

Anti-CD20 monoclonal antibodies: historical and future perspectives

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ABSTRACT

Antibodies to CD20 have confirmed the hypothesis that monoclonal reagents can be given *in vivo* to alleviate human diseases. The targeting of CD20 on normal, malignant and auto-immune B-lymphocytes by rituximab has demonstrated substantial benefits for patients with a variety of B-cell lymphomas, as well as some with autoimmune disorders. There has been a notable increase in the survival rates from B-cell lymphoma in the decade since anti-CD20 therapy was introduced.

Key words: CD20, monoclonal antibodies, lymphoma, immunotherapy.

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Introduction

Monoclonal antibody (mAb) therapy with the anti-CD20 mAb rituximab represents one of the most important advances in the treatment of lymphoproliferative disorders in the last 30 years. Prior to its introduction, there had been only modest improvement in the treatment outcome of diseases such as follicular (FL) and diffuse large B-cell lymphoma (DLBCL).¹⁻³ However, the use of rituximab, particularly in combination with various chemotherapy/radiotherapy regimes, has significantly improved all aspects of the survival statistics for these patients. In addition, rituximab is approved, or being investigated for the treatment of many other hematologic disorders ranging from other malignancies, such as chronic lymphocytic leukemia (CLL), to autoimmune disorders, such as immune and thrombotic thrombocytopenic purpura and rheumatoid arthritis. This review considers why CD20 is such an effective target and outlines a range of new CD20 mAb that should improve efficacy in the future.

Progress in developing CD20 mAb

The last three decades have seen considerable progress in our understanding of the structure and function of the CD20 molecule and in the development of engineered anti-CD20 mAb. Table 1 and Figure 1 chart these advancements, giving the key discoveries leading to the translation of this knowledge to the clinic. In particular, pre-clinical work has investigated the extent to which CD20 mAb engage the main effector pathways commonly employed by mAb, i.e. comple-

ment-dependent cytotoxicity (CDC), programmed cell death (PCD) and Fc:FcR dependent mechanisms, with passive immunization a potential fourth mechanism. While it is widely accepted that Fc-Fcγ receptor (FcγR) interactions are critical,²⁴ the role of CDC and PCD is still disputed.²⁵ As these have been discussed in detail elsewhere,^{25,26} here we will underline only the critical and recent evidence regarding each mechanism.

CDC

Rituximab was originally shown to be capable of binding C1q and inducing complement-mediated cell lysis.²⁷ Subsequent work confirmed this and it is clear that CD20 is an excellent target for CDC against numerous cell types *in vitro*.²⁸⁻³⁰ probably, at least in part, because of its high expression and the proximity of the mAb-binding-epitope to the plasma membrane.³¹ Furthermore, rituximab's ability to redistribute CD20 into Tx-100 insoluble lipid rafts appears to cluster the mAb and greatly enhances its ability to capture C1q and elicit CDC.^{29,32} Support for CDC as a key effector mechanism comes from studies demonstrating that expression of complement-defence molecules is associated with rituximab resistance,^{33,34} that complement is consumed *in vivo* following rituximab infusion, and that replacement of the consumed components restores the activity of *ex vivo* rituximab in CDC assays³⁵ and might benefit patients.³⁶ Similarly, a number of animal models have clearly shown that complement inactivation/deficiency results in reduced anti-CD20 mAb activity *in vivo*.^{32,37} However, it should be noted that these early models were not ideal²⁵ and animal models of normal B-cell depletion

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Table 1. The current development status of anti-CD20 mAb and the key effector mechanisms selected/modified.

