Drugs of the Future 2011, 36(9): 657-662

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.9.1688499



MONOGRAPH

# OBINUTUZUMAB

Rec INN; USAN

*Humanized Anti-CD20 Monoclonal Antibody*
*Apoptosis Inducer*
*Oncolytic*

Afutuzumab (previous name)
GA101
R-7159
RG-7159
RO-5072759

Immunoglobulin $G_1$, anti-[Homo sapiens CD20 (membrane-spanning 4-domains subfamily A member 1, MS4A1, B lymphocyte surface antigen B1, Leu-16, Bp35)], humanized monoclonal antibody, GA101; γ1 heavy chain (1-448) [humanized VH (Homo sapiens FR/Mus musculus CDR, Homo sapiens IGHJ4*01) [8.8.12] (1-119)-Homo sapiens IGHG1*01 (120-448)], (222-219')-disulfide with κ light chain (1'-219') [humanized V-KAPPA (Homo sapiens FR/Mus musculus CDR, Homo sapiens IGKJ4*01) [11.3.9] (1'-112')-Homo sapiens IGKC*01 (113'-219')]; (228-228'':231-231'')-bisdisulfide dimer
Immunoglobulin $G_1$, anti-(human CD20 (antigen)) (human-mouse monoclonal GA101 heavy chain), disulfide with human-mouse monoclonal GA101 κ-chain, dimer
Immunoglobulin $G_1$, anti-(human B-lymphocyte antigen CD20 (membrane-spanning 4-domains subfamily A member 1, B-lymphocyte surface antigen B1, Leu-16 or Bp35)), humanized mouse monoclonal GA101 des-CH3107-K-γ1 heavy chain (222-219')-disulfide with humanized mouse monoclonal GA101 κ light chain dimer (228-228'':231- 231'')-bisdisulfide

CAS: 949142-50-1
EN: 382400

**SUMMARY**

*The emergence of anti-CD20 monoclonal antibodies (MAbs), especially rituximab, has dramatically improved the treatment of B-cell malignancies during the past 10 years. However, many patients still experience refractory forms and short-term relapses. Obinutuzumab (GA101; formerly afutuzumab) is the first humanized and glycoengineered type II anti-CD20 MAb to enter clinical development. As illustrated in recent biological studies, obinutuzumab exhibits much stronger activity than rituximab against non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) cells. Despite inferior complement-dependent cytotoxicity, it demonstrates enhanced direct cell death and antibody-dependent cellular cytotoxicity mechanisms, suggesting the possibility to overcome resistance to rituximab. The first early clinical trials of obinutuzumab in relapsed/refractory indolent and aggressive NHL and in CLL patients showed significant overall response rates and a good tolerability profile. Phase III trials are currently being conducted to assess the role of obinutuzumab in combination with conventional therapy in B-cell malignancies.*

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**BACKGROUND**

Over the last decade, the addition of rituximab, a chimeric $IgG_1$ anti-CD20 monoclonal antibody (MAb), to conventional chemotherapy, has dramatically improved the management of B-cell malignancies, increasing response rates, progression-free survival and overall survival (1-3). Thus, it is now widely used in diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and also chronic lymphocytic leukemia (CLL). However, a substantial number of patients fail to achieve complete remission or tend to relapse and become refractory to rituximab, what is often synonymous of poor prognosis, particularly in DLBCL (4). Therefore, there is a need to develop new agents with improved efficacy that are able to delay the onset of relapse and obtain durable remissions.

Nowadays, various therapeutic approaches are being evaluated based on specific signaling pathway inhibitors or using molecules directed against other new targets on B cells (5). Unfortunately, clinical benefit often remains to be formally demonstrated, and some of these agents exhibit poor tolerability profiles.

