Anti-CD20 monoclonal antibodies: reviewing a revolution

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ABSTRACT
Since the inception of rituximab in the 1990s, anti-CD20 monoclonal antibodies have revolutionised the treatment of B cell hematological malignancies and have become a cornerstone of modern gold-standard practice. Additionally, the potent efficacy of these agents in depleting the B cell compartment has been used in the management of a broad array of autoimmune diseases. Multiple iterations of these agents have been investigated and are routinely used in clinical practice. In this review, we will discuss the physiology of CD20 and its attractiveness as a therapeutic target, as well as the pharmacology, preclinical and clinical data for the major anti-CD20 monoclonal antibodies: rituximab, obinutuzumab and ofatumumab.

INTRODUCTION
Few recent medical advances rival the development of monoclonal antibodies against CD20 in terms of impact on treatment paradigms. Since the FDA approval of rituximab in 1997, anti-CD20 therapy has defined a new epoch, particularly in the management of B cell malignancies, such that the term “pre-rituximab era” and “rituximab era” are common place in the vernacular of hematology and oncology. Although the precise physiological role of CD20 remains incompletely defined, it is clear that targeting this molecule is a highly effective means of depleting the B cell compartment, and thus it has become a cornerstone of the management B cell lymphoma. Beyond malignant disease, anti-CD20 therapies play an important role in the treatment of inflammatory and autoimmune diseases. This review will seek to explore the physiological and pathophysiological underpinnings of CD20 monoclonal antibodies (mAbs), the history of their development, their pharmacological properties and the difference between various iterations of anti-CD20 agents. Focusing on malignant conditions, the review will then summarise the extant scientific evidence for the role of these drugs in the management of various diseases. Though a multitude of CD20 mAbs have been produced and utilised to various extents, this review will focus on the dominant agents, rituximab, obinutuzumab and ofatumumab.

CD20 in health and disease
CD20 is a non-glycosylated surface phosphoprotein that is found on most healthy and malignant B cells. Although CD20 is not expressed on precursor B cells, it appears early in the B cell maturation pathway, but is ultimately lost from fully differentiated plasma cells. A member of the membrane spanning 4-A protein family, the CD20 molecule includes two extracellular loops (a large loop and a small loop) within which the epitope binding sites for the anti-CD20 mAbs are located. The natural ligand of CD20 continues to elude detection, however its association with the B cell receptor (BCR) complex suggests a role in BCR signalling. Evidence suggesting structural similarities with known ion channels lead to the supposition that CD20 was involved in calcium flux, and indeed, subsequent studies of cell lines transfected with CD20 revealed increased ion shift when compared to untransfected cells; the increased conductance being abrogated by the addition of a calcium chelator. For many years, the function of CD20 in normal immune physiology remained abstruse, though some data have demonstrated a role in the generation of maximal humoral responses. CD20 deficiency in mice and humans has been shown to cause modest quantitative deficiencies in antibody production and germinal centre formation, though data are somewhat inconsistent and a thorough model of the mechanistic details remains incomplete.

CD20’s attractiveness as a therapeutic target is underpinned by several properties related to its structure and expression. Clearly, the conservation of CD20 expression in virtually all mature B cell lymphoid malignancies is chief amongst its allures as a treatment target. Its distinct absence from pre-B hematopoietic stem cells and terminally differentiated plasma cells limits off target toxicity, and conserves the stem cell pool, which is important for B cell regeneration following therapy. CD20 undergoes little post-translational structural modification, thereby maintaining predictable binding epitopes, and the absence of a natural ligand means mAbs have no known endogenous binding competitors. CD20 is not normally shed from the cell surface, nor does it internalise upon ligand binding. Importantly, the degree of expression...
of CD20 on cancerous B cells compared to normal B cells is also relatively constant, and this feature in conjunction with the close proximity of CD20’s extracellular epitope components to the cell membrane also enhance its appeal as a therapeutic target, particularly in relation to complement-mediated killing and antibody-dependent cellular cytotoxicity (ADCC). Few antigens boast such a panoply of characteristics favourable to antibody binding.

**Mechanism of action of CD20 mAbs**

CD20 mAbs are postulated to destroy B cells via several distinct mechanisms, with observable differences between the various mAbs that can be explained by examination of their structural variations and the resultant differences in CD20 binding. Broadly, mAbs targeting CD20 can be subclassified into two major categories: type 1 and type 2, determined by their relative ability to induce the redistribution of CD20 into lipid rafts within the plasma membrane. Found inside the bilayer of cellular membranes, lipid rafts are defined as heterogenous, small and dynamic lipid-protein microdomains that serve as platforms for signal transduction. Lipid rafts facilitate cellular processes via colocalization of receptors and effector molecules; this has been shown to be important in BCR signalling. Such translocation of CD20 (caused by type 1 mAbs but not type 2) has been demonstrated to have significant implications for mAb effector functions. Beyond differences in the mode of cell killing, the subtypes also display variable susceptibilities to mechanisms of mAb resistance. Type 1 antibodies bind twice as many molecules per target cell when compared to type 2 mAbs, but this increased density appears to make them more vulnerable to internalisation and therefore to proteolytic degradation and downregulation of CD20 expression, so called “antigen modulation”. It is important to appreciate that such subtle differences in structure, or even the spatial configuration in which the antibodies bind to CD20, and this feature in conjunction with the cell membrane also enhance its appeal as a therapeutic target, particularly in relation to complement-mediated killing and antibody-dependent cellular cytotoxicity (ADCC) 

**Complement dependent cytotoxicity**

Originally thought to be the predominant mechanism of anti-tumour activity, rituximab was demonstrated to bind C1q, leading to complement pathway activation and complement-dependent cytotoxicity (CDC). This manifests as enhanced phagocytosis via opsonisation, cell-busting membrane attack complex formation, and upregulated recruitment of other immune effector cells. Type 1 antibodies, such as rituximab and ofatumumab, are more effective in activating the classical complement cascade by virtue of their ability to redistribute CD20 into lipid rafts. However, superior complement activation may be at least partially explained by the arrangement of Type 1 CD20 mAbs on the surface of the target cell. Tight clustering of mAbs allows for better interaction with C1q, which requires at least double-headed binding for activation. Ofatumumab demonstrates even more potent stimulation of the complement pathway than its fellow type 1 mAb rituximab, and this is thought to be related to the finding that ofatumumab binds to both extracellular loops of CD20, achieving a closer proximity to the cell surface membrane. This is likely critical to optimising the Fc:Fc interactions that benefit the recruitment of effector molecules such as C1q. Further evidence for the importance of structural antibody configuration in complement activation comes from a study demonstrating the formation of IgG hexamers between neighboring Fc regions, maximising interaction with C1q. This finding prompted engineered mutational modification of mAbs to induce hexameric formation on target cells to enhance complement activation. Such modifications may also serve to augment the limited CDC of type 2 mAbs, at least in in vitro models. However, what remains to be proven, is whether such antibody upgrades will deliver superior efficacy and clinical outcomes.

**Fcγ receptor mediated effects**

Expressed on many immune cells including neutrophils, natural killer (NK) cells and macrophages, Fcγ receptors interact with IgG antibodies, and are the key mediator by which antibodies trigger cellular immune responses. NK cells are powerful effector cells and following stimulation via FcγRIIIa, attack opsonized targets by releasing cytolytic compounds like granzyme B and perforin in a process known as antibody-dependent cell-mediated cytotoxicity (ADCC). Additionally, once opsonized, signaling through FcγR on macrophages, neutrophils and monocytes stimulates engulfment and ultimately destruction of the target cell following fusion of the phagosome with the effector cell’s lysosome, a process that has come to be known as antibody-dependent cell-mediated phagocytosis (ADCP). In addition to ADCC and ADCP, type 1 CD20 mAbs can produce a form of caspase-dependent apoptotic direct cell death, resultant from a phenomenon termed “hypercrosslinking”. A further form of direct killing that is also caused by hypercrosslinking but appears to be caspase-independent and related to extracellular calcium influx and reactive oxygen species generation has also been described. Although the relative importance of the various FcγR dependent pathways on in vivo mAb efficacy remain unclear, the centrality of FcγR-mediated mechanisms to CD20 mAb function has been demonstrated in numerous studies. Both ADCC and ADCP activity were nullified in genetically modified mice with absent or dysfunctional FcγR signaling, and target cell killing was enhanced in mice with FcγR signaling inhibitors knocked out. In humans, some clinical trials have revealed the presence of an FcγR polymorphism that confers increased binding affinity to IgG to be correlated with improved clinical response to mAb therapy, although this finding has not been consistently reproduced in other studies.