mAb	Format	Indication	Manufacturer	Comments	Phase of development
Rituximab ⁴	Chimeric IgG1	NHL/RA	Genentech/Biogen		Approved 1997
Y ⁹⁰ -Ibritumomab tiuxetan ⁵	Murine (90Y)	NHL	Biogen/IDEC	Low ADCC	Approved 2002
I ¹³¹ tositumomab ⁵	Murine (I31I)	NHL	GSK	Low CDC	Approved 2003
Ofatumumab ^{6,7}	Human IgG1	NHL/RA	Genmab AC/GSK	High CDC and ADCC	Phase III trials
Ocrelizumab ⁸	Humanized IgG1	NHL/RA	Genentech/Roche/Biogen		Phase III trials
TRU-015 ^{9,10}	SMIP ^a	RA	Trubion Pharma/Wyeth	High ADCC Low CDC	Phase I/II
Veltuzumab ^{11,12}	Humanized	NHL and ITP	Immunomedics		Phase I/II IgG1
AME-133v ^{13,14}	Humanized IgG1	Relapsed NHL	Applied Molecular Evolution/Eli Lilly	High ADCC	Phase I/II
PRO131921 humanized (Version 114) ¹⁵	IgG1	CLL and NHL	Genentech	High CDC and ADCC	Phase I/II
GA101 ¹⁶	Humanized IgG1	CLL and NHL	Glycart/Roche	High PCD and ADCC Low CDC	Phase I/II

^aSmall modular immunopharmaceutical (SMIPSM) drug composed of human IgG1 Fc and hinge regions (hinge, CH₁, and CH₂) linked directly to an anti-CD20 scFv.

show little to no requirement for functional complement in the activity of rituximab.^{38,39} Furthermore, Weng and Levy⁴⁰ demonstrated that the level of expression of the complement defence molecules CD45, CD55 and CD59 on FL cells did not correlate with responses to rituximab. Together these data suggest that many factors influence the efficacy of complement on target cells. For example, the level of complement defence molecules on normal and malignant B cells may often be too high to allow complement to play a prominent role in anti-CD20 mAb immunotherapy. Alternatively, as suggested by Taylor and colleagues,⁴¹ certain complement components which are essential for therapy may become depleted when patients with bulky disease are treated with large doses of mAb. To add a further layer of complexity, recent evidence suggests that complement may actually be disadvantageous to the efficacy of rituximab. First, Li *et al.*⁴² showed that deposition of active complement components facilitated the removal of rituximab: CD20 complexes from the lymphoma cells by FcR-expressing macrophages through the process of *shaving*,^{35,43,44} a phenomenon which seems to be exacerbated by the addition of C3b. Second, C3b deposition has also been shown to block the interaction between the Fc domain of rituximab and CD16 (FcγRIIIA) on NK cells, hence impairing ADCC.⁴⁵ Finally, evidence comes from a recent hypothesis-generating study⁴⁶ investigating the impact of C1qA polymorphisms on the efficacy of rituximab. In this study of 133 patients, expression of the A allele which leads to low C1q levels was shown to correlate with enhanced rituximab responses in FL, compared to those patients with the G allele (high C1q expressing).

Although it is tempting to speculate that these effects are solely due to differences in complement activation, it should be noted that C1q has numerous other effects *in vivo* including a critical role in the phagocytosis of apoptotic bodies⁴⁷ and effects on APC maturation and function.^{48,49} On consideration of all of these data, it appears that although complement can evoke potent CDC responses

both *in vitro* and with xenografts *in vivo*, there is little direct evidence to suggest that this activity provides a substantial positive effect on rituximab-mediated depletion of B cells in humans.

Programmed cell death

It has also been proposed that mAb binding of CD20 can directly transmit intracellular signals that lead to PCD.^{25,26} This was based on early observations of changes in cell growth, including growth arrest with anti-CD20 mAb.⁵⁰ Since then PCD has been demonstrated with a range of lymphoma cell lines, but rarely on primary tumors, and has generally been shown to depend on further anti-CD20 mAb crosslinking.^{29,51,52} Furthermore, not all B-cell lines are sensitive^{28,53} and the cell death pathway evoked is clearly cell-line and stimulus dependent - apparently varying with both the mAb chosen and the degree of hyper-crosslinking delivered. When rituximab is sufficiently cross-linked it is capable of eliciting potent apoptotic responses in sensitive cell-lines via the intrinsic mitochondrial pathway.^{54,55} However, cell death induced by non-hyper-crosslinked anti-CD20 mAb appears to be non-apoptotic and varies considerably depending on the mAb used, rituximab being relatively weak and tositumomab strong at inducing PCD.⁵⁶

