 mo (1.0–33.9+) FL BOR = 26% (5/14)	IRRs in 73% of patients; 2 grade 3–4 in 1600/800 mg group 12 SAEs in 9 patients; 4 treatment-related (herpes zoster infection, neutropenia, febrile neutropenia, pancreatitis)
		22 (20 FL; 2 others)	G 1600/800 mg	2 CR/CRu (9%); 10 PR (46%) FL ETR = 50% (10/20)	5 CR/CRu (23%); 9 PR (41%) Median PFS = 11.9 mo (1.8–33.9 +) FL BOR = 60% (12/20)	
Morschhauser et al. [72] aggressive NHL	Phase II; mc, ol, r	21 (10 DLBCL; 11 MCL)	G 400/400 mg	3 CR/CRu (15%); 2 PR (10%) ETR = 24%	3 CR/CRu (15%); 2 PR (10%) BOR = 24% Median PFS = 2.6 mo (0.3–32.7)	IRRs in 75% of patients (3 grade 3–4) 21 SAEs; 7 treatment-related (3 IRRs; 2 TLS; 1 bradycardia; 1 pyrexia)
		19 (15 DLBCL; 4 MCL)	G 1600/800 mg	6 PR (32%) ETR = 32%	3 CR (16%); 4 PR (21%) BOR = 37% Median PFS = 2.7 mo (0.2–32.7)	

Table 1 continued

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses		AEs
				ETR	BOR	
Cartron et al. [73] r/r CLL	Phase I; mc, ol, dose escalation	13	G 400/800–1200/2000 mg (1000/1000 mg added subsequently)	8 PR (62%)	8 PR (62%)	IRRs in all patients; 2 grade 3 episodes 7 patients with grade 3–4 neutropenia (not dose-related)
	Phase II; mc, ol, r	20	G 1000/1000 mg	3 PR (15%)	1 CR; 5 PR BOR = 30% Median PFS = 10.7 mo	IRRs in 19/20 patients (95%); 5/20 (25%) grade 3 27 grade 3–4 AEs in 15 patients Febrile neutropenia in 2/20 (10%)
GAUSS						
Sehn et al. [74] relapsed iNHL	Phase II; mc, ol, r	74 FL; 14 others	G 1000 mg weekly × 4, then maintenance (if CR/PR/SD) × 2 yrs or until PD	4 CR/CRu (5%); 29 PR (39%)[b] FL ETR = 45% ($p = 0.01$ vs. RIT)	28 CR/CRu (38%); 19 PR (26%)[c] FL BOR = 64% ($p = 0.04$ vs. RIT) Median PFS = 17.6 mo	IRRs in 74% of patients; cough in 24% (safety population $n = 87$) SAEs in 15% 18 deaths
		75 FL; 12 others	RIT 375 mg/m^2 weekly × 4, then maintenance (if CR/PR/SD) × 2 yrs or until PD	3 CR/CRu (4%); 17 PR (23%)[b] FL ETR = 27%	20 CR/CRu (27%); 17 PR (23%)[c] FL BOR = 49% Median PFS 25.4 mo	IRRs in 51% of patients; cough in 9% (safety population $n = 86$) SAEs in 15% 11 deaths

Table 1 continued

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses		AEs
				ETR	BOR	
Japanese phase I						
Ogura et al. [75] r/r NHL	Phase I; mc, ol, dose escalation	12 (8 FL; 2 SLL; 1 MZL; 1 other)	G 200/400–1200/2000 mg	2 CR (17%); 5 PR (42%) ETR = 58%	NR	All patients had ≥1 IRR; grade 3 in 2 patients 91% of all AEs = grade 1 or 2 2 patients had leukopenia/neutropenia leading to withdrawal

AEs adverse events, *BOR* best overall response, *CLL* chronic lymphocytic leukemia, *CR* complete response, *CRu* unconfirmed complete response, *DLBCL* diffuse large B-cell lymphoma, *DLTs* dose-limiting toxicities, *ETR* end of treatment response, *FL* follicular lymphoma, *G* obinutuzumab (GA101), *iNHL* indolent non-Hodgkin lymphoma, *IRRs* infusion-related reactions, *mc* multicenter, *MCL* mantle cell lymphoma, *mo* months, *MZL* marginal zone lymphoma, *NHL* non-Hodgkin lymphoma, *NR* not reported, *ol* open-label, *PD* progressive disease, *PFS* progression-free survival, *PR* partial response, *r* randomized, *RIT* rituximab, *r/r* relapsed/refractory, *SAEs* serious adverse events, *SD* stable disease, *SLL* small lymphocytic lymphoma, *TLS* tumor lysis syndrome, *yrs* years

[a] Where two G doses separated by a forward slash are shown, the first-mentioned dose was given on day 1 and the second dose on day 8 of the first cycle; the second dose was then given on day 1 of the remaining cycles. Unless stated otherwise, eight 21-day cycles of treatment were given (total of nine intravenous infusions)

[b] Response at end of induction; independent review facility assessment. Patients with FL only

[c] Best response noted over entire treatment period; independent review facility assessment. Patients with FL only