As a common cell surface antigen expressed on B cells, CD20 has become the most suitable antibody target for the treatment of B-cell malignancies (6). It is a nonglycosylated protein of 33-35 kDa, whose expression is restricted to the B-cell hematopoietic lineage, and it is found in a large majority of B-cell neoplasms (7). As illustrated in Figure 1, it is expressed on B-cell precursors and mature B cells, but neither on hematopoietic stem cells nor plasma cells. Therefore, anti-CD20 MAbs usually do not significantly affect immunoglobulin

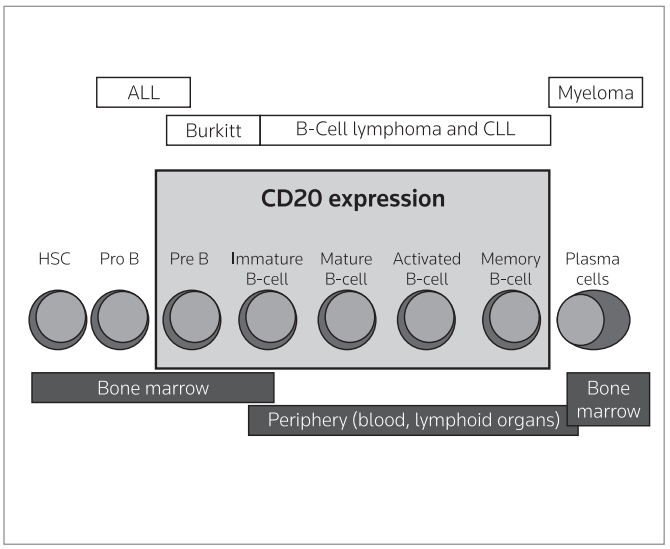


**Figure 1.** Schematic representation of B-cell differentiation, with potential B-cell-derived malignancies on the top. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; HSC, hematopoietc stem cells.

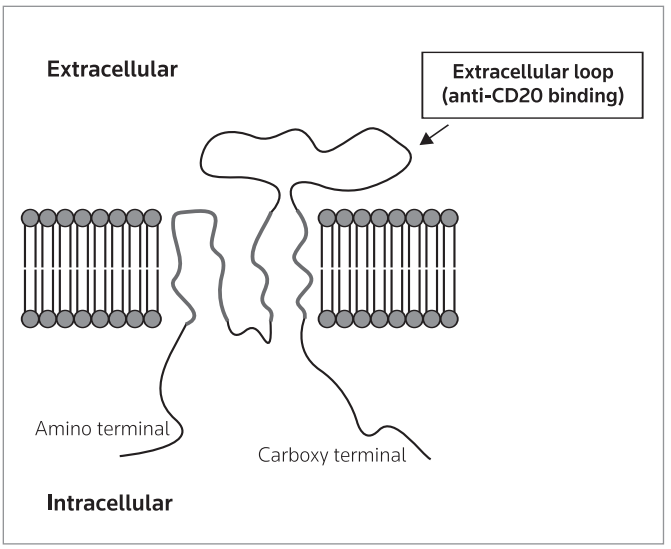


**Figure 2.** Schematic representation of the CD20 molecule.

serum concentrations. In light of these considerations, CD20 represents an attractive therapeutic target in B-cell malignancies.

CD20 has four transmembrane domains, two intracellular segments corresponding to the amino and carboxy termini and two extracellular parts which are ideal binding targets for anti-CD20 MAbs (Fig. 2) (7, 8). This configuration makes CD20 deeply anchored in the membrane, protecting it from antigen shedding.

Various effector pathways have been proposed to be involved in the antitumor effect of anti-CD20 MAbs. These include direct cell death (DCD) (resulting from regulation of cell cycle or induction of apoptosis), complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) (Fig. 3).

In DCD mechanisms, binding of antibodies to CD20 has been reported to cause a rapid distribution of CD20 molecules into lipid rafts. These lipid rafts represent specialized microdomains of the plasma membrane, highly enriched in sphingolipids and cholesterol, and are involved in numerous membrane-associated signaling pathways. As demonstrated in vitro, this distribution could subsequently lead to an increased intracellular calcium level and downstream apoptotic signaling (9).