It has never been formally shown what molecular process *in vivo* might mimic the high affinity crosslinking achieved with mAb reagents *in vitro*, although it is postulated that this could be performed by FcγR-bearing effector cells.²⁵ Perhaps the best evidence that PCD may operate *in vivo* on primary tumor cells comes from a study in which both caspase-3 and caspase-9 activation, taken to signify classical apoptosis, was observed in 10 patients with CLL treated with rituximab,⁵⁷ although there are alternative explanations for these data.²⁵ More recently, Stolz *et al.*⁵⁴ demonstrated evidence of caspase activation and apoptosis in xenografted B-cell lymphomas in mice treated with rituximab. Interestingly, rituximab insensitivity in this model was associated with increased expression

of anti-apoptotic Bcl-2 family proteins, which could be overcome with the BH3-mimetic ABT-737. Although interesting, these results only reflect effects in cell lines as opposed to *bona fide* tumor cells, and do not demonstrate that apoptosis is an important effector mechanism for *in vivo* depletion of primary lymphoma cells. In our most recent studies, we have compared the ability of rituximab to deplete human CD20 transgenic mouse B cells *in vivo* in the presence or absence of a second transgene encoding high levels of Bcl-2 which blocks the intrinsic apoptosis pathway.⁵⁸ Although B cells expressing the Bcl-2 transgene are relatively resistant to apoptotic stimuli, such as cyclophosphamide, etoposide and dexamethasone *in vitro*, *in vivo* they are just as susceptible to rituximab as B cells lacking the transgene (Beers *et al.*, unpublished observations, 2009). Clearly, in this fully syngeneic model, induction of the intrinsic apoptosis pathway is not important for subsequent B-cell depletion. By contrast, tositumomab appears (without further crosslinking) to promote a cytoplasmic form of death, involving lysosomes, which is able to bypass the apoptotic inhibition provided by high levels of Bcl-2 both in the presence and absence of radiation^{56,59,60} perhaps explaining the efficacy of the I-¹³¹ radioimmunoconjugate, I¹³¹-tositumomab, even in cases refractory to both chemotherapy and rituximab.⁶¹ Thus, as with CDC, the support for rituximab promoting cell death is apparent, but whether this mechanism is critical for the depletion of CD20 positive target cells *in vivo* remains to be proven.

Fc:FcR dependent mechanisms

Although the evidence regarding the involvement of CDC and PCD remains inconclusive, it is clear that

Fc:FcR interactions are critical for the success of anti-CD20 immunotherapy. FcγR are expressed on immune cells such as monocytes, macrophages, natural killer cells and neutrophils. FcγR-dependent activation of these immune effectors potentially leads to the release of inflammatory mediators and/or killing/direct phagocytosis of the opsonized target cells.²⁵ However, the outcome of these mAb:effector cell interactions varies markedly, dependent on both the cell type and balance of activatory and inhibitory FcγR signaling induced.^{62,63} The first evidence that Fc:FcR interactions are critical for the efficacy of anti-CD20 mAb came from the seminal paper of Clynes and Ravetch⁶⁴ showing that rituximab treatment of subcutaneous Raji xenografts is fully dependent upon the γ chain-associated activatory FcγR. However, some of the best evidence comes from clinical studies where patients with the higher affinity allelic variants of CD16 (FcγRIIIA) respond better to treatment with rituximab.^{62,63,65} Polymorphisms in FcγRIIa⁶⁵ have also been found to influence responses in FL. Interestingly, and in marked contrast to the above, no association between FcγR polymorphic variation and response was shown in CLL patients,⁶⁶ indicating that the requirement for Fc:FcR interaction varies between diseases, as may the dominant effector mechanisms.

In syngeneic mouse model systems, using either mouse anti-mouse CD20 mAb in wild-type mice⁶⁷ or anti-human CD20 mAb in human CD20 transgenic mice³⁸ (also Beers *et al.*, unpublished observations, 2009), a complete absence of normal B-cell depletion has been observed in mice lacking the common γ chain, indicating an absolute requirement *in vivo* for activatory FcγR interactions. Recently, the ability of anti-mouse CD20 mAb to deplete syngeneic E -Myc