**Cellular vaccine effect**

In addition to the aforementioned modes of CD20 mAb activity, there are data that suggest these agents are capable
of inducing long term changes in specific immunity by promoting antigen presentation and thereby activation of effector T cells directed against tumour antigens. This mechanism of action may explain the delayed responses that have been observed in some patients with lymphoproliferative disorders, and can occur despite clearance of the mAb. While there are limited data from human studies to support this concept, it has been shown that follicular lymphoma idotype-specific T cells are increased in patients following treatment with rituximab. There is also growing evidence of the importance of T cell responses as mediators of tumour cell killing with mAbs in animal models.

Controversies and future directions

Though our understanding has improved considerably, the differences between in vitro versus in vivo effector functions, as well as physiologic differences between murine and human models confound interpretation. Adding further complexity are findings that support interactions between both antagonistic and synergistic effector pathways. Specifically, complement activation can enhance FcyR-mediated cellular killing via anaphylatoxin generation, but conversely, some studies have demonstrated that complement fixation may reduce ADCC. It has been shown that increased deposition of C3b can mitigate NK cell activity, and that depleting C3 using cobra venom factor can abrogate this inhibitory effect. Additionally, a C1qa polymorphism that reduces C1q levels, has been correlated with superior responsiveness to rituximab in follicular lymphoma patients. These findings have led many to question the relative impact of complement to therapeutic efficacy in vivo, despite strong evidence of its role in vitro, but the truth may simply be more complex and nuanced than current data can reveal.

As our understanding of the numerous effector pathways continues to grow, there is burgeoning interest in methods to modulate the characteristics of new anti-CD20 mAbs, aiming to enhance both complement-mediated and FcyR-mediated killing. Obinutuzumab is an example of such efforts, having been de-fucosylated based on data demonstrating that this modification greatly augments IgG1 affinity for CD16a Fc receptor. While further exploration of potential molecular modifications is beyond the scope of this article (and has been reviewed recently by Kellner et al), these developments offer promise for overcoming resistance to extant mAbs, but many are yet to prove their efficacy in the clinical arena.

Rituximab

In 1997, intravenous rituximab was the first monoclonal antibody therapy approved for cancer treatment by the FDA, its European sanction following the year after. Rituximab’s inception was a herald of a new era of “biological” therapeutics that have transformed modern hematology and oncology practice and have become an essential cornerstone in the management of many cancers. Rituximab is a chimeric human/murine IgG kappa immunoglobulin, with murine 2B8 light and heavy chain variable region sequences combined with human kappa and IgG1 constant region sequences. The origins of rituximab can be traced to the original Nobel prize-winning development of hybridoma technology, which enabled production of clonal antibodies from a single B cell. Therapeutic applications of these antibodies were first tested in the 1980, and work by the Nadler and Levy groups proved that antibody therapies were highly active against lymphoma cells. These early efforts with patient-specific antibodies that were unsuitable for commercialization, were contemporaneous with other work exploring the expression of cell surface antigens using monoclonal antibodies. In 1987 Press et al tested a murine monoclonal antibody with specificity for the antigen that would later be renamed CD20, and demonstrated the mAb’s ability to deplete malignant B-cells from patients with refractory B-cell lymphomas with impressive, albeit ephemeral, clinical responses. However, murine antibodies are immunogenic in humans, and thus survive only briefly in vivo; they also have a reduced capacity for complement fixation and weakened ADCC. The advent of recombinant DNA technology allowed these shortcomings to be overcome through the production of a murine-human chimeric mAb against CD20. In 1994 Reff et al reported on the activity of another chimeric CD20 mAb, IDEC-C2B8, that was able to stimulate complement and antibody-dependent cytolysis of human B cell-lymphoma cells lines in vitro, and could deplete 95% of bone marrow and lymph node B cells from macaques with minimal toxicity. 3 years later, rituximab became the fourth monoclonal antibody approved by the FDA, and the first for treatment of a malignancy. Approval from European regulators followed in 1998.

Despite the array of clinical studies utilizing rituximab (outlined in the following section), some aspects of its use remain uncertain. The complex pharmacokinetics of rituximab have been explored but clinical use of the drug has not necessarily been optimized as a result. Rituximab disposition shows a non-linear, 2-exponential decay pattern with an elimination half-life of approximately 3 weeks; the antibody being cleared rapidly from the circulation by target binding, and more slowly by catabolism. The pivotal initial study of rituximab that justified its regulatory approval used a 375mg/m² dose. Modern dosing is still based on this initial trial although a number of factors have been shown to alter the pharmacokinetics of rituximab. Tumour burden has been shown relate inversely to circulating concentrations of rituximab, which is significant as circulating rituximab levels have been correlated with patient response. Specifically, maintain trough levels of rituximab above 25mcg/ml appears to be an important threshold concentration for optimal patient response. This “tumour sink” phenomenon can also vary with the type of disease, for example, clearance of rituximab has been shown to be significantly accelerated in patients with chronic lymphocytic leukemia (CLL) compared to non-Hodgkin lymphoma (NHL), likely because of the higher number of malignant cells present in CLL. As logically follows, the clearance of rituximab tends to reduce in line with disease burden through progressive therapeutic cycles, and this could have significant implications for dosing. A further important contributor to rituximab pharmacokinetics is gender, and several studies have shown that the elimination half-life is...
longer in female patients. The resultant increased drug exposure is thought to underpin the gender difference in outcomes shown in the RICOVER-60 trial, in which female gender correlated with superior progression-free survival (PFS). A similar outcome was found in the CORAL trial, in which women attained superior event-free survival (EFS) compared to men. To address this, the SEXIE-R-CHOP trial administered a higher dose of rituximab (500mg/m²) to elderly men, whilst maintaining traditional dosing for women. In this study, rituximab trough levels, overall survival (OS) and PFS were no different between men and women, and no excess toxicity for the higher dose of rituximab was documented; suggesting that perhaps pharmacokinetic-considered dosing could abrogate gender differential outcomes shown in other studies.

A further development is the advent of subcutaneously administered rituximab, which reduces the demand on healthcare resources and can potentially improve access to treatment, especially in resource-limited settings, while also improving the patient’s experience and quality of life. The subcutaneous preparation contains human recombinant DNA-derived hyaluronidase which breaks down the subcutaneous matrix enabling larger proteins such as immunoglobulin to be absorbed. Preclinical studies in xenograft and animal models showed the subcutaneous formulation to have equivalence in therapeutic efficacy and pharmacokinetics. Thereafter, trials to demonstrate safety and pharmacokinetic equivalence were undertaken in patients with CLL, follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). These studies consistently demonstrated the non-inferiority of the subcutaneous formulation for maintaining adequate trough levels of rituximab. Clinical efficacy and safety were confirmed by several large phase III trials, including the MabEASE, SABRINA and SAWYER studies, which demonstrated no difference in clinical outcomes between intravenous and subcutaneous routes of rituximab administration. Safety findings appeared comparable as well, and no unexpected safety signals have been generated. However, the subcutaneous formulation has been associated with a slightly increased risk of administration reactions in some trials, as well as local injection site reactions, but these seem to be generally mild and easily managed. Examination of the patient experience has generally favored subcutaneous rituximab, which is associated with a much lower median administration time than intravenous (6 minutes vs 170–240 minutes).

**Indolent hematological malignancies**

**Follicular lymphoma**

Rituximab’s initial regulatory approval was predicated on early studies demonstrating efficacy in relapsed/refractory low grade lymphoma, in which rituximab monotherapy produced overall response rates (ORRs) in the order of 38–57%. More contemporary phase III studies have now indisputably established the activity of rituximab monotherapy in both the upfront and relapsed settings.

The establishment of rituximab’s activity as a single agent in FL, logically prompted investigation of its effect when combined with conventional chemotherapy drugs. That rituximab enhances outcomes for FL patients receiving chemotherapy has been conclusively established through many studies, including a plethora of randomised controlled trials (RCTs) that have examined the use of rituximab as therapy for both upfront and relapsed disease and in combination with a variety of different chemotherapy backbones. A detailed analysis of each of these trials is beyond the scope of this article, and the trials have been summarized in Table 1.

In addition to its utility in both induction and salvage therapy for FL, rituximab has also been established as useful for maintenance therapy, prolonging time to next treatment and enhancing PFS outcomes. However, studies have not consistently demonstrated an OS benefit, and use of maintenance is associated with a modest increase in the risk of infection.