Several in vitro studies in B-lymphoma cell lines have demonstrated that anti-CD20 MAbs, and particularly rituximab, are able to bind C1q and subsequently to activate the complement cascade, leading to cell membrane disruption (10). Noteworthy, the ability of the different anti-CD20 MAbs to induce complement-mediated lysis correlates with the level of CD20 antigen expression, but also with their capacity to translocate CD20 into lipid rafts, an effect that could be dependent on the epitope recognized by the MAb (8, 11). However, the importance of this mechanism in B-cell depletion remains controversial, and some data even suggest that complement activation could have deleterious effects, since it inhibits ADCC and natural killer (NK) cell activation (12).

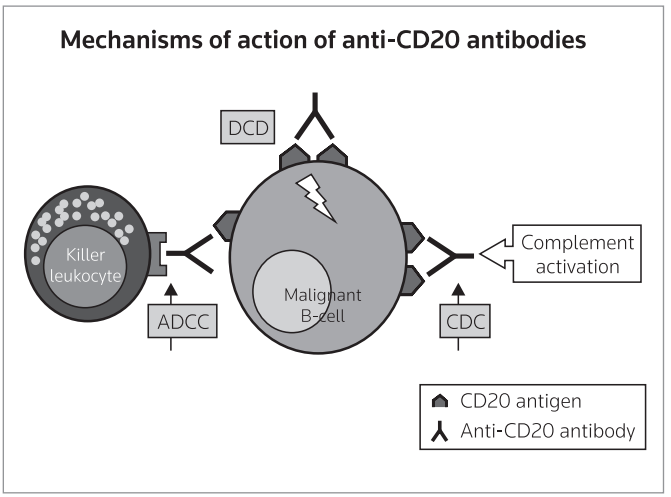


**Figure 3.** Main effector pathways involved in the antitumor effect of anti-CD20 monoclonal antibodies (MAbs). DCD, direct cell death; CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cell-mediated cytotoxicity.

Finally, ADCC mechanisms, mediated by effector cells expressing FcγRI, FcγRII, and mainly FCγRIII (CD16), displayed on NK cells, have also been suggested to take part in the therapeutic effect of anti-CD20 MAbs, since those receptors are able to recognize the Fc portion of rituximab (13). It appears that NK cells, and also as recently suggested macrophages, represent the main effector cells responsible for ADCC, and that the level of CD20 expression does not correlate with the degree of ADCC efficacy (7, 14). In contrast, in follicular lymphoma patients treated with rituximab as a single agent, the efficacy of the antibody appeared to be affected by an inherited genetic variation in the gene encoding the FcγRIIIA receptor, known to influence the binding affinity of $IgG_1$ to this receptor. Patients who

were homozygous carriers of a valine residue (instead of a phenylalanine) at position 158 had a higher rate of clinical and molecular response (15). This observation indicated that ADCC is at least partly responsible for the therapeutic activity of rituximab, and prompted several attempts to enhance it by modifying the Fc portion of the antibody (amino acid substitution or glycosylation modification) to improve its affinity for the FcγRIIIA receptor and therefore the activation of effector cells.

The relative contribution of each of these mechanisms is dependent on the type of anti-CD20 MAb (but may also depend on antibody dose and schedule) (8). Anti-CD20 MAbs are classified according to their mode of B-cell depletion. Type I antibodies have a particular potential to induce a translocation and thus to stabilize CD20 into lipid rafts, this binding mode leading to stronger C1q recruitment and potent induction of CDC, but only low levels of direct cell death (16). Regarding the mechanisms of type I and particularly rituximab resistance, it has recently been postulated that rituximab could induce loss of CD20 by "shaving" of rituximab/CD20 complexes by phagocytic cells, but also through internalization of CD20 (14, 17). On the other hand, type II antibodies do not stabilize CD20 on lipid rafts, resulting in a reduced C1q binding and thus lower levels of CDC, but improved direct cell death (18, 19). Furthermore, type II antibodies appear to induce significantly less antigenic internalization and could therefore result in less resistance to therapy. Although other mechanisms may account for clinical resistance observed with type I anti-CD20, to date, the majority of CD20 antibodies commercialized or under development are of type I, including rituximab, veltuzumab, ocrelizumab and ofatumumab, whereas the prototype of type II antibody is the murine antibody B1 (tositumomab) (Table I) (16).