History of anti-CD20 mAb in clinical translation

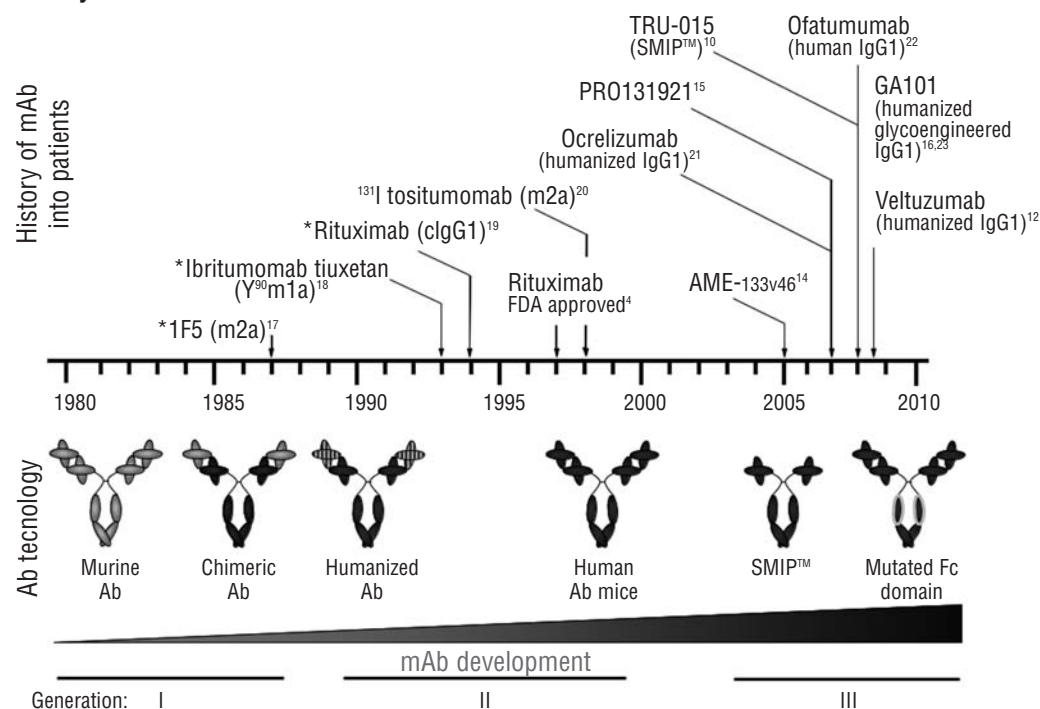


Figure 1. History of CD20 mAb in clinical translation. The timeline describes the chronological introduction over the last three decades of respective anti-CD20 mAb in human trials, as well as the corresponding progress in Ab technology from 1st through to 3rd generation reagents: generation Ab are murine or human/mouse chimeric Ab, 2nd generation Ab are either humanized or fully human and 3rd generation Ab have further modifications to the Ab structure e.g. mutation or a-fucosylation of the Fc domain for enhanced FcR binding profiles.

tumor cells was also shown to be dependent on activatory FcγR.⁶⁸ However, it still remains to be determined which of the FcγR-expressing immune effector cells are critical. In the mouse, there is good agreement that monocytes/macrophages are the key effectors when deleting either normal or malignant B cells with anti-CD20 mAb.^{38,67,68} Depleting macrophages using liposome-encapsulated clodronate⁶⁹ results in decreased mAb efficacy^{38,67,68} (also Beers *et al.*, unpublished observations, 2009), but the removal of neutrophils or natural killer cells has no impact. Gong *et al.*³⁸ also investigated the relative importance of the splenic and liver compartments of the reticuloendothelial system. They showed that surgical limitation of the hepatic blood supply correlated with lower B-cell depletion, underscoring the role of hepatic Kupffer cells and the need for an intact reticuloendothelial system for maximal mAb response. They also postulated that differences in depletion kinetics between tissues were, for the most part, simply a reflection of the access of those B cells to the vasculature and that targets with slower recirculation kinetics were more resistant to depletion simply due to reduced access to the reticuloendothelial system's effector cell populations. Similar studies are clearly impossible in humans, so it is not known whether the same systems operate. However, the need for lymphoma cells to traffic out of solid tumor deposits and pass over the reticuloendothelial system might help explain some of the slow and late responses to rituximab. This provides a logical alternative to the immunization effect (see below) used to explain late responses to rituximab.