The largest study to investigate rituximab maintenance in frontline FL management was the "Primary Rituximab and Maintenance" (PRIMA) trial, a phase III study of 1019 patients with advanced FL, who were randomised to 8-weekly rituximab maintenance (375mg/m²) for 2 years or to observation alone. This occurred after induction treatment with a physician’s choice rituximab-containing regimen, including R-CVP, R-CHOP and R-FCM. In the most recent release of data from this trial with a median of 9 years of follow-up, median PFS in those receiving maintenance was 10.49 years compared to 4.06 years for observation, with this benefit extending to all subgroups of patients who achieved at least a partial response to induction (although statistically significance was not recached for those treated with R-CVP). OS at 10 years, was however, identical at 80% in each arm.

The RESORT study, run by the Eastern Cooperative Oncology Group (ECOG) compared rituximab maintenance to a re-treatment strategy in patients with low tumour burden (by GELF criteria), untreated FL. The primary endpoint of this study was time to treatment failure, and ultimately no difference in this metric was demonstrable between the two groups. 3-year freedom from cytotoxic therapy was better in the maintenance group (95% vs 84%), but the median number of rituximab doses was far greater in the maintenance arm (18 vs 4). The efficacy of maintenance therapy following treatment for relapsed disease has also been studied by several groups, and the largest trial performed by van Oers and colleagues showed that the 334 patients who received maintenance (3 monthly rituximab at 375mg/m² for 2 years) benefited from greater median PFS (3.7 years vs 1.3 years), which was observed in patients who were treated with either CHOP or R-CHOP. Those who received R-CHOP induction had a median PFS of 4.4 years with maintenance and 1.9 years with observation alone. However, once again, even a long-term follow-up study did not show a statistically significant benefit for OS, with a 5 year OS of 74.3% in the maintenance arm vs 64.7% in the observation arm (p = 0.07). Given the equivocal impact of maintenance therapy on OS, Vidal et al undertook, and recently updated, a meta-analysis that pooled individual patient data from seven maintenance trials. This analysis does in fact show a benefit in OS, but this was limited to patients receiving maintenance following treatment for relapsed disease or for those who had not received rituximab during their induction regimen (hazard ratio [HR] 0.79, 95%
CI 0.66–0.96). However, the study also enumerates the price of maintenance in terms of infection risk, with grade 3 or 4 infections being significantly more frequent in those receiving maintenance (relative risk [RR] 1.67, 95% CI 1.4–2.0). This risk when considered in conjunction with the unclear effect on OS, the cost of providing ongoing rituximab and the absence of data showing improved quality-of-life has led a minority of experts to question the virtue of maintenance therapy, particularly after induction with rituximab-containing therapy.

Chronic lymphocytic leukemia (CLL)

Several phase I/II studies published at the beginning of the century generated substantial interest in a potential role for rituximab in CLL, demonstrating both efficacy and safety in this population when used as a single agent or when combined with other CLL-active therapies.82–85 However the evidentiary foundations for rituximab in CLL were two large RCTs that tested rituximab in addition to fludarabine and cyclophosphamide (FCR) against FC alone. The CLL-8 study was the first randomised trial exploring FCR vs FC in 817 treatment naïve CLL patients.86 At 3 years, PFS was significantly better for FCR (PFS, 65% vs 45%). Additionally, FCR bested FC in terms of OS (HR 0.67, P = 0.012), and ORR and CR rate (P < 0.0001). Recipients of FCR were more likely to experience grade 3 or 4 neutropenia and leukopenia (P < 0.001), but no difference in other toxicities, including severe infections was detected. A recent update of these data reveals an even greater benefit to rituximab with prolonged follow-up, as patients treated with FCR enjoyed almost double the length of PFS compared to FC recipients (56.8 months vs 32.9 months), as well as greater median OS (FCR: not reached, FC 86 months).87 Follow up studies also demonstrated striking results for a subset of patients with favourable disease characteristics (absence of del17p, mutated immunoglobulin heavy chain variable region), many of which achieved extremely durable remissions from FCR.87,88

However, CLL is a disease of older adults with a median age at presentation of 72 years and the use of FC-chemotherapy backbone in younger patients was associated with a 3–5% treatment related mortality.86,89 The CLL11 study examined the use of CD20 monoclonal antibodies in combination with chlorambucil which is better tolerated in older patients. In this study (which also examined obinutuzumab), the addition of rituximab to chlorambucil was associated with a 56% reduction in the risk of progression or death (HR: 0.44; 95% CI: 0.34;0.56; p < 0.0001) and prolonged OS (HR: 0.60, 95% CI: 0.38;0.94; p = 0.0242) compared to next treatment, MZL marginal zone lymphoma, NHL non-Hodgkin lymphoma, NR not reported, PD progressive disease, PFS progression-free survival, PR partial response, R rituximab, R/R relapsed/refractory, SD stable disease, SLL small lymphocytic lymphoma, TF treatment failure

### Table 1. Major trials of rituximab in Follicular lymphoma.

| Reference | Line of therapy | No of patients | Regimen | ORR | CR | Outcome | OS |
|-----------|---------------|---------------|---------|-----|----|---------|----|
| GLLSG     | 1L            | 428           | R-CHOP vs CHOP | 96% vs 20% vs 90% vs 17% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| East German Study Group | 1L | 201 | R-MCP vs MCP | 92% vs 50% vs 75% vs 25% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| Marcus et al | 1L | 321 | R-CVP vs CVP | 81% vs 41% vs 57% vs 10% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| GELA-GOELAMS FL2000 | 1L | 358 | R-CHVP + INF vs CHVP + INF | 81% vs 51% vs 72% vs 39% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| FOLL05 | 1L | 504 | R-CVP vs R-CHOP vs R-FM | 88% vs 67% vs 93% vs 73% vs 91% vs 72% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| Rummel et al. | 1L | 514 (420 NHL inc 279 FL, 94 MCL) | R-benda vs R-CHOP | 93% vs 40% vs 91% vs 30% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| van Oers et al | R/R | 465 | R-CHOP vs CHOP | 85.1% vs 29.5% vs 72.3% vs 15.6% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| PRIMA | 1L (maintenance) | 1018 | R maintenance vs observation | 3-yr PFS: 74.9% vs 57.6% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |
| RESORT | 1L | 228 | R maintenance vs retreatment | 6-yr PFS: 59.2% vs 42.7% | mOFS | (median observation time 18 months) ; 12.6% vs 29.8% | Deaths: 2.7% vs 8.3% |

1L, first-line; aNHL, aggressive non-Hodgkin lymphoma; Chl, chlorambucil; CLL, chronic lymphocytic leukemia; CR, complete response; DLBCL, diffuse large B-cell lymphoma; EFS, event-free survival; FL, follicular lymphoma; G obinutuzumab (GA101); HR, hazard ratio; iNHL, indolent non-Hodgkin lymphoma; MCL, mantle cell lymphoma; mDOR, median duration of response; mFU, median follow-up; mOS, median overall survival; mPFS, median progression-free survival; mTTNT, median time to next treatment; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; NR, not reported; PD, progressive disease; PFS, progression-free survival; PR, partial response; R, rituximab; R/R, relapsed/refractory; SD, stable disease; SLL, small lymphocytic lymphoma; TF, treatment failure
in an indolent disease as fewer than 10% of patients died during this period.\textsuperscript{92} Thereafter, rituximab was shown to be effective in CLL when combined with other chemotherapy partners including bendamustine and chlorambucil,\textsuperscript{89,93,94} and at least at present, rituximab continues to be a cornerstone of CLL treatment in modern practice. The emergence of new targeted therapies in CLL such as inhibitors of Bruton’s tyrosine kinase (BTK), BCL2 and PI3K may be the herald of another revolution in CLL care, and the potential synergy with CD20 mAbs is the subject of a number of clinical trials currently in progress.