Recent efforts in the field of anti-CD20 MAbs have been made to improve their efficacy by increasing DCD capacity through modifications of variable regions, but also to increase immune effector functions, such as ADCC, by engineering the Fc region of the antibody. This is perfectly illustrated by the works of Mössner et al., who designed and reported preclinical data for obinutuzumab (GA101), a third-generation, humanized and glycoengineered CD20 $IgG_1$ type II antibody that was derived from the parental murine antibody B-ly1 (16). In order to obtain a humanized MAb with effective human CD20 binding, cDNAs encoding variable heavy- ($V_H$) and light- ($V_L$) chain regions were first cloned from the B-ly1 hybridoma, and their complementarity-determining regions were grafted onto different human $V_H$ and $V_L$ acceptor frameworks, providing numerous humanized Ab variants of these anti-CD20 antibodies. Those were expressed in CHO cells modified to alter the glycosylation of the Fc portion of the antibody (through constitutive engeneering of wild-type β-1,4-*N*-acetyl-glucosaminyltransferase III and wild-type Golgi α-mannosidase II expression) and the antibodies were purified using protein A and ion-exchange chromatographic techniques (16). The second step was to obtain a functional type II MAb by screening the resulting variants for their ability to induce DCD in human lymphoma cell lines. It appeared that the most active variants were characterized by a sequence alteration in the elbow hinge region of the $IgG_1$, an area known to affect the flexibility of the Fab domain (20). The most active MAbs, including obinutuzumab, harbored a human germline $V_H$ framework 1 with a valine instead of the leucine residue present in the murine Ab.

***Table I.*** *Differences between type I and type II anti-CD20 monoclonal antibodies (MAbs).*

| Type I MAbs | Type II MAbs |
|---|---|
| *Molecules* | |
| Rituximab<br>Veltuzumab<br>Ocrelizumab<br>Ofatumumab | Obinutuzumab<br>Tositumomab |
| *Properties* | |
| Stabilize CD20 into lipid rafts<br>Full binding to CD20<br>Weak homotypic aggregation | Do not stabilize CD20 on lipid rafts<br>50% inferior binding to CD20<br>Strong homotypic aggregation<br>Glycoengineered, afucosylated Fc segment *<br>(* in obinutuzumab) |
| *Resulting effects* | |
| High CDC<br>Low DCD<br>ADCC + | Low CDC<br>Improved DCD<br>ADCC ++ (obinutuzumab) |

CDC, complement-dependent cytotoxicity; DCD, direct cell death; ADCC, antibody-dependent cell-mediated cytotoxicity.

## PRECLINICAL PHARMACOLOGY

Mössner et al. then compared the in vitro activity of obinutuzumab and rituximab in different non-Hodgkin's lymphoma (NHL) cell lines. As expected, obinutuzumab exhibits typical characteristics of a type II anti-CD20 antibody, namely, a 50% inferior binding to CD20 at saturating concentrations compared to rituximab, no mobilization of CD20 into lipid rafts, leading to an inferior CDC, but a stronger homotypic aggregation of B cells in vitro. Thus, using an apoptosis assay, it was shown that obinutuzumab induces superior phosphatidylserine exposure and much superior cell death compared with rituximab in a panel of NHL cell lines of different origin, resulting in a 5- to 100-fold greater DCD (21). Interestingly, these type II characteristics (apoptosis induction) were partially lost after reversion of mutation of the elbow hinge region. In addition to modifications of its variable region, obinutuzumab also harbors a glycoengineered, afucosylated Fc segment, resulting in much greater binding affinity for FcγRIIIa than rituximab, translating into an increased induction of ADCC (22). This effect is particularly interesting, since various data indicate that enhancing ADCC results in improved clinical efficacy of MAbs.

As a result, and compared to rituximab, Mössner et al. showed for obinutuzumab a significantly higher B-cell depletion capacity in whole blood from healthy donors, but also in blood samples from CLL patients, and a superior in vivo efficacy in human lymphoma xenograft models, resulting in complete tumor regression in all animals at a dose of 30 mg/kg. Finally, more recent work showed that obinutuzumab had the ability to induce nonapoptotic, lysosome-mediated cell death, suggesting possible efficacy in cells rendered resistant to chemotherapy-induced apoptosis (23). Recently, it has also been shown, using fine epitope mapping, that obinutuzumab binds CD20 in a completely different orientation than type I antibod-

ies despite the recognition of an overlapping epitope (24). The spatial configuration and membrane distribution of CD20 molecules bound to obinutuzumab are therefore distinct compared to that observed with rituximab (24), a potential explanation for the different signaling into B cells induced by these interactions.