9.8 months; there was no relevant difference in progression-free survival (PFS) between dosage groups after a median of 14.2 months (range 0.3–36.1 months) of observation. Based on both these results and on pharmacokinetic modeling, which showed that obinutuzumab 1000 mg per cycle with additional 1000 mg doses on days 8 and 15 of cycle 1 can achieve exposures similar to the 1600/800 mg regimen used in GAUGUIN [80], a simplified flat-dose 1000 mg schedule was adopted for subsequent phase II and III investigation [81]. It has been hypothesized that differences in CD20 binding, activation of biological pathways, and underlying mechanisms of action compared with type I anti-CD20 mAbs permit the use of flat dosing for obinutuzumab rather than conventional body surface area-based dosing [80]. The 1000-mg flat-dose schedule for obinutuzumab, which has been implemented as the standard dose in clinical trials, rapidly achieves CD20 target saturation in all patients tested, with serum concentrations maintained at this therapeutic level throughout the treatment course [80].

Among 13 phase I patients with relapsed/refractory CLL in the separately reported GAUGUIN CLL study, there was a median response duration of 10.5 months (range 8.5–37 months) in eight partial responders (for a best ORR of 62%; Table 1) [73]. Among 20 patients with CLL who were recruited to phase II, the best ORR was substantially lower (6/20; 30%). This has been linked to a higher baseline tumor burden and consequent lower treatment exposure than in phase I [73]. Median response duration was 8.9 months (range 0.8–26.1 months). Tolerability was acceptable in other groups of patients, with IRRs being the most common AEs (Table 1). Most notably, all CLL patients treated in the GAUGUIN study experienced a rapid and sustained elimination of B-cells in the peripheral blood, which was independent of the dose applied. These findings provided the rationale for investigating obinutuzumab in the phase III CLL11 trial. Data from CLL11 and other dedicated studies in patients with CLL are presented later in this review.

Further data in larger numbers of patients with relapsed iNHL were obtained from the multicenter GAUSS study (NCT00576758) [74]. The initial phase I component of this trial evaluated obinutuzumab doses of 200–2000 mg given once weekly for 4 weeks (induction) followed by maintenance therapy every 3 months for 2 years in 22 patients with relapsed B-cell NHL (including 10 with FL) or CLL [82]. The best ORR was 32%, with a response observed in 15% of rituximab-refractory patients. The maximum tolerated dose was not reached. A flat dose of 1000 mg was selected for the phase II part of GAUSS based on the phase I data and other clinical experience. As summarized in Table 1, 175 patients with relapsed iNHL were enrolled to phase II, of whom 149 had FL. The ORR at the end of induction was higher with obinutuzumab than with rituximab, as shown by investigator assessments at the end of the induction period (45% vs. 33%; $p = 0.08$) and by a blinded independent review panel (45% vs. 27%; $p = 0.01$) (Table 1). There was no significant difference between treatments for the secondary endpoint of PFS, but the study was not powered to detect differences. AEs were balanced between the two groups, but there were more IRRs and coughs in the obinutuzumab group (Table 1) [74].

An additional phase I study in 12 Japanese patients, of whom 8 had FL, also showed no DLTs [75]. B-cell depletion was seen in all patients and persisted for the duration of treatment. No disease progression was observed during the treatment period.

Moreover, the majority of AEs were of grade 1 or 2 in severity; as in other studies, IRRs predominated (Table 1).

COMBINATION THERAPY

Phase I and II

Following the promising activity of obinutuzumab in phase I and II single-agent studies, a number of studies have been carried out in the combination therapy setting. The phase Ib GAUDI study (NCT00825149) examined the safety and antitumor activity of two doses of obinutuzumab (G) (400/400 mg or 1600/800 mg) combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP; G-CHOP), or with fludarabine and cyclophosphamide (FC; G-FC), as induction therapy in 56 patients with relapsed/refractory FL (Table 2) [83]. These patients had received up to six prior treatments. Patients were allocated to either CHOP or FC by their physicians on the basis of clinical need and the physician's judgment; allocation to the two obinutuzumab dose regimens was randomized. As seen in the previously described phase I and II monotherapy studies, IRRs were the most common AEs and were predominantly of mild severity (Table 2). Two patients from the G-FC 400/400 mg arm and three from the G-FC 1600/800 mg arm discontinued treatment because of AEs; one other patient in the latter group discontinued because of insufficient response. All rituximab-refractory patients responded to treatment, and the high ORRs noted (Table 2) supported future phase III investigation [83].

As G-CHOP was seen to have a safety profile similar to R-CHOP (rituximab combined with CHOP), the design of the GAUDI study was amended to compare the safety of obinutuzumab plus CHOP or bendamustine in 81 treatment-naïve patients with FL (Table 2) [84]. After 2 years' maintenance in initial responders who went on to receive obinutuzumab 1000 mg as monotherapy, high CR rates were seen in both treatment arms and opportunistic infections were infrequent [84].