\section*{Aggressive hematological malignancies}

\subsection*{Diffuse large B cell lymphoma}

Rituximab is considered the standard of care for patients receiving treatment for new diagnoses of DLBCL, as well as in combination salvage regimens for relapsed disease.\textsuperscript{95} Vose et al published the first Phase II data establishing efficacy and safety for rituximab in untreated DLBCL in 2001, demonstrating an ORR of 94% (61% CR).\textsuperscript{96} Several landmark randomised trials followed on from this study, beginning with studies of elderly patients with DLBCL, in which participants were randomised to R-CHOP or CHOP alone.\textsuperscript{97,98} The French GELA group published the first randomised trial of patients aged 60–80 and showed the superiority of the addition of rituximab in terms of CR rate (76% vs 63%), 2-year event free survival (EFS, defined as disease progression, new treatment or death from any cause) (57% vs 38%) and 2 year OS (70% vs 57%).\textsuperscript{97} A longer term (median of 5 years) follow-up from this cohort continued to affirm the benefits of rituximab, showing median EFS to be 3.8 years in the R-CHOP arm against 1.1 years in the CHOP group.\textsuperscript{99} At 10 years, those who had rituximab continued to demonstrate a survival advantage (OS 44% vs 28%).\textsuperscript{100} Perhaps more meaningful, particularly in an elderly study population, the 5-year median PFS and OS remained in favor of rituximab: 54% vs 30%, and 58% vs 45%.\textsuperscript{99} A second phase III randomised trial also demonstrated outcome benefits for elderly patients receiving rituximab, and interestingly demonstrated no benefit of post induction rituximab maintenance therapy for those in the R-CHOP cohort.\textsuperscript{98} The pivotal MINT trial helped to shift the paradigm for younger patients with favorable disease characteristics, randomizing patients aged 18–60 (median age 47) and aaIPI of 0 or 1 to CHOP-like chemotherapy with or without the addition of rituximab.\textsuperscript{101} Whilst this study was limited to patients with favorable prognostic features, a clear benefit for rituximab-containing therapy was evident in 3-year EFS (79% vs 59%), PFS (85% vs 68%) and OS (93% vs 84%).\textsuperscript{101} A follow-up study again demonstrated that this benefit was sustained in the longer term, with a 6-year OS rate of 90% vs 80%.\textsuperscript{102} The evidence for rituximab in patients with relapsed/refractory DLBCL is less robust than in the upfront setting, particularly because randomised trials are few, and include a low proportion of patients with previous rituximab exposure. Given the near universal adoption of up-front rituximab in the modern era, the benefit of this agent in the relapsed setting is less certain. A HOVON group trial of 239 patients with relapsed aggressive B-cell lymphomas randomised patients to chemotherapy with dexamethasone, high-dose cytarabine and cisplatin (DHAP) and etoposide, ifosfamide and methotrexate (VIM), delivered as DHAP-VIM-DHAP, with or without rituximab.\textsuperscript{103} Approximately 90% of patients in both arms had DLBCL. The results favored the addition of rituximab in terms of failure-free survival (FFS) at 24 months (50% vs 24%), PFS at 24 months (52% vs 31%), with cox-regression showing an OS benefit at the same time point (HR 0.60 [95%CI 0.41–0.89] vs 0.76 [0.52–1.10]). However, of the 225 patients evaluable for analysis, only 4% had ever been exposed to rituximab previously, significantly reducing the applicability of these findings to modern practice.\textsuperscript{103} There have been no other randomised trials that directly compare rituximab-containing regimens to chemotherapy-only regimens, but several studies of salvage combinations that include the CD20 mAbs have outcomes that compare favorably against historical control data; thereby allowing more relapsed/refractory patients to proceed to autologous stem cell transplant.\textsuperscript{104,105}

\subsection*{Burkitt lymphoma}

In the management of Burkitt lymphoma (BL), a number of prospective but uncontrolled trials have examined incorporating rituximab into conventional chemotherapy regimens, and have consistently impressive outcomes, suggesting an advantage was derived from the addition of the mAb.\textsuperscript{106–109} Ribrag and colleagues subsequently published the only randomised controlled trial of rituximab in BL, recruiting 260 HIV negative BL patients from French centers and randomly assigning them to a dose-dense chemotherapy regimen with or without added rituximab.\textsuperscript{110} At a median follow-up of 38 months, the outcomes were significantly better for patients who were treated with rituximab; 3-year EFS was 75% vs 62% and 3-year OS was 83% vs 70%. This translates to EFS and OS hazard ratios of 0.59 (95% CI 0.38–0.94) and 0.51 (0.30–0.86) respectively. Importantly, safety outcomes were not significantly different between the rituximab and no rituximab groups, with similar rates of infectious and hematological toxicity and there was no increase in deaths from treatment toxicity amongst those treated with rituximab.\textsuperscript{110}

\subsection*{B-cell acute lymphoblastic leukemia}

CD20 is expressed in approximately 30%–40% of B-cell acute lymphoblastic leukemia (B-ALL), and unsurprisingly there has been significant interest in the potential utility of anti-CD20 mAbs in these cases.\textsuperscript{111,112} Thomas et al studied 216 patients with Philadelphia chromosome negative (Ph-) pre B-ALL, who received two doses of rituximab to complement a Hyper-CVAD chemotherapy backbone, and found improved OS at 3 years compared to historical controls, most evident in those under 60 years of age (3 year OS 71% vs 47%).\textsuperscript{113} A further non-randomised study from the GMALL group also demonstrated a benefit in 263 patients receiving induction chemotherapy with added rituximab over historical outcomes, showing higher CR rates and improved 5-year OS for both standard and high-risk patients.\textsuperscript{114} However, the veracity of conclusions based on comparison to historical data is far from ideal. Consequently, the GRALL-
2005 study, undertaken from 2006 to 2014, is the only randomised trial of rituximab in the CD20+ (>20% by immunohistochemistry) Ph-ve B-ALL setting. 209 patients were randomised and the 105 patients in the rituximab arm received 16–18 doses of rituximab (375mg/m²) across induction, consolidation and maintenance phases of therapy. The primary endpoint, EFS, was significantly superior for the rituximab group at 2 years (65% vs 52%), predominantly due to a reduced rate of relapse (18% vs 30.5%). However, these benefits were not shown to translate into increased rate of CR, minimal residual disease (MRD) negativity or evidence of enhanced OS.

Obinutuzumab

Obinutuzumab is a fully humanized, de-fucosylated IgG1 type 2 monoclonal antibody against CD20. Obinutuzumab recognizes a unique, exposed epitope of CD20 and has a modified elbow-hinge amino acid sequence (substitution of leucine by valine) compared to type 1 agents, resulting in spatial alterations to the CD20-mAb assembly complex on B-cells. De-fucosylation of the Fc region enhances its binding affinity to the FcγRIII receptor leading to increased direct cell death induction and enhanced ADCC and ADCP compared to rituximab. Conversely, CDC is reduced (up to 100-fold compared to type 1 antibodies), as type 2 antibodies do not localize the antibody-antigen complex into lipid rafts. However, this has the effect of reducing FcγRIIl-mediated CD20 internalization, leading to increased binding capacity and perhaps reducing the risk of antigen modulation.

In preclinical studies, obinutuzumab caused increased depletion of normal B-cells from the blood of healthy volunteers compared to rituximab, as well as increased depletion of malignant B-cells from the blood of patients with CLL. Obinutuzumab has demonstrated improved efficacy compared to rituximab in human lymphoma xenograft models, inducing complete tumour regression in an aggressive DLBCL model while rituximab only inhibited further growth. Superiority of obinutuzumab has also been reported in similar xenograft models of advanced mantle cell lymphoma and rituximab-refractory DLBCL.

Obinutuzumab was the first cancer drug to receive FDA approval with the breakthrough therapy designation on November 1, 2013, for the treatment of previously untreated CLL. Breakthrough therapy–designated drugs must show a substantial improvement of outcomes over current therapies according to the FDA Safety and Innovation Act enacted in 2012. Obinutuzumab was subsequently licensed in Europe in July 2014 following the first published data from the phase III CLL11 trial, in combination with chlorambucil for the treatment of adult patients with previously untreated CLL and comorbidities, unsuitable for full dose fludarabine-based therapy. This study also identified the issue of obinutuzumab infusion-related reaction (IRR), occurring mainly with the first dose of administration. While there were no IRR-related deaths, IRR of grade 3 or more occurred in 20% of patients with 7% withdrawal due to adverse event (AE) compared to rates of 4% and <1% respectively in the rituximab plus chlorambucil arm.

Obinutuzumab has since been studied extensively across a range of B cell malignancies (see Table 2).

Indolent hematological malignancies

Indolent NHL

Single agent obinutuzumab was initially evaluated in pre-treated patients with indolent NHL, aggressive NHL and CLL in the multicentre phase I-II GAUGUIN study. The phase I stage aimed to investigate the safety and tolerability of escalating doses of obinutuzumab monotherapy and 21 patients with relapsed/refractory indolent NHL were enrolled. The overall response rate (ORR) at end of treatment was 33%; responses were only obtained in the follicular lymphoma (FL) patients, resulting in an ORR of 54% in this subgroup (31% CR). Based on the phase I results, two dosing regimens (400/400mg and 1600/800mg) were compared in the phase II stage which enrolled 40 patients with relapsed/refractory indolent NHL, most with FL. 90% had stage 3–4 disease and 55% were refractory to prior rituximab therapy. End of treatment ORR in rituximab-refractory group was 8% in the low dose arm and 50% in the high dose arm, with 2 CRs in the high dose arm. Subsequently, 175 patients with relapsed indolent NHL (149 patients with FL) were randomly assigned to induction followed by maintenance therapy with either single-agent obinutuzumab or rituximab. The investigator-assessed ORR was higher with obinutuzumab compared with rituximab (43.2 vs 35.6% in the overall population, 43.2 vs 38.7% in patients with FL). There was no difference in the secondary endpoint of PFS, although the trial was not powered for this outcome. These early phase trials established the acceptable safety profile and tolerability of obinutuzumab. IRRs were the most common adverse events, nearly all of which were grade 1–2 in severity.