In humans, *in vitro* experiments with blood borne effector cells point to the NK cell as a prominent effector in ADCC,^{30,70} but whether this is also true in tissues is unclear. Similarly, whether FcγRIIIb-expressing neutrophils, the predominant leukocyte in peripheral blood, play a role in providing therapy *in vivo* remains to be clarified. Cartron *et al.*⁷¹ found no correlation between neutrophil phagocytosis (from patients with different FcγRIIIb polymorphisms) and response to rituximab. However, they did find a high level of response in FL patients given GM-CSF plus rituximab, possibly associated with increases in monocyte, granulocyte, and dendritic cell populations.⁷² Recently, Shibata-Koyama *et al.*⁷³ demonstrated enhanced phagocytosis of lymphoma cells in human whole blood using a modified non-fucosylated rituximab reagent with enhanced affinity for FcγRIIIb on neutrophils. Although it is possible that neutrophils have a role in the functioning of rituximab *in vivo*, which may be boosted by additional manipulations, such as G-CSF treatment or a-fucosylation of the mAb Fc domain,⁷⁴ definitive proof is currently lacking.

Immunization

Mechanisms such as CDC, ADCC and PCD are considered to be immediate and comparatively short-acting, but the clinical response to a single course of mAb can be late acting and prolonged. This has led to the suggestion that anti-CD20 mAb could also have an immunization effect.⁷⁵ Rituximab-induced cell death, by the three main pathways described, will result in release of tumor antigens and changes in localized inflammation. Such an environment promotes the uptake of tumor-associated antigens

by dendritic cells and cross-presentation to T lymphocytes, providing the potential for cell mediated immunity.⁷⁶⁻⁷⁸

That this might occur during therapy was demonstrated recently in a small proof of principle study which showed an increase in FL idiotype specific T cells after rituximab monotherapy.⁷⁹ However, due to the size of the study, it is not known whether this immunization effect correlates with clinical outcome. Moreover, whether this vaccine effect is specific to therapeutic mAb in general or any cell-killing modality is currently unclear.⁸⁰ Alternative explanations also exist, such as whether the mAb and/or chemotherapy alters the immunogenicity of the tumor cells as suggested by Zitvogel and Kroemer⁸¹ and Haynes.⁸²

Future anti-CD20 mAb

The success of rituximab has stimulated considerable efforts to develop improved reagents and there are now at least 7 CD20 mAb in clinical development with many more in pre-clinical evaluation (Table 1). These new mAb are engineered for potential benefits over the 1st generation rituximab, the modifications include: 2nd generation reagents where the IgG1 mAb is humanized or fully human to reduce immunogenicity, but with an unmodified Fc region; and 3rd generation mAb which are humanized and have an engineered Fc region designed to improve therapeutic performance by adapting their effector functions.

The former (2nd generation) include ocrelizumab, vel-tuzumab and ofatumumab, and the latter (3rd generation) includes, TRU-015, AME133V, Pro13192 and GA101 (Table 1 and Figure 1). Clinically, ofatumumab is the most advanced of these reagents in that it will be the first to seek FDA and EMEA approval for the treatment of CLL. Its most notable features are its slow off rate, unusual CD20 epitope specificity and high CDC activity.³⁰ This latter feature is probably related to the slow off rate and/or unusual binding specificity, resulting in lysis of rituximab resistant CLL targets. It will be interesting to know if such potency of lysis can be achieved *in vivo* where complement availability may be limited as discussed. Interestingly, this ability to activate complement has not been associated with more toxicity in patients, which was a potential concern knowing the toxicity associated with systemic complement activation. The clinical efficacy and safety of single-agent ofatumumab have now been reported in two phase I-II trials in relapsed/refractory CLL and FL with phase III trials ongoing.^{6,22} Moreover, ofatumumab was effective in a group of fludarabine- and alemtuzumab-resistant CLL patients, known to have a poor prognosis.⁷

It will be interesting to see if combination chemotherapy with ofatumumab will also produce higher responses than those observed with rituximab.

The other two 2nd generation mAb are very similar to rituximab in both their structure and potency, and advantages over rituximab will probably come from their immunogenicity and alternative routes of administration. The 3rd generation mAb, AME133V, Pro13192 and GA101 are all modified either by amino-acid substitution or by glycoengineering to promote interaction with FcγR, particularly FcγRIIIa. As discussed, considerable clinical data

suggest that high affinity interaction with FcγRIIIa is beneficial for FL treatment and patients with the low affinity allele, 158F, are less sensitive to rituximab treatment. Using these 3rd generation mAb should overcome this difficulty. The final 3rd generation mAb, TRU-015, is slightly smaller than IgG, has low complement activating ability and is currently under development for RA.