G-CHOP was also investigated in the first-line setting in 80 patients with advanced DLBCL in the GATHER study (NCT01414855) (Table 2) [85]. The ORR and CR rates as determined by investigators were 83% and 55%, respectively (Table 2). IRRs, most of which were grade 1–2 in intensity, were typically observed during the first cycle.

Phase III

The phase III GADOLIN study (NCT01059630) (Table 2) compared bendamustine monotherapy (120 mg/m^2 on day 1 and 2, for up to six 28-day cycles) with obinutuzumab (1000 mg on day 1, 8, and 15 of cycle 1 and on day 1 of cycles 2–6 in 28-day cycles) plus bendamustine (90 mg/m^2 on day 1 and 2, for up to six 28-day cycles) induction and obinutuzumab maintenance (1000 mg every 2 months for 2 years or until progression in patients achieving stable disease or better with induction) in 396 patients with rituximab-refractory iNHL who had received a median of two prior treatments [86]. At a preplanned interim analysis for efficacy, enrollment was stopped and the trial was analyzed in full, as the primary endpoint had been met. Median observation time was 20.3 months for bendamustine alone and 21.9 months for obinutuzumab plus bendamustine. Recruitment had been ongoing between data cutoff and interim analysis, and a significant number of patients were still undergoing treatment. PFS was significantly longer with combination therapy (median not reached) than with bendamustine alone

Table 2 Combination trials of obinutuzumab plus chemotherapy in patients with B-cell NHL

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses	AEs
GAUDI					
Radford et al. [83] r/r FL	Phase Ib; mc, ol, r	14	CHOP q3wk × 6–8 cycles + G 400/400 mg	2 CR (14%); 11 PR (79%) ORR = 93%	Grade 1–2 IRRs were most common: CHOP regimens 68%, FC regimens 82% Grade 3–4 IRRs = 7% (restricted to first infusion) Neutropenia: CHOP regimens 43%, FC regimens 50% All patients received the planned G dose
		14	CHOP q3wk × 6–8 cycles + G 1600/800 mg	9 CR (64%); 5 PR (36%) ORR = 100%	
		14	FC q4wk × 4–6 cycles + G 400/400 mg	11 CR (79%); 3 PR (21%) ORR = 100%	
		14	FC q4wk × 4–6 cycles + G 1600/800 mg	3 CR (21%); 9 PR (64%) ORR = 86%	
GAUDI (first-line)					
Dyer et al. [84] FL (first-line)	Phase Ib; mc, ol, r	40	CHOP q3wk × 6–8 cycles + G 1000 mg, then G maintenance (if CR/PR) every 3 mo × 2 yrs or until PD	CR = 70% PFS (32 mo) = 84%	G-CHOP 78% (31% grade ≥3); cough 11%; dose delay 6%; 1 death due to G-related RTI + lactic acidosis G-BEN: 100% (44% grade ≥3); cough 17%; dose delay 17% Grade ≥3 events mainly infections and cytopenia
		41	BEN 90 mg/m^2 q4wk × 4–6 cycles + G 1000 mg, then G maintenance (if CR/PR) every 3 mo × 2 yrs or until PD	CR = 61% PFS (32 mo) = 92%	

Table 2 continued

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses	AEs
GATHER					
Zelenetz et al. [85] DLBCL (first-line advanced)	Phase II; mc, ol	80	CHOP cycles 1–6 + G 1000 mg d1, d8, and d15 of cycle 1, then d1 cycles 2–8 SDI (120 or 90 min) permitted from cycle 2 for patients with no grade ≥3 and/or serious IRRs and lymphocyte count ≤5000/μl in cycle 1	44 CR (55%) 22 PR (28%) ORR = 83%	Most AEs were grade 1–2 IRRs in 64%; 2 grade 3 and no grade 4 During 288 SDI infusions, there were 3 grade 1–2 IRRs (no grade ≥3 IRRs) Neutropenia: 13% grade 3, 21% grade 4
GADOLIN					
Sehn et al. [86] RIT-r iNHL	Phase III; mc, ol, r	202 (198 treated)	BEN 120 mg/m^2 d1 + 2 cycles 1–6	23 CR(12%)[b,c] ORR[b] = 63% Median PFS =15 mo Deaths = 41	Grade ≥3 = 62% Neutropenia = 26% IRRs = 6% Thrombocytopenia = 16% Anemia = 10%
		194	BEN 90 mg/m^2 d1 + 2 cycles 1–6 + G 1000 mg d1, d8, and d15 cycle 1, then d1 cycles 2–6 and maintenance 1000 mg every 2 mo (if no PD) for 2 yrs or until PD[d]	21 CR(11%)[b,c] ORR[b] = 69% Median PFS = NR (HR 0.55, $p = 0.0001$ vs. BEN) Deaths = 34	Grade ≥3 = 68% Neutropenia = 33% IRRs = 11% Thrombocytopenia = 11% Anemia = 8%