Dose-finding studies such as GAUGUIN demonstrated that plasma concentrations increased more rapidly upon administration of a 1600/800mg dose than with lower doses, leading to a steady state indicative of CD20 target saturation. Pharmacokinetic modelling showed that 1000mg on days 1, 8 and 15 of cycle 1 could achieve similar exposures. A simplified fixed dose schedule of obinutuzumab 1000mg on days 1, 8 and 15 of the first 21-day cycle and day 1 of subsequent 21-day cycles was selected to achieve adequate exposure levels in subsequent phase II and III trials. Pharmacokinetic studies showed that the flat-dose schedule rapidly achieved CD20 target saturation and serum drug concentrations were maintained at the therapeutic level throughout the treatment course.

Based on rituximab-based combination regimens, obinutuzumab was evaluated in combination with either CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or FC (fludarabine, cyclophosphamide) in patients with relapsed/refractory FL in the phase 1b GAUDI study. There were no dose-limiting toxicities (DLT) or unexpected AEs. This study was the first to demonstrate the benefit of obinutuzumab-based therapy in rituximab-refractory disease.

Obinutuzumab in combination with CHOP or bendamustine
Table 2. Trials of obinutuzumab.

| Phase | Reference | Population | No of patients | Regimen | ORR | CR | PR | Other |
|-------|-----------|------------|----------------|---------|-----|----|----|-------|
| GAUGUIN | Phase I | Salles et al | Relapsed/refractory NHL | 34 | 12 FL, 4 MCL, 1 DLBCL, 3 others | 60% | 100% | 1200/2000mg | 21.3 months \(\text{mPFS}\) 100% \(\text{mOS}\) 28% \(\text{HR}\) 0.66 \(\text{p} < 0.05\) |
| Phase II | Salles et al | Relapsed/refractory NHL | 40 | 18 (14 FL, 4 others) 22 (20 FL, 2 others) | 44% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase III | Morschhauser et al | Relapsed/refractory aNHL | 40 | 10 DLBCL, 11 MCL | 44% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase I | Cartron et al | Relapsed/refractory CLL | 13 | G 800 – 1200/2000mg | 62% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase II | Cartron et al | Relapsed/refractory CLL | 20 | G 1000/1000mg | 62% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| GAUSS | Phase I | Sehn et al | Relapsed/refractory NHL | 22 | Including 5 CLL, 2 SLL | 64% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase II | Sehn et al | Relapsed/refractory NHL | 175 | 74 FL, 14 others | 64% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Japanese study | Phase I | Ogura et al | Relapsed/refractory INHL | 8 | 2 FL, 1 MZL, 1 other | 58% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase II | Byrd et al | Symptomatic, untreated CLL | 20 | | 49% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase III | Goede et al | Untreated CLL in elderly/ comorbidities | 781 | | 49% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| GAUDI | Phase Ib | Radford et al | R/R FL | 56 | 14 | 93% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase II | Zelenetz et al | Untreated advanced DLBCL | 80 | | 83% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase III | Sehn et al | Rituximab-refractory INHL | 198 | 194 | 83% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| GATHER | Phase II | Zelenetz et al | Untreated advanced DLBCL | 1202 | 601 | 88.5% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase III | Marcus et al | Untreated INHL (FL and MZL) | 1202 | 601 | 88.5% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| GOYA | Phase III | Vitolo et al | Untreated DLBCL | 1418 | 706 | 77% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |
| Phase III | Stilgenbauer et al | Untreated and R/R CLL | 712 | 158 | 78% \(\text{mPFS}\) 18 months 83% \(\text{mOS}\) 11.9 months 22% \(\text{mDOR}\) 10.5 months 10% \(\text{mTTNT}\) 11.1 months \(\text{HR}\) 0.54 \(\text{p} < 0.001\) |

1L. first line, aNHL aggressive non-Hodgkin lymphoma, CHL chlorambucil, CLL chronic lymphocytic leukemia, CR complete response, DLBCL diffuse large B-cell lymphoma, EFS event-free survival, FL follicular lymphoma, G obinutuzumab (GA101), HR hazard ratio, iNHL indolent non-Hodgkin lymphoma, MCL mantle cell lymphoma, mDOR median duration of response, mFU median follow-up, mOS median overall survival, mPSF median progression-free survival, mTTNT median time to next treatment, MZL marginal zone lymphoma, NHL non-Hodgkin lymphoma, NR not reported, PD progressive disease, PFS progression-free survival, PR partial response, R rituximab, R/R relapsed/refractory, SD stable disease, SLL small lymphocytic lymphoma, TF treatment failure
was also evaluated in 81 treatment-naïve FL patients; patients with an end-of-treatment response were eligible for obinutuzumab maintenance therapy for 2 years or until disease progression. IRRs were once again the most common AE (58%) with the majority being grade 1/2. Grade 3/4 neutropenia was the most common hematologic AE (36% during induction and 7% during maintenance). ORR at the end of induction was 94% with CR rate of 36%. Estimated 3-yr PFS was 90% in the obinutuzumab plus bendamustine group and 84% in the obinutuzumab plus CHOP group.126

The GADOLIN trial was the first randomized phase III study to demonstrate the efficacy of an alternative anti-CD20 monoclonal antibody in rituximab refractory indolent NHL. Obinutuzumab plus bendamustine (G-B) followed by obinutuzumab maintenance was compared to single-agent bendamustine induction in patients who had been either refractory to a rituximab-containing regimen or had experienced progression during or within 6 months of receiving rituximab. Most patients had FL and were refractory to chemoimmunotherapy; 72–78% had intermediate or high risk FLIPI. In the primary analysis, the median PFS was longer in the G-B arm (not reached) than in the bendamustine monotherapy arm (14.9 months) as assessed by independent review committee with a 45% reduction in risk of progression or death. Updated results with 17 more patients and an additional 10 months of follow-up demonstrated superior median PFS in the FL cohort of 25 months in the G-B arm compared to 14 months in the bendamustine arm; median OS had not been reached in the G-B arm at the time of updated analysis compared to 54 months. Toxicities were manageable and comparable between both groups.127,128 Following the results of the primary analysis, the FDA granted approval in 2016 for the use of obinutuzumab (in combination with bendamustine) for treatment of relapsed or refractory FL following a rituximab-containing regimen.

In the phase III GALLIUM trial, patients with untreated indolent NHL (FL or marginal zone lymphoma) were randomly assigned to receive obinutuzumab or rituximab as part of induction chemoimmunotherapy in combination with CHOP, CVP or bendamustine, followed by maintenance for two years with the same anti-CD20 monoclonal antibody. In the 1202 patients with FL, median investigator-assessed 3-year PFS was 80% with obinutuzumab compared to 73% with rituximab, producing a clinically meaningful reduction in the risk of progression by 34%. The time to next treatment also favoured the obinutuzumab arm. There was no difference in OS at 3 years however it will be several more years before the OS data is mature given the long natural history of FL. Adverse events mostly related to cytopenias and IRRs, and grade 3–5 AEs were slightly more frequent in the obinutuzumab arm, although the incidence of treatment discontinuation was relatively similar between arms.129 The GALLIUM data support the use of obinutuzumab-based regimens as frontline therapy for FL. However given a median PFS of 10 years or more with rituximab based induction and maintenance, it remains uncertain whether obinutuzumab is best used in the frontline or relapsed and refractory setting.