In summary, obinutuzumab is an original anti-CD20 antibody, with properties related to type II antibodies that were enhanced by a mutational process in the elbow hinge region, and with improved binding to Fc receptors via glycoengeneering. With this improved direct cell-killing activity together with increased ADCC activity, it therefore represents an optimized new anti-CD20, fairly distinct from those currently under development (25).

Numerous confirmatory biological works were recently presented at the latest American Society of Hematology (ASH) meetings. Reslan et al. have compared the effect of obinutuzumab and rituximab at a concentration of 10 μg/mL on patients' freshly isolated CLL cells and showed after 24 hours of exposure an average decrease in viability of 37.6% for obinutuzumab compared to 28.8% for rituximab (26). In this study, obinutuzumab, unlike rituximab, induced DCD through a caspase-dependent mechanism involving loss of mitochondrial membrane potential and the production of reactive oxygen species. Similar results were reported in a French study by Ysebaert et al. (27). However, contrasting with these observations, Jak et al. demonstrated a rather lysosome-dependent DCD mechanism for obinutuzumab, as described above (28). Interestingly, a synergistic effect for obinutuzumab and cytotoxic drugs, such as purine analogues and alkylating agents, was also demonstrated. Overall, obinutuzumab has more potent efficacy against CLL cells than rituximab (29).

Heinrich et al. presented in vitro data on the efficacy of obinutuzumab on two sensitive mantle cell lymphoma (MCL) cell lines. After 4 hours of exposure, they found a 70% and 40% cell reduction, respectively, for the two cell lines with obinutuzumab versus 25% and 5%, respectively, with rituximab (30). Pievani et al. evaluated the effect of the addition of the anti-CD20 MAb on NK cells exposed to B-cell NHL cell lines and showed an increase in NK cell activation that was significantly greater with obinutuzumab than with rituximab (31). Interestingly, it has recently been shown that obinutuzumab could also significantly increase the ADCC of γδ T cells against FL cells (32). Finally, in vivo studies using NHL xenograft models have shown the superiority of the association of obinutuzumab and bendamustine over the association of rituximab and bendamustine, with tumor growth inhibition of 72% and 42%, respectively, and also the superiority of obinutuzumab plus fludarabine over rituximab plus fludarabine, with tumor growth inhibition of 100% and 85%, respectively (33). These authors also showed a potentially additive effect for obinutuzumab and navitoclax, an apoptosis regulator Bcl-2 family inhibitor currently being evaluated in phase I/II clinical trials. More recently, confirmatory preclinical studies have also been published (34, 35).

## CLINICAL STUDIES

To date, no clinical trials have been fully published, but phase I and phase II studies have been reported at the annual meetings of the ASH over the last 3 years. We presented at ASH 2008 and updated in 2009 the preliminary results of a phase I/II study of obinutuzumab administered as a single agent to patients with relapsing $CD20^+$ hematological malignancies for whom no other therapy was available (36). All had previously been exposed to rituximab and had received a median of four (range 1-7) prior regimens. Obinutuzumab was delivered at doses ranging from 50 to 2000 mg according to a safety-driven dose-escalation design on days 1, 8 and 22, and subsequently every 3 weeks for a total of 9 infusions. Data collected from the first 12 evaluable patients (FL = 9; DLBCL = 1; CLL = 1; Waldenström's macroglobulinemia [WM] = 1) showed that obinutuzumab was well tolerated, with no dose-limiting toxicities observed. The most common adverse events were grade 1 or 2 infusion-related reactions, characterized by fever, chills, hypo- or hypertension, nausea and vomiting. In the majority of patients, they were limited to the first infusion. Pharmacokinetic studies showed that obinutuzumab led to rapid and sustained circulating B-cell (CD19) depletion, and no significant changes in baseline immunoglobulin levels were observed. Measurement of plasma cytokines and complement during and immediately after the first infusion showed an increase in IL-6 and IL-8, with a smaller increase in IL-10 and TNF. No change in complement fractions was observed, which is consistent with in vitro preclinical data. The updated results on a total of 21 patients (FL = 13; mantle cell lymphoma = 4; DLBCL = 1; CLL = 1; WM = 1; lymphoplasmacytoid lymphoma = 1) confirmed the good tolerability of the drug (36). Only 1 tumor lysis syndrome was observed. Responses occurred at all dose levels and across all FcγIIIRA genotypes, with an overall relative response (RR) of 43% (5 complete responses [CR]; 4 partial responses [PR]) and a sustained response (duration ranging from 7.5 to 17 months) in 6 of these 9 responding patients, all having FL histology.