Table 2 continued

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses	AEs
Trněny et al. [87] RIT-r FL (subanalysis of FL patients)	Phase III, mc, ol, r	166	BEN 120 mg/m^2 d1 + 2 cycles 1–6	CR[b] = 14%[c] ORR[b] = 63%[c] Median PFS =13.8 mo[c]	Grade ≥3 = 59%
		155	BEN 90 mg/m^2 d1 + 2 cycles 1–6 + G 1000 mg d1, d8, and d15 cycle 1, then d1 cycles 2–6 and maintenance 1000 mg every 2 mo (if no PD) for 2 yrs or until PD[d]	CR[b] = 9%[c] ORR[b] = 71%[c] Median PFS = NR (HR 0.48 vs. BEN)[c]	Grade ≥3 = 66%
GALLIUM					
FL and MZL (first-line)	Phase III, mc, ol, r	699	CHOP q3wk × 6 cycles, or CVP q3wk × 8 cycles, or BEN q4wk (d1 + 2) × 6 cycles + G 1000 mg d1, d8, and d15 cycle 1, then d1 cycles 2–6 or 2–8, then G maintenance 1000 mg every 2 mo (if CR/PR) for 2 yrs or until PD	Primary endpoint: investigator-assessed PFS in FL patients Results due to be presented late 2016	
		702	CHOP q3wk × 6 cycles, or CVP q3wk × 8 cycles, or BEN q4wk (d1 + 2) × 6 cycles + RIT 375 mg/m^2 d1 cycles 1–6 or 1–8, then R maintenance 375 mg/m^2 every 2 mo (if CR/PR) for 2 yrs or until PD		
GOYA					
DLBCL (first-line)	Phase III, mc, ol, r	706	CHOP q3wk × 6–8 cycles + G 1000 mg d1, d8, and d15 cycle 1, then d1 cycles 2–8	Primary endpoint: investigator-assessed PFS Results due to be presented late 2016	
		712	CHOP q3wk × 6–8 cycles + RIT 375 mg/m^2 d1 cycles 1–8		

Table 2 continued

Reference and type of disease	Study phase and details	No. of patients	Regimens[a]	Responses	AEs
GATS					
FL, DLBCL, and MZL (first-line)	Phase II, mc, ol	36	CHOP q3wk × 6 cycles + G 1000 mg d1, d8, and d15 cycle 1, then d1 cycles 2–8 SDI (90 min) permitted from cycle 2 for patients with no grade ≥3 and/or serious IRRs and lymphocyte count ≤5000/μl in cycle 1	Primary endpoint: tolerability, PK, and time-course cytokines Results due to be presented late 2016	

AEs adverse events, *BEN* bendamustine, *CHOP* cyclophosphamide, doxorubicin, vincristine, and prednisone, *CVP* cyclophosphamide, vincristine, and prednisone, *CR* complete response, *d* day, *DLBCL* diffuse large B-cell lymphoma, *FC* fludarabine and cyclophosphamide, *FL* follicular lymphoma, *G* obinutuzumab (GA101), *HR* hazard ratio, *iNHL* indolent non-Hodgkin lymphoma, *IRRs* infusion-related reactions, *NHL* non-Hodgkin lymphoma, *NR* not reached, *mc* multicenter, *min* minutes, *mo* months, *MZL* marginal zone lymphoma, *ol* open-label, *ORR* overall response rate, *PD* progressive disease, *PFS* progression-free survival, PK pharmacokinetics, *PR* partial response, *q3wk* every 3 weeks, *q4wk* every 4 weeks, *r* randomized, *r/r* relapsed/refractory, *RIT* rituximab, RIT-r rituximab-refractory, *RTI* respiratory tract infection, *SDI* shorter duration of infusion, *yrs* years

[a] Where two G doses separated by a forward slash are shown, the first-mentioned dose was given on d1 and the second dose on d8 of the first cycle; the second dose was then given on d1 of the remaining cycles. Unless stated otherwise, eight 21-day cycles of treatment were given (total of nine intravenous infusions)

[b] End of induction

[c] Independent review facility assessment

[d] Twenty-eight-day cycles. Non-progressing patients in the BEN + G arm went on to receive G monotherapy every 2 months for up to 2 years. PFS estimation was by independent review

(median 14.9 months) as assessed by an IRF [primary endpoint; hazard ratio (HR) 0.55; 95% confidence interval (CI) 0.40–0.74; $p = 0.0001$].

Subgroup analyses and secondary endpoints (including PFS by investigator and OS) were consistent and supportive of the primary endpoint. There were no significant differences in IRF-assessed ORR or CR rate at the end of induction. Whether this was due to the differences between groups in bendamustine dosage or to the different modes of action of obinutuzumab and bendamustine was uncertain (Table 2). The prognostic relevance of minimal residual disease (MRD) status in 93 biomarker-evaluable patients with FL in GADOLIN was also investigated [88]. MRD analysis measures the small number of malignant cells that remain after treatment. MRD status was associated with clinical CR rate and improved PFS, and significantly more patients were MRD-negative after induction with obinutuzumab plus bendamustine than after bendamustine alone (82% vs. 43%; $p < 0.0001$). Median OS was not reached in either arm at the time of reporting, but there were more deaths due to disease progression with bendamustine alone than with obinutuzumab plus bendamustine [86]. Overall, grade ≥3 AEs were more common in the obinutuzumab plus bendamustine group, but grade ≥3 thrombocytopenia and anemia were more frequent in the bendamustine-only group (Table 2).