All but one of these mAb (GA101) are so-called Type I mAb; characterized by their ability to redistribute CD20 into Tx-100 insoluble lipid rafts and induce potent CDC,^{29,32,56} unlike Type II mAb which instead induce homotypic adhesion and PCD. GA101 was derived from the murine mAb Bly1 and converted from Type I to Type II during humanization.⁸³ This is the first time that an unconjugated humanized Type II mAb has been investigated clinically (B1/tositumomab the other established Type II is only used as an I¹³¹ radiolabeled format) and it will be extremely interesting to see how it performs compared with both rituximab and the other optimized Type I reagents.

Given the dependency of anti-CD20 mAb on Fc-FcγR interactions, and that both types appear equally effective at binding opsonized targets to macrophages³⁹ and eliciting ADCC⁸⁴, it might be expected that both mAb Types would perform equivalently *in vivo*. However, in xenograft tumor studies³² and more recently syngeneic models of normal B-cell depletion³⁹ (also Beers *et al.*, unpublished observations, 2009) we have observed that Type II mAb are notably more effective. In support of this, pre-clinical studies with GA101 indicate that it outperforms rituximab in a number of assays including *in vivo* xenograft models.^{16,23} We are currently exploring whether the superior performance of Type II mAb is due to their direct cell killing by PCD, their failure to promote CDC when compared with Type I mAb, or other as yet undefined differences between the two classes.

Anti-CD20 mAb in combination therapy

As detailed earlier, the main success of anti-CD20 mAb has been in combination with chemo- or radiotherapy.

Although single-agent rituximab, in patients with relapsed or refractory low-grade NHL demonstrated overall response rates (ORR) of 40-50%, with median time to progression (TTP) of approximately nine months,⁸⁵⁻⁸⁸ combined rituximab and CHOP chemotherapy (R-CHOP) produced a higher ORR of 95%, with median TTP of 82 months.^{89,90} Addition of rituximab to standard front-line chemotherapy regimens significantly improves ORR, CR and OS in low-grade NHL⁹¹⁻⁹³ and newly diagnosed patients with DLBCL.^{94,95}

The mechanism of this synergistic activity is not clear. Demidem *et al.*⁹⁶ showed *in vitro* that a lymphoma cell line that was resistant to some cytotoxic agents could be sensitized by pre-treatment with rituximab, with some evidence of apoptosis.

It is well recognized that anti-apoptotic Bcl-2 is over-expressed in lymphoid malignancies⁹⁷ and a link with chemosensitization by rituximab was first established by Alas *et al.*⁹⁸ who showed that rituximab down-regulated IL-10 in AIDS-related lymphoma cells, where IL-10 is a recognized anti-apoptotic factor, and a promoter of Bcl-2 expression. Further studies showed that rituximab down-

regulated both Bcl-2 and IL-10 expression,⁹⁹ via the p38MAPK signaling pathway.¹⁰⁰

In lymphoma cell lines such as Daudi, Raji and Ramos, a different mechanism appears to be involved. Here, rituximab apparently chemosensitizes cells to drug-induced apoptosis through downregulation of another anti-apoptotic member of the Bcl-2 family, Bcl-xL.¹⁰¹ The expression of Bcl-xL is regulated by nuclear factor κB (NF-κB) and extracellular signal regulated kinase 1/2 (ERK 1/2). *In vitro* experiments with Daudi and Raji cell lines show that rituximab blocks NF-κB and ERK 1/2 signaling, as well as PI3K-Akt activity, leading to reduced Bcl-xL expression.^{102,103}