In a subsequent phase II study, 41 indolent NHL patients were randomized to receive, according to the same scheme of treatment, either a low dose (400 mg per infusion; 9 infusions) (n = 18) or a high dose (1600 mg on days 1 and 8 and 800 mg x 7 thereafter) of obinutuzumab (37). In the pharmacokinetic study, the high-dose group demonstrated higher obinutuzumab plasma concentrations and more sustained target saturation. Interestingly, responding patients also showed higher plasma concentrations and a slower elimination of obinutuzumab as compared to nonresponding patients. This translated into an improved overall response rate after completion of treatment in the high-dose group (55%, including 2 CR and 10 PR) compared with the low-dose group (17%, with only 3 PR) (38). Interestingly, in 11 patients defined as rituximab-refractory in the high-dose cohort, the response rate was found to be 50%. Transient neutropenia were observed in 3 of 22 patients in the high-dose group, as opposed to none in the low-dose group (18 patients). These early results support a dose–effect relationship for obinutuzumab, a property that has not been fully investigated or documented so far with rituximab, providing a rationale for its use at different doses or schedules. Consistently, although to a lesser extent, some responses were observed in relapsing aggressive NHL (DLBCL and MCL) (39).

Similar safety and efficacy profiles were reported from a Canadian phase I/II study (40). Four weekly infusions of escalating doses of obinutuzumab, followed by a 3-monthly maintenance in responding patients, were administered to 22 patients with relapsing $CD20^+$ malignancies (FL = 10; DLBCL = 3; CLL/small lymphocytic lymphoma [SLL] = 7; MCL = 1; marginal zone lymphoma [MZL] = 1),

mostly rituximab-refractory. The objective response rate was 25%, and 60% of patients had stable disease. Of note, detailed reconstitution of the B cells is still unknown and long-term safety remains to be established.

## CONCLUSIONS/FUTURE DIRECTIONS

Based on these preliminary data, the novel type II glycoengineered anti-CD20 MAb obinutuzumab can be considered as a promising treatment for B-cell malignancies, both indolent NHL and CLL and aggressive lymphomas. Its stronger preclinical efficacy as compared to rituximab, thanks to an improved DCD and ADCC, provides a rationale for its use in relapsing and refractory forms of these diseases, but maybe also in frail patients not able to support conventional chemotherapy. Therefore, clinical trials on obinutuzumab in $CD20^+$ hematological malignancies are currently ongoing; among these are a randomized phase III study of bendamustine alone versus bendamustine + obinutuzumab in relapsed/refractory indolent NHL previously treated with a rituximab-based regimen, and the German CLL Study Group has initiated a multicenter, international, randomized trial comparing chlorambucil alone with chlorambucil plus rituximab or obinutuzumab in comorbid CLL patients (41). Other studies comparing rituximab and obinutuzumab head to head, either as single agents or in combination with chemotherapy, have been initiated to better define the potential of this antibody in the therapeutic treatment of B-cell malignancies.

## SOURCE

Roche (CH); developed in the U.S. by Genentech, a member of the Roche Group.

## DISCLOSURES

G. Salles has received research grant support, honoraria and has served on the Advisory Board of Roche-Genentech; L. Karlin states no conflicts of interest.

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