Emerging data from GADOLIN also suggest improvements in health-related quality of life [89]. The time taken to a ≥6-point worsening from baseline on the Functional Assessment of Cancer Treatment-Lymphoma Trial Outcome Index (FACT-Lym TOI) was 8.0 months in the combination therapy arm and 4.6 months with bendamustine alone. A higher proportion of patients reported meaningful improvements on the lymphoma subscale, lymphoma TOI, and total score with obinutuzumab plus bendamustine than with bendamustine alone [89].

Ongoing phase III combination therapy studies with obinutuzumab include GALLIUM (first-line advanced iNHL; NCT01332968) and GOYA (first-line DLBCL; NCT01287741). GALLIUM aims to assess the efficacy and safety of obinutuzumab plus chemotherapy versus rituximab plus chemotherapy followed by maintenance immunotherapy. After induction, responders will progress to maintenance therapy with their randomized antibody treatment alone, given every 2 months until disease progression or for a maximum of 2 years. In May 2016, at a prespecified interim analysis, the GALLIUM Independent Data Monitoring Committee recommended analysis of the study data as the primary endpoint of investigator-reported PFS had been met [90]. This is the second head-to-head comparative trial against rituximab that has shown a positive result for obinutuzumab, the first being the CLL11 study (described below). In the phase III GOYA trial, previously untreated patients with DLBCL were randomized to obinutuzumab 1000 mg every 21 days (with two additional doses on day 8 and day 15 of cycle 1) or rituximab 375 mg/m^2 every 21 days for eight cycles in addition to 6–8 cycles of CHOP chemotherapy. Recruitment of 1418 patients was completed in 2014. After the final analysis in July 2016, Roche issued a press release to say that the study's primary endpoint of improvement in investigator-assessed PFS had not been met. Detailed results are expected to be announced at ASH 2016.

CLINICAL TRIALS OF OBINUTUZUMAB IN CLL

Phase I and II

A number of studies have investigated the use of obinutuzumab in patients with CLL. The GAGE study (NCT01414205), a randomized, phase II study in 80 symptomatic, untreated CLL patients, demonstrated significant antitumor activity with both 1000 mg and 2000 mg dosages of obinutuzumab. End of induction response was superior with the higher dosage (Table 3) [91]. The results suggested a possible dose-response relationship, but this requires further investigation and longer follow-up. Obinutuzumab has also shown manageable toxicity and promising activity in combination with either bendamustine or FC in the phase Ib GALTON study (NCT01300247) in 41 patients with previously untreated CLL [92].

Phase III

In the phase III CLL11 study (Table 3), 781 patients with previously untreated CLL and a score higher than 6 on the Cumulative Illness Rating Scale or an estimated creatinine clearance of 30–69 ml/min were randomized to chlorambucil alone, obinutuzumab plus chlorambucil, or rituximab plus chlorambucil for six 28-day cycles [93]. The main study followed a safety run-in designed to ensure that chlorambucil-containing chemoimmunotherapy regimens were safe in recruited older patients with comorbidities [94]. IRRs and neutropenia were identified as potential risks during the run-in, but none of the specified stopping criteria were met, and the main study was opened for randomization in April 2010.

Both combinations increased PFS significantly over chlorambucil monotherapy; obinutuzumab plus chlorambucil, but not rituximab plus chlorambucil, prolonged OS significantly (Table 3) [95]. Obinutuzumab plus chlorambucil also conferred longer PFS (Table 3) [93, 95] and a higher CR rate (21% vs. 7%) [93] than rituximab plus chlorambucil, and it was associated with significantly and substantially increased time to next treatment (Table 3) [95].

AEs were reported at higher frequencies with obinutuzumab plus chlorambucil than with either of the other two treatments, but toxicities were manageable and the risk of infection was not increased over rituximab plus chlorambucil or chlorambucil alone. Among patients for whom MRD data were available, the proportion who were MRD-negative in bone marrow and peripheral blood at the end of treatment was markedly higher with obinutuzumab plus chlorambucil than with rituximab plus chlorambucil treatment (bone marrow, 18% vs. 3%; peripheral blood, 36% vs. 3%) [96]. MRD in peripheral blood at the end of treatment was an independent prognostic factor for both PFS (HR 5.29; 95% CI 3.48–8.04; $p < 0.001$) and OS (HR 3.04; 95% CI 1.53–6.03; $p = 0.002$).

The GREEN study (NCT01905943) is an ongoing, open-label, multicenter, phase IIIb study in patients with previously untreated or relapsed/refractory CLL receiving obinutuzumab alone or in combination with chemotherapy (bendamustine, FC, or chlorambucil). One of the objectives of this study is to investigate the potential of alternative obinutuzumab administration protocols for reducing IRRs. Emerging results suggest that the safety profile of obinutuzumab plus bendamustine is manageable if the appropriate measures are taken (e.g., monitoring at-risk patients for tumor lysis syndrome after the first infusion) [97, 98].