Our own studies have shown little potentiation of cell death by rituximab and other Type I mAb in combination with radiation. In contrast, we have observed potent additive effects with radiation and Type II mAb⁵⁹ in an ERK-dependent mechanism. This is independent of apoptotic cell death as caspase inhibition and/or Bcl-2 overexpression are unable to block the potentiation, perhaps explaining the potency of ¹³¹I-tositumomab in the treatment of chemoresistant FL (which over-express Bcl-2). Unfortunately, as with the PCD experiments detailed above, these experiments are performed in sensitive, predominantly Burkitt's lymphoma, highly adapted *in vitro* cell lines as opposed to bone fide tumors *in vivo*. We are currently designing novel mouse models to address these questions more appropriately. Despite the efficacy of chemo-immunotherapy, a significant number of patients remain resistant to such combination therapy and so novel combinations are currently being investigated. Many of these make the assumption that resistance arises from a blockade of apoptosis and attempt to overcome this using strategies to down-regulate or block anti-apoptotic proteins in the tumor cells. For example, Vega *et al.*¹⁰⁴ showed that rituximab-resistant cell lines which expressed high levels of Bcl-xL (produced by repeated treatment with rituximab) were sensitized to death by bortezomib (a proteasome inhibitor) and DHMEQ, (a specific inhibitor of NF-κB), an observation correlated with the downregulation of Bcl-xL. As mentioned earlier, Stolz *et al.*⁵⁴ also showed that Bcl-xL was over-expressed in lymphoma lines that were resistant to rituximab-induced cell death, and showed that this could be overcome by combined use of rituximab and the BH3-mimetic ABT-737. Similar activity was seen by combining rituximab AT-101 a less well-defined BH3-mimetic.¹⁰⁵ Whether these approaches will be successful and well-tolerated in the clinic remains to be seen, although at least pre-clinical drug combinations with BH3 mimetics appear extremely promising.¹⁰⁶

Thalidomide and its more potent 2nd generation derivative, lenalidomide are an entirely different class of drugs being explored in combination studies. These immunomodulatory (IMiD) agents possess a multitude of biological effects ranging from modulation of cell-mediated immunity and alteration of cytokine responses through to anti-angiogenic properties.¹⁰⁷ Importantly they display single agent activity in both indolent and aggressive lymphomas.^{108,109} Based on this and their non-overlapping spectrum of activities it was anticipated that IMiD would complement rituximab, and in lymphoma-bearing SCID mice survival was prolonged when lenalidomide was

combined with rituximab.¹¹⁰ However, Lapalombella *et al.*¹¹¹ recently demonstrated that lenalidomide down-regulated CD20 expression in CLL cells, resulting in diminished apoptosis and ADCC, which may in fact reduce its efficacy.

In another approach, Zhao *et al.*¹¹² have combined rituximab with histone deacetylase inhibitors (HDACi). HDACi alter transcription regulation and hence expression of genes involved in cellular differentiation, proliferation and apoptosis.¹¹³ In *in vitro* experiments using lymphoma cell lines, murine models and primary tumor from patients with relapsed B-NHL previously treated with rituximab, Zhao *et al.*¹¹² showed that the combination of an HDACi and rituximab promoted tumor cell apoptosis through enhanced downregulation of Bcl-2 and Bcl-xL via NF- κ B inactivation. Phase II trial data have been reported on the use of single-agent HDACi in relapsed/refractory B-NHL resistant to rituximab, with an ORR of 29%, indicating that these agents may have utility in this context.¹¹⁴

Conclusion

More than a decade after the introduction of rituximab, anti-CD20 mAb have become mainstream treatment for many B-cell disorders. Even so, questions remain as to the

best use of anti-CD20 mAb, optimization of dosing, why its activity is limited as a single agent, and its exact mode of action, both alone and in combination with chemotherapy. In spite of these unresolved issues, there are multiple new anti-CD20 mAb which will soon be reaching clinical practice. These offer numerous advantages over rituximab and it will be extremely interesting to observe how these compare clinically. In addition to the obvious importance to patient treatment, the range of engineered modifications should help to guide our understanding of the critical effector mechanisms used by anti-CD20, allowing us to optimize these reagents even further. It is likely to be some time before we have determined the optimal anti-CD20 mAb for a given disease, a task that becomes more difficult if different diseases or different tissue compartments require different effector mechanisms for optimal B-cell depletion. However, by combining basic cancer cell biology, appropriate *in vivo* models, and well designed clinical trials we hope to be able to address these issues.

Authorship and Disclosures

All authors co-wrote and edited the manuscript.

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