**CLL**

Thirteen patients with relapsed/refractory CLL were given single-agent obinutuzumab (ranging from 400-1200mg) in the initial phase I dose-escalation stage of the GAUGUIN study. Obinutuzumab was subsequently administered at a fixed dose of 1000mg to 20 relapsed/refractory CLL patients in the phase II stage. Interestingly, the end-of-treatment ORR in the phase I cohort was 62%, but only 15% in the phase II cohort (best overall response 62% vs. 30%). The authors postulated that this may be related to higher baseline tumour burden in the fixed-dose group, resulting in lower exposure to treatment.130

The GALTON study evaluated obinutuzumab in combination with chemotherapy of the investigator’s choice (either fludarabine/cyclophosphamide or bendamustine) given to 41 treatment-naïve CLL patients. The ORR was higher in the bendamustine arm (G-B, 90%) compared to the FC arm (G-FC, 62%), as was the CR rate (20% compared to 10%). Toxicity was manageable, with IRRs being the most common AE (88%, grade 3–4 20%) and grade 3–4 neutropenia in 48% with G-FC and 55% with G-B.131

Goede and colleagues from the German CLL Study Group (GCLLSG) published the first phase III clinical trial data for obinutuzumab in 2014 from the pivotal CLL11 trial, which sought to establish the role of obinutuzumab as part of frontline treatment for CLL in patients with significant comorbidities (defined as a Cumulative Index Rating Scale score > 6 and/or an estimated creatinine clearance of 30–69 ml/min). At the time of study initiation, the standard of care for this patient group was largely undefined with chlorambucil monotherapy established as the comparator based on the GCLLSG CLL5 study.132 The median age of patients was 73 years with most having more than three comorbidities. The first stage of the trial demonstrated the superiority of chlorambucil with either rituximab or obinutuzumab over chlorambucil alone. Patients receiving chlorambucil monotherapy who progressed during or after treatment (n = 30) were permitted to cross over to the G-Clb group. Following accrual of additional patients, the second stage directly compared R-Clb (330 patients) with G-Clb (total of 333 patients). Treatment was administered every 28-days for six cycles.

Both antibody-containing combinations significantly improved PFS compared to chlorambucil alone. However G-Clb demonstrated a superior median PFS (26.7 vs. 15.2 months), CR rate (21% vs. 7%), and rates of MRD negativity in both peripheral blood and bone marrow in comparison to R-Clb.90 AEs were more frequent with G-Clb, with IRRs being the most common (20% grade 3 or greater) mainly occurring with the first infusion. While neutropenia was more frequent, rates of infections were not increased.133 Additionally, a recently presented update of this trial, encompassing an additional 2 years of follow-up has demonstrated a significant OS benefit for patients treated with G-Clb compared to those receiving R-Clb (median OS not reached vs 73.1months, HR 0.76, 95% CI 0.60–0.97, p = 0.0245).134

Higher rates of IRR have been consistently reported with obinutuzumab compared to rituximab. The higher affinity of obinutuzumab for FcγRIII leads to stronger FcγR activation and faster recruitment and activation of immune effector cells,
particularly in the peripheral blood, causing strong cytokine release. Immediate and marked release of IL-6 and IL-8 with the first infusion is accompanied by rapid, profound depletion of circulating B-cells and disappearance of NK cells from blood.\textsuperscript{135} Preliminary data indicate correlation between CD20 surface expression on CLL cells, FcγRIII polymorphisms, and risk of developing any grade of IRR with first infusion of rituximab or obinutuzumab. Trisomy 12 is associated with higher levels of CD20 expression and was found to be a risk factor for occurrence of IRR.\textsuperscript{136}

The GREEN study, an open-label, multicentre phase IIIb safety trial, enrolled both fit and unfit patients with previously untreated and relapsed/refractory CLL to receive obinutuzumab alone or in combination with chemotherapy (FC, bendamustine, or chlorambucil). The study aimed to reduce IRRs on the first day of administration by using a lower dose and slower infusion rate; in contrast to CLL11, the first dose was split (25mg on day 1 at 12.5mg/hour and 975mg on day 2). Although preliminary safety data indicated a safety profile similar to previous studies, with more manageable IRRs, later analysis did not show any substantial reduction in the incidence of IRRs compared to CLL11.\textsuperscript{137,138}

Building on the CLL11 trial which established G-Clb as the standard of care for older comorbid patients, the combination of obinutuzumab and venetoclax was evaluated in the CLL14 study in comparison to G-Clb. Data from the run-in phase demonstrated an ORR of 100% in 13 patients who completed therapy (median age of 75 years). This cohort included 2 patients with 17p deletion. After the fixed duration treatment of 12 cycles with median follow-up of 29.6 months, 80.2% were progression-free and 92.3% were still alive. 92% achieved MRD negativity in peripheral blood within 3 months of completion of 12 month therapy; at 18 months of follow-up, 64% remained MRD negative.\textsuperscript{139} Analysis of the randomized phase, which subsequently recruited 432 patients, is currently awaited. This combination is also undergoing study in the CLL2-BAG trial (NCT02401503), which is evaluating a sequential regimen of bendamustine debulking followed by induction with obinutuzumab and venetoclax and maintenance for up to 24 months, in both treatment-naïve and pre-treated CLL patients.\textsuperscript{140} Other combinations currently under investigation in phase II trials include obinutuzumab and idelalisib in the CLL2-BCG trial (NCT02445131) and obinutuzumab and ibrutinib in the CLL2-BIG trial (NCT02345863).\textsuperscript{140}

**Aggressive B-cell lymphomas**

The rationale for investigating obinutuzumab in aggressive lymphoma was based on the GAUGUIN study which included 40 patients with relapsed/refractory DLBCL (25) and MCL (15); 63% were rituximab-refractory. Five out of 25 rituximab-refractory patients had objective responses to induction with single agent obinutuzumab with the majority occurring in the high dose arm.\textsuperscript{141} The combination of obinutuzumab with CHOP chemotherapy (G-CHOP) in the frontline was first evaluated in the phase II GATHER study. Obinutuzumab dosing was based on early PK studies and designed to achieve saturation of CD20 binding during the first cycle. An ORR of 83% was documented with a CR rate of 55%.\textsuperscript{142} However, the subsequent phase 3 GOYA trial that included 1418 patients failed to demonstrate the superiority of G-CHOP over R-CHOP. The primary end-point of investigator-assessed PFS was not met with 66% in R-CHOP arm and 69% in G-CHOP arm being progression free after a median follow-up of 29 months (HR 0.92 (0.76, 1.11), p = 0.3868). Grade 3/4 AEs and fatal AEs were more common in the G-CHOP group, as were treatment discontinuations, dose reductions, and dose interruptions due to AEs.\textsuperscript{143}

**Ofatumumab**

Ofatumumab is a fully humanised type 1 monoclonal antibody against CD20, binding to an epitope that incorporates components of both the small extracellular loop and the N-terminal region of the large extracellular loop.\textsuperscript{21} Ofatumumab shows tighter binding to CD20 than rituximab as well as a slower dissociation rate with a half-life of three hours. While ofatumumab is a type 1 antibody, it does not induce apoptotic B cell death upon binding as rituximab does.\textsuperscript{144} Ofatumumab’s structural characteristics enable to bind to CD20 at closer proximity to the cell membrane surface in comparison to rituximab.\textsuperscript{144} This feature in addition to more avid binding to C1q and a seemingly reduced impact of complement regulatory proteins, contribute to ofatumumab’s superior CDC compared with rituximab.\textsuperscript{145} Regarding ADCC, most data demonstrate relative equivalence between rituximab and ofatumumab, though some studies suggest that ofatumumab displays greater efficacy in this modality also.\textsuperscript{146,147} Preclinical studies of ofatumumab demonstrated superior in vitro activity in comparison to rituximab providing justification for further clinical trials.\textsuperscript{146,148}

**Indolent hematological malignancies**

**Follicular lymphoma**

The first study of ofatumumab in FL reported single-agent activity in 40 patients with relapsed/refractory, grade 1/2 FL. Patients received four weekly doses of either 300mg, 500mg, 700mg or 1000mg with response rates of 63%, 33%, 20% and 50% at the respective doses. 15 of the patients had been previously exposed to rituximab, and of these, 64% showed a response, including 3 out 4 patients that were judged to be rituximab refractory. The median time to progression (TTP) was 8.8 months and the median duration of response was 29.9 months.\textsuperscript{149} Based on this demonstration of ofatumumab’s activity, a subsequent trial was undertaken in 116 rituximab refractory FL patients, in which patients received 8 weekly treatments of ofatumumab, with 300mg in cycle one, followed by random allocation to 500mg or 1000mg in subsequent cycles.\textsuperscript{150} 86% of these patients had advanced stage disease, and approximately half were high risk according to the FL international prognostic index (FLIPI). Ofatumumab once again proved to be exceedingly well tolerated, however its efficacy as a single agent in this trial was at best modest, with an overall response rate (ORR) of 11% and no significant difference between dosing levels. The median progression free survival (PFS) proved only to be 5.8 months. Ofatumumab at
500mg or 1000mg was subsequently tested in the upfront setting in FL grades 1–3 and was delivered in combination with CHOP chemotherapy for six cycles.\textsuperscript{151} 29 patients were treated in each dose group, with an ORR in the 500mg group of 90% with 24% complete response (CR); in the 1000mg group the ORR was 100% with 38% in CR. Curiously, the CR rate was greatest (76%) in those with high risk FLIPI scores, but limited follow-up precludes determination as to whether this translates into a survival benefit. Overall, these results were considered comparable to pre-existing data for rituximab in combination with both bendamustine and CHOP.\textsuperscript{74,152}