Table 3 Trials of obinutuzumab in patients with CLL

References (setting)	Study phase and details	No. of patients	Regimens	Responses	AEs
GAGE					
Byrd et al. [91] (first-line)	Phase II; mc, ol, r	41	G 1000 mg d1–2,[a] d8, and d15 cycle 1, then d1 cycles 2–8	2 CR (5%); 18 PR (44%) ORR = 49% 18-mo PFS = 59%[b]	IRRs[c] in 28 patients (70%); 1 grade 3–4 Neutropenia = 38% (30% grade 3–4) Thrombocytopenia = 25% (15% grade 3–4)
		39	G 2000 mg d1–3,[a] d8, and d15 cycle 1, then d1 cycles 2–8	8 CR/CRi (20%); 18 PR (46%) ORR = 67% 18-mo PFS = 83%[b]	IRRs[c] in 24 patients (63%); 0 grade 3–4 Neutropenia = 32% (all grade 3–4) Thrombocytopenia = 16% (11% grade 3–4)
GALTON					
Brown et al. [92] (first-line)	Phase Ib; mc, ol	21	G 1000 mg d1–2,[a] d8, and d15 cycle 1, then d1 cycles 2–6 + F 25 mg/m^2 + C 250 mg/m^2 d2–4 cycle 1, then d1–3 cycles 2–6 (28-day cycles)	5 CR/CRi (24%); 8 PR (38%) ORR = 62% No relapses or deaths after median F/U 20.7 mo	IRRs[c] in 91% of patients (none as SAEs) Grade 3–4 AE in 86% SAEs in 29% (including 3 febrile neutropenia + 1 neutropenia + 3 infections)
		20	G 1000 mg d1–2,[a] d8, and d15 cycle 1, then d1 cycles 2–6 + BEN 90 mg/m^2 d2–3 cycle 1, then d1–2 cycles 2–6 (28-day cycles)	9 CR/CRi (45%); 9 PR (45%) ORR = 90% No relapses or deaths after median F/U 23.5 mo	IRRs[c] in 90% of patients (4 as SAEs) Grade 3–4 AEs in 85% SAEs in 45% (including 2 febrile neutropenia + 1 infection)

Table 3 continued

References (setting)	Study phase and details	No. of patients	Regimens	Responses	AEs
CLL11					
Goede et al. [93–95] (first-line)	Phase III; mc, ol, r	781	CHL 0.5 mg/kg d1 and d15 × 6 28-day cycles G 1000 mg d1–2,[a] d8, and d15 cycle 1, then d1 cycles 2–6 + CHL 0.5 mg/kg d1 and d15 (28-day cycles) RIT 375 mg/m^2 d1 cycle 1, then 500 mg/m^2 d1 cycles 2–6 + CHL 0.5 mg/kg d1 and d15 (28-day cycles)	G + CHL vs. CHL comparison: Median PFS = 29.9 vs. 11.1 mo (HR 0.18; $p < 0.001$). Median OS = NR vs. NR (HR 0.47; $p = 0.0014$) G + CHL vs. RIT + CHL comparison: Median PFS = 29.2 vs. 15.4 mo (HR 0.40; $p < 0.001$). Median OS = NR vs. NR (HR 0.70; $p = 0.0632$). Median TTNT 42.7 vs. 32.7 mo (HR 0.54; $p < 0.001$)	AEs more frequent with G + CHL than in other groups, but no increase in infection risk G + CHL vs. RIT + CHL comparison of IRRs: Any grade = 66% vs. 38% Grade ≥3 = 20% (restricted to first infusion) vs. 4% Serious = 10% vs. 2%

AEs adverse events, *BEN* bendamustine, *C* cyclophosphamide, *CHL* chlorambucil, *CLL* chronic lymphocytic leukemia, *CR* complete response, *CR* complete response with incomplete marrow recovery, *d* day, *F* fludarabine, F/U, follow-up, *G* obinutuzumab (GA101), *HR* hazard ratio, *IRRs* infusion-related reactions, *mc* multicenter, *mo* month, *NR* not reached, *ol* open-label, *ORR* overall response rate, *OS* overall survival, *PFS* progression-free survival, *PR* partial response, *r* randomized, *RIT* rituximab, *SAEs* serious adverse events, *TTNT* time to next treatment

[a] The first dose was split over 2 or 3 days; 100 + 900 mg (= 1000 mg) or 100 + 900 + 1000 mg (= 2000 mg); 28-day cycles

[b] Note that 95% confidence intervals overlapped for these PFS results (39–79% and 67–99%) and survival curves merged after month 18, indicating equivalence of treatments

[c] IRR = any AE during or within 24 h after the infusion that was related to study medication

DOSE RATIONALE

In all phase III trials, obinutuzumab was, or is being, administered at the aforementioned rationally optimized 1000-mg flat-dose schedule [80], compared with the standard, approved 375 mg/m^2 dose of rituximab in iNHL/DLBCL or 500 mg/m^2 in CLL. A comparison of equal doses of the two mAbs was not feasible in the phase III trials because of the requirement for a third treatment arm and the associated need to recruit many additional patients. However, while there is controversy over whether the approved dose of rituximab is optimal, or which patients may benefit from a higher dose [99], there is no clinical evidence proving that increasing the dose of rituximab leads to better long-term outcomes for patients [100, 101]. Furthermore, differences between the obinutuzumab and rituximab arms in the CLL11 trial in terms of the kinetics of peripheral B-cell depletion [102] and achievement of MRD negativity [96] imply that the distinct biology of the two mAbs has a greater influence on the clinical results than the different dose schedules, and as such, increasing the dose of rituximab would be unlikely to result in comparable outcomes to obinutuzumab.

SHORTER DURATION OF INFUSION

Reducing the duration of infusion for intravenous drugs has potential advantages in terms of patient and physician burden. Long infusion times and frequent infusion rate changes result in lengthy observation times, increased nursing and administration staff workloads, and inconvenience to patients [103].

A notable early example of research to balance the benefits of shorter duration of infusion (SDI) against the potentially increased risk of hypersensitivity reactions was the effort made in the 1990s to reduce paclitaxel infusion times from 24 to 3 h [104]. Since then, SDI with rituximab has been investigated in 351 patients with rheumatoid arthritis in the RATE-RA study (NCT01382940): there was no increase in the rate or severity of IRRs when infusion times were reduced from the standard 4.25 to 2 h [103]. SDI with rituximab was subsequently assessed in a phase III study in 425 patients with DLBCL or FL [105]. Reduction of the infusion time from a median of 240 min to a fixed time of 90 min was found to be feasible in patients who tolerated an initial infusion at the longer standard rate. These observations led to an increase in the recommended infusion rate for rituximab to a maximum of 400 mg/h [106] and to the investigation of SDIs in patients receiving obinutuzumab. In the studies in which SDI was investigated, patients received obinutuzumab at the regular infusion rate for the first cycle to establish safety (i.e., no IRRs of grade $\geq$3) and activity (lymphocyte count $\leq$5000/μl), after which SDI could be started from cycle 2.

GATHER

The safety of SDI of obinutuzumab after cycle 1 was evaluated in the GATHER study (first-line advanced DLBCL) (Table 2) [85]. After treatment at the regular infusion rate was deemed safe, based on 20 patients who received the standard-rate infusion with G-CHOP therapy, patients who met the SDI inclusion criteria (who had not experienced a serious and/or grade $\geq$3 IRR and had a lymphocyte count $\leq$5000/μl) were treated at the SDI rate. Initially three patients in the cohort received obinutuzumab over 120 min; if none of the three experienced IRRs of grade $\geq$3, the 90-min infusion was then tested for the

remaining patients. Overall, 51 of 80 patients (64%) experienced IRRs, most of which were of grade 1–2 in severity and reported in cycle 1. There were no IRRs of grade ≥3 with the 90-min infusion [85].

GATS Study (JapicCTI-152848)

SDI with obinutuzumab in combination with chemotherapy (CHOP) is also being investigated in a phase II study in Japanese patients (Table 2) receiving first-line therapy for various types of CD20-positive B-cell NHL. In cycle 1, all patients receive obinutuzumab 1000 mg on day 1 over 4.25 h and on days 8 and 15 over 3.25 h. Patients who meet the criteria for SDI (no serious and/or grade ≥3 IRRs in the first three infusions and lymphocyte count ≤5000/μl prior to SDI) receive their next obinutuzumab infusion over 1.5 h. The primary objectives of the study are to evaluate infusion tolerability, pharmacokinetics, and change in cytokine levels over time.

FUTURE PERSPECTIVES

Since the introduction of rituximab in 1997, great advances have been made in the management of lymphoma and lymphocytic leukemia of B-cell origin. The formulation of truly novel treatment paradigms has been made possible, and multiple avenues of research are now being explored to find new and more effective ways of applying targeted therapy to maximize tumor responses and OS, while minimizing AEs and patient discomfort. This includes the expansion of combination therapy choices to include multiple targeted agents with the potential for reducing reliance on chemotherapy. Chemotherapy-free combinations have the potential to further optimize effector functions, and anti-CD20 therapy can reasonably be considered as a base treatment option. Anti-CD20 therapy holds great promise for the future management of B-cell NHL, and several novel entities are under development.

Several trials are now evaluating obinutuzumab in combination with other targeted therapies in B-cell NHL. The phase Ib/II GALEN study (NCT01582776) is evaluating obinutuzumab in combination with lenalidomide in relapsed/refractory and first-line FL patients and in patients with aggressive B-cell lymphomas (DLBCL and MCL). Phase Ib data from this study have already shown that the combination is effective, with an objective response in 13 of 19 evaluable patients, including 7 CRs [107]. Other similar studies include the GO27834 (ROMULUS) study (NCT01691898), a phase II study in which combinations of obinutuzumab or rituximab with the anti-CD79b antibody–drug conjugate, polatuzumab vedotin, are being tested in patients with relapsed/refractory B-cell NHL. In addition, the phase II GO29365 study (NCT02257567) is evaluating polatuzumab vedotin in combination with rituximab or obinutuzumab and bendamustine in patients with relapsed/refractory FL or DLBCL. The novel oral BCL-2 inhibitor, GDC-0199 (ABT-199; venetoclax), is being investigated in a phase I/II study (GO27878; NCT02055820) in combination with rituximab or obinutuzumab plus CHOP in patients with B-cell NHL, while the phase I GO29383 study (NCT02220842) is assessing obinutuzumab in combination with atezolizumab (MPDL3280A) in relapsed/refractory FL and DLBCL. Atezolizumab is a fully humanized antibody that acts against the protein ligand PD-L1.