**CLL**

The utility of single agent ofatumumab in relapsed/refractory CLL was demonstrated in a study of 33 heavily pretreated patients. Each patient received four doses of ofatumumab administered weekly, at one of three escalating dose regimens. At the highest dose level (500mg for one week, followed by 3x 2000mg), the ORR was 50%, although no patient achieved a CR. Therapy was well tolerated, however 51% of patients developed an infection, with the vast majority being either grade 1 or 2.\textsuperscript{153} Based on these initial data, a larger phase II trial of ofatumumab monotherapy was conducted in 138 patients with either alemtuzumab and fludarabine refractory or bulky fludarabine refractory disease, in which participants were treated over 24 weeks with initial 8 weekly doses of ofatumumab, consolidated with 4 monthly infusions.\textsuperscript{154} The ORR in the alemtuzumab and fludarabine refractory group and the bulky fludarabine refractory group was 58% and 47% respectively. With median PFS of 5.7 and 5.9 months, and OS of 15.4 and 13.7 months respectively. The results of this trial prompted the FDA and the European Medicines agency (EMA) to approve the use of ofatumumab in fludarabine and alemtuzumab refractory cases in 2009 and 2010 respectively.\textsuperscript{155}

Investigators have subsequently examined single agent ofatumumab as the comparator for ibrutinib in phase 3 studies of CLL with ibrutinib demonstrating superiority in regard to PFS (8.1 months vs not reached), and 12-month OS (81% vs 90%) and ORR (4.1% vs 42.6%) with a comparable rate of serious adverse events (SAEs).\textsuperscript{156} The notably lower response rate in ofatumumab arm when compared to previous studies was attributed to the use of CT guided response assessment, which was not included in the landmark study.

The GIMEMA group explored ofatumumab in combination with bendamustine in refractory CLL, yielding an ORR of 72.3% with 17% of patients achieving CR.\textsuperscript{157} The PFS and OS at a median follow-up of 24.2 months were 49.6% and 83.6% respectively. Although significant myelosuppression was common (grade 3 or 4 neutropenia occurred in 61.7% of patients) this did not translate into a high rate of serious infections (grade 3 or 4 infections occurred in 6% of patients). The COMPLEMENT-2 trial recently reported the efficacy of ofatumumab with fludarabine and cyclophosphamide (FC) in comparison to FC alone in the relapsed setting. Patients who received ofatumumab had greater PFS (28.9 months vs 18.8 months) and ORR (84% vs 68%), though no statistically significant increase in OS was observed (56.4 months vs 45.8 months, 95%CI 0.56–1.09).\textsuperscript{158}

Further studies of ofatumumab in combination with novel agents have demonstrated efficacy with lenalidomide,\textsuperscript{159} ibrutinib,\textsuperscript{160} and idelalisib.\textsuperscript{161} However, the latter combination was associated with increased rates of serious infections and death.

Frontline studies of single-agent ofatumumab in CLL have confirmed activity, but CR rates were low (~2.5%), suggesting that it may be better used in combination.\textsuperscript{162} Induction therapy with fludarabine, cyclophosphamide and ofatumumab (FCO) has been investigated by Weirda et al in a randomised trial.\textsuperscript{163} 61 patients were treated in two dosing cohorts; both groups received standard FC plus ofatumumab 300mg (first dose), thereafter the dose of ofatumumab varied; group A received 500mg and group B received 1000mg for the remaining 5 cycles. The ORR and CR rate were 77% and 32% in group A and 73% and 50% in group B, respectively.\textsuperscript{155} FCO was tolerated well, with the most frequent AE being grade 1 or 2 infusion reactions. Grade 3 or 4 neutropenia did occur in 48%, but only 8% of patients suffered grade 3 or 4 infections. For those patients deemed unsuitable for fludarabine-based therapy, ofatumumab was investigated in combination with chlorambucil in the COMPLEMENT-1 study.\textsuperscript{164} Patients were randomised to chlorambucil and ofatumumab or chlorambucil alone. The ORR and CR rate for the dual therapy arm were 82% and 12%, respectively, as opposed to 69% and 1% for chlorambucil monotherapy. Median PFS was significantly greater for those who received ofatumumab (22.4 vs 13.1 months). OS was not reached for either group at the time of follow-up and the frequency of severe infections was similar between the two arms.\textsuperscript{164} These data led both the FDA and EMA to approve the use of this combination as upfront management of patients unfit for fludarabine-based induction regimens.

Another potential role for ofatumumab is as maintenance therapy for those who had a complete or partial response following second or third line CLL therapy. This application was studied in the PROLONG trial, in which patients were treated with 8-weekly ofatumumab (1000mg) for up to 2 years and compared to observation alone.\textsuperscript{165} Median PFS was longer for the maintenance cohort (29.4 vs 15.2 months), though no difference in OS was evident. Both PFS and time to next treatment declined rapidly after discontinuation of ofatumumab, perhaps suggesting the possibility of further benefit with more prolonged maintenance. However, again in the era of novel therapies and novel drug combinations, the role of this drug at all stages of CLL management remains uncertain.

**Aggressive hematological malignancies**

There is a relative paucity of data on ofatumumab in aggressive hematological malignancy. A study of ofatumumab in a group of heavily pre-treated patients with DLBCL relapsing post, or ineligible for autologous stem cell transplant, achieved a meagre ORR of 14% and median PFS of 2.6 months.\textsuperscript{166} A subsequent phase II trial of ofatumumab in combination with ICE or DHAP as second line treatment in relapsed DLBCL, (mirroring the CORAL study which examined the same agents in combination with rituximab) demonstrated a similar ORR of 61% with a CR rate of 30% to the rituximab based
Rituximab in autoimmune diseases

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune condition characterised by inflammatory arthritis affecting multiple joints of the body. In the absence of treatment, RA commonly leads to pain, loss of function and premature mortality in affected individuals. However, the prognosis of rheumatoid arthritis has drastically improved in recent decades due to the introduction of biologic therapies targeting specific components of pathogenesis, including tumour necrosis factor (TNF) inhibition and B cell depletion amongst several other targets. Biologic agents are typically used in conjunction with more traditional synthetic disease modifying anti-rheumatic drugs (DMARDs) such as methotrexate.

The pathogenesis of RA is complex, and the precise contribution of B lymphocytes to RA pathogenesis is not well defined. B cells have several potential roles, including acting as antigen presenting cells, activating T lymphocytes, secretion of proinflammatory cytokines such as TNF and the production of rheumatoid factor and other autoantibodies. However, the strongest evidence for the role of B lymphocytes in RA pathogenesis comes from several trials of rituximab, which report successful control of disease activity following B lymphocyte depletion.

The first randomised control trial (RCT) of rituximab in RA, published in 2004, involved 161 patients with active RA, and compared two doses of 1000mg rituximab either as a single agent or in combination with methotrexate or cyclophosphamide, with methotrexate alone. The primary outcome was the American College of Rheumatology (ACR) response criteria, which is a composite measure including patient and physician assessments, pain scales, functional questionnaires and acute phase reactants. All treatment protocols containing rituximab showed superiority to methotrexate alone at 24 weeks, with the rituximab/methotrexate and rituximab/cyclophosphamide combination groups showing superiority out to 48 weeks. Further studies, designed predominantly to identify optimal dosing regimens, have repeatedly confirmed these results.

Rituximab efficacy has now been well established in patients not previously exposed to other biologic agents such as TNF inhibitors, as well as those patients who have previously failed these therapies. Beyond clinical symptoms, imaging studies have also reported reduction of joint damage in patients treated with rituximab. Currently, rituximab is typically prescribed for RA patients who have had inadequate responses to synthetic DMARDs and TNF inhibitors. It is generally prescribed in combination with methotrexate therapy, with large registries suggesting better response rates with combination therapy compared to rituximab monotherapy. It is overall well tolerated, with infection rates and adverse events similar to many other commonly prescribed biologic agents in RA patients.

ANCA associated vasculitis

The anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides include granulomatosis with polyangiitis (GPA, previously known as Wegener’s granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA, previously known as Churg Strauss). These are rare conditions in which autoimmune mediated small vessel inflammation and necrosis occurs in one or multiple organs, often leading to rapid organ failure and death without treatment. For many years, the mainstay of induction therapy for these diseases has been cyclophosphamide, with chronic exposure leading to an array of unwanted side effects including infertility and bladder malignancy.