There are also two phase III studies ongoing in first-line CLL. The CLL14 study (NCT02242942) will compare the efficacy and safety of a combined regimen of obinutuzumab

and venetoclax versus obinutuzumab plus chlorambucil in patients with CLL and coexisting medical conditions. The anticipated time on study treatment is approximately 1 year, with a follow-up period of up to 5 years. The second study is evaluating the combination of obinutuzumab and ibrutinib, a Bruton's tyrosine kinase inhibitor, compared with obinutuzumab plus chlorambucil in patients with CLL or small lymphocytic leukemia (NCT02264574). Follow-up is planned for up to 3 years.

Other open-label, multicenter phase Ib/II studies in which obinutuzumab is being used in combination with other investigational agents include atezolizumab plus obinutuzumab and CHOP or bendamustine in first-line FL and DLBCL (NCT02596971); atezolizumab plus obinutuzumab and lenalidomide in relapsed/refractory FL (NCT02631577); atezolizumab plus obinutuzumab and polatuzumab vedotin in relapsed/refractory FL and DLBCL (NCT02729896); obinutuzumab plus polatuzumab vedotin and lenalidomide in relapsed/refractory FL and DLBCL (NCT02600897); obinutuzumab plus polatuzumab vedotin and venetoclax in relapsed/refractory FL and DLBCL (NCT02611323); and obinutuzumab plus idasanutlin (an MDM2 antagonist) in relapsed/refractory FL and DLBCL (NCT02624986). A phase Ib study examining the combination of obinutuzumab plus idasanutlin and venetoclax is also planned.

A large number of other molecular targets are being explored for their therapeutic potential in hematologic malignancies; these are beyond the scope of this review and are discussed elsewhere [45]. However, novel approaches designed to promote targeting of tumors by T-cells are also being investigated. Bispecific T-cell engagers (BiTE) contain the variable domains of two antibodies joined together, one of which binds CD19, while the other binds T-cell CD3 [108]. The creation of a CD19/CD3 complex brings tumor and T-cells together in close proximity, which in turn activates the T-cell and causes it to destroy the tumor cell via perforin-mediated apoptosis [45]. Other researchers are exploring the potential of anti-CD20/CD3 bispecific antibodies to overcome problems of short half-life in vivo, structural instability, and poor solubility of anti-CD19 BiTEs [109–111]. Another approach is modification with chimeric antigen receptors (CARs) of T-cells to confer tumor-specific cytotoxicity [112]. Preclinical and clinical studies of CD19 CAR-T-cells have shown encouraging results in a variety of cancers, particularly B-cell hematologic malignancies [113].

There is also a need to explore the potential applicability of surrogate endpoints in hematologic malignancies to help direct therapy and to expedite the conduct of clinical trials. Positron emission tomography using 18-fluoro-2-deoxyglucose has become a standard clinical tool for staging and response assessment in aggressive lymphomas, and results from a number of studies indicate its potential utility in predicting outcomes in patients with DLBCL and FL [114, 115]. Another approach is to measure MRD (as described above for GADOLIN and CLL11) [88, 96], which can be used for evaluation of treatment effectiveness, risk stratification, and long-term outcome prediction. While multicolor flow cytometry and polymerase chain reaction-based methods are currently the two most commonly used techniques for assessing MRD, next-generation sequencing is likely to be more widely employed in the future [116].

PFS is the standard endpoint for assessing new drugs in first-line FL, but the increasing efficacy of new treatments and the indolent nature of FL demand extended patient follow-up in clinical trials [117]. There is therefore interest in whether CR at 30 months (CR30) could accurately predict likely treatment effects on PFS. A recent meta-analysis of data from 13 randomized, first-line trials in 3837 patients with individual patient data has supported this hypothesis [117]: an absolute improvement in CR30 of ≥10% over a control CR30 of 50% predicted a significant improvement in PFS. This suggests that CR30 has utility as a surrogate for PFS in first-line FL trials and supports its use to facilitate treatment development.

In conclusion, researchers are continuing their efforts to develop increasingly efficient therapies that provide long treatment-free periods for patients with relapsing or refractory disease, to identify the most effective combination therapies (notably triplet combinations) and, as discussed above, to develop chemotherapy-free regimens based on immunotherapy. The advances with obinutuzumab described here against the background of the many other therapeutic approaches that are underway are evidence of marked and significant progress in the development and application of immunotherapy against lymphocytic leukemia and B-cell lymphoma.

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