Like RA, the aetiology and pathogenesis of ANCA associated vasculitis is complex and poorly defined. However, evidence suggests B lymphocytes are involved in the process. Active B lymphocytes are present in the blood of patients with GPA in greater quantities than in healthy individuals. Cyclophosphamide, a drug known to be successful in the treatment of ANCA associated vasculitis, has been shown to have an inhibitory influence on B cell activation and function.

In 2010, on a background of several positive uncontrolled studies, an RCT comparing rituximab therapy with cyclophosphamide in the 197 patients with either GPA or MPA showed that rituximab was non-inferior to cyclophosphamide as an induction agent, with superiority suggested in patients with relapsing disease. A more recent RCT has also reported that rituximab has efficacy as a maintenance agent above that of the traditionally used DMARD azathioprine.

Rituximab is now commonly used in the management of GPA and MPA, particularly in younger patients who wish to preserve fertility, with cost being the limiting factor to access for all patients. An RCT of the efficacy of rituximab in EGPA is currently underway.

Other autoimmune conditions

Despite a lack of RCT evidence, there are several other autoimmune conditions in which off-label rituximab is used in some circumstances.

Rituximab has been trialled in systemic lupus erythematosus (SLE), an autoimmune condition characterised by multi-organ inflammation, polyclonal B cell activation and the presence of multiple autoantibodies. Despite the role of B cells in the pathophysiology of this illness, two RCTs assessing both renal and non-renal SLE failed to show benefit of rituximab above that of standard therapy. However, there are case reports and case series suggesting efficacy in a subset of patients with SLE, and rituximab is sometimes prescribed in patients who have failed more mainstream therapies.
Similarly, despite positive uncontrolled study reports, an RCT evaluating the efficacy of rituximab as a second line therapy in adult immune thrombocytopenic purpura (ITP) also failed to show benefit over placebo in patients previously treated with corticosteroids. However, there was a trend towards benefit with higher response rates that did not reach statistical significance in this study. Additionally, Tran et al published a relatively large prospective trial of 122 patients with chronic, relapsed or refractory ITP showed an ORR of 44% with excellent safety outcomes.

Other autoimmune conditions with uncontrolled case report or case series data suggesting benefit of rituximab include antiphospholipid syndrome, blistering diseases of the skin such as pemphigus and pemphigoid, myasthenia gravis, neuro-myelitis optica and the inflammatory myopathies. Within the realm of hematology, similarly limited evidence exists in the support of the use of rituximab either alone or in combination with other immunosuppressants in acquired hemophilia, autoimmune hemolytic anaemia (including a small randomised controlled trial), and thrombotic thrombocytopenic purpura. Further controlled trials are required to more definitively establish efficacy in these conditions, although the rarity of several of these diseases poses a significant challenge to trial recruitment.

Other anti-CD20 mAbs in autoimmune disease

Numerous trials utilising new generation anti-CD20 mAbs have been undertaken or remain ongoing in autoimmune disease. These agents include ofatumumab, obinutuzumab, ocrelizumab and veltuzumab. Some of these drugs have demonstrated safety and efficacy in these settings, though an exploration of these data is beyond the scope of this article and has been recently reviewed by Du and colleagues. Nonetheless it is important to note that there are no published data comparing these agents to rituximab directly, hence it is difficult to determine where these mAbs may be most usefully deployed in clinical practice, and the advantages of these drugs over rituximab remain to be proven.

Anti-drug antibodies

Monoclonal antibodies can be immunogenic and provoke the formation of anti-drug antibodies (ADAs). ADAs can affect the pharmacokinetic, efficacy and toxicity profile of mAbs, but there is considerable variability between different drugs. As these agents have evolved from murine to chimeric to humanised and fully human iterations, there has been a reduction in the frequency of ADAs detected. Nonetheless, ADAs may still form in up to 26.3% of patients treated with fully human mAbs. The presence and impact of ADAs have not been routinely reported in large clinical trials of CD20 mAbs, and data correlating ADAs with patient outcomes is extremely scarce. A recent review by van Brummelen that examined data from EMA and FDA drug reports, showed that ADAs formed in 1–2% of patients treated with rituximab, 6% of patients receiving obinutuzumab, but no data were available for ofatumumab. Interestingly, there are no data that show the presence of ADAs significantly impacts efficacy, toxicity or pharmacokinetics in the anti-CD20 agents in the setting of cancer treatment. A recent study of 339 patients with multiple sclerosis (MS) treated with rituximab showed a much higher rate of ADA formation, occurring in 37% of relapsing-remitting MS patients and 26% of progressive MS patients. However, although the presence of ADAs was associated with incomplete B cell depletion, no relationship could be established with adverse events or patient outcomes. Accordingly, it remains unclear how important an issue ADAs are for anti-CD20 mAbs, and further study is required to clarify the significance of this phenomenon.

Conclusion

Extensive though this review may be, it cannot provide a panoptical analysis of all scientific and clinical data for all anti-CD20 mAbs. However, the profound and revolutionary impact of these agents on modern medical therapeutics is surely undisputed. The prospect of an ongoing role for CD20 mAbs is relatively assured in the short-term, and new developments in this arena abound. Modern pharmaceutical engineering methods are achieving impressive and targeted modulation of mAb properties that may augment their clinical efficacy and safety, and lead to new generations of CD20 targeted drugs. Indeed, a variety of other anti-CD20 mAbs not discussed in this article have been trialled in numerous diseases. Novel therapy combinations also offer potential synergistic benefits to overcome resistant disease or improve response rates. An intriguing development, beyond the scope of our article, is the production of so-called “biosimilars” in the wake of patent expirations. These copies of the aforementioned mAbs represent a chance for a larger number of patients to access these therapies that can be prohibitively expensive in resource-limited settings, but also pose difficulties in the critical task of establishing therapeutic equivalence. While work continues to decipher the remaining mysteries of CD20 physiology and the mechanisms of action of the mAbs, the potential applications of targeting this antigen may yet to be fully realised and will hopefully continue the revolution sparked by rituximab’s emergence, now over twenty years ago.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| aaIPI | age-adjusted international prognostic index |
| ACR | American College of Rheumatology |
| ADA | anti-drug antibody |
| ADCC | antibody-dependent cell-mediated cytotoxicity |
| ADCP | antibody-dependent cell-mediated phagocytosis |
| AE | adverse event |
| ANCA | anti-neutrophil cytoplasmic antibody |
| B-ALL | B-cell acute lymphoblastic leukemia |
| BCR | B-cell receptor |
| BL | Burkitt lymphoma |
| BTK | Bruton tyrosine kinase |
| CDC | complement-dependent cytotoxicity |
| CHOP | cyclophosphamide, doxorubicin, vincristine, prednisone |
| CI | confidence interval |
| Clb | chlorambucil |
| CLL | chronic lymphocytic leukemia |
| CR | complete response |
CVP cyclophosphamide, vincristine, prednisone
DHAP dexamethasone, high-dose cytarabine and cisplatin
DLBCL diffuse large B-cell lymphoma
DLT dose-limiting toxicity
DMAR Dds ease modifying anti-rheumatic drug
ECOG Eastern Cooperative Oncology Group
EFS event-free survival
EGPA eosinophilic granulomatosis with polyangiitis
EMA European Medicines Agency
FC fludarabine and cyclophosphamide
FCM fludarabine, cyclophosphamide and mitoxantrone
FCO fludarabine, cyclophosphamide and ofatumumab
FCR fludarabine, cyclophosphamide and rituximab
FCO fludarabine, cyclophosphamide and ofatumumab
FDA Food and Drug Administration
FFS failure-free survival
FL follicular lymphoma
FLPI follicular lymphoma international prognostic index
G obinutuzumab (GA-101)
GELF Groupe d’Etude des Lymphomes Folliculaires
GPA granulomatosis with polyangiitis
HIV human immunodeficiency virus
HR hazard ratio
IRR infusion-related reaction
ITP immune thrombocytopenic purpura
mAb monoclonal antibody
MCL mantle cell lymphoma
MPA microscopic polyangiitis
MRD minimal residual disease
MS multiple sclerosis
NHL non-Hodgkin lymphoma
NK natural killer
ORR overall response rate
OS overall survival
PFS progression-free survival
Ph- Philadelphia chromosome negative
R rituximab
RA rheumatoid arthritis
RCT randomized controlled trial
RR relative risk
SAE serious adverse event
SLE systemic lupus erythematosus
TNF tumour necrosis factor
TPP time to progression
VIM etoposide, ifosfamide and methotrexate

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Disclosure of potential conflicts of interest

In accordance with Taylor & Francis policy and our ethical obligations as researchers, we report that Prof Opat:

(1) Consulted for Roche, Ranssens, Celgene, Takeda, Novartis, Abbvie, Gilead, Bristol-Myers Squib, Merck, Sanofi, Mundipharma
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