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.

Citation: Lim SH, Beers SA, French RR, Johnson PWM, Glennie MJ, and Cragg MS. Anti-CD20 monoclonal antibodies: historical and future perspectives. Haematologica. 2010; 95:135-143. doi:10.3324/haematol.2008.001628

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. complement-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 assays[35] 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 ideal[25] and animal models of normal B-cell depletion

Funding: SHL is funded by the MRC as a Clinical Research Fellow. MSC is funded by a Fellowship from Leukaemia Research.
Acknowledgments: the authors would like to thank all of the members of the Tenovus Research Laboratory and, in particular, members of the CD20 team, past and present. We also apologize to those authors whose work has not been cited in this review due to space limitations.
Manuscript received on June 1, 2009. Manuscript accepted on July 8, 2009.
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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 (131I)	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*	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

**Small modular immunopharmaceutical (SMIP™) drug composed of human IgG1 Fc and hinge regions (hinge, CH_2, and CH_3) 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 Levy[40] 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 study[46] 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 bodies[47] 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, I^{131}-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:FcγR 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:FcγR interactions are critical for the efficacy of anti-CD20 mAb came from the seminal paper of Clynes and Ravetch[64] 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 FcγRIIa[63] 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:FcγR 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 mice[67] or anti-human CD20 mAb in human CD20 transgenic mice[38] (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 Eμ-Myc

History of anti-CD20 mAb in clinical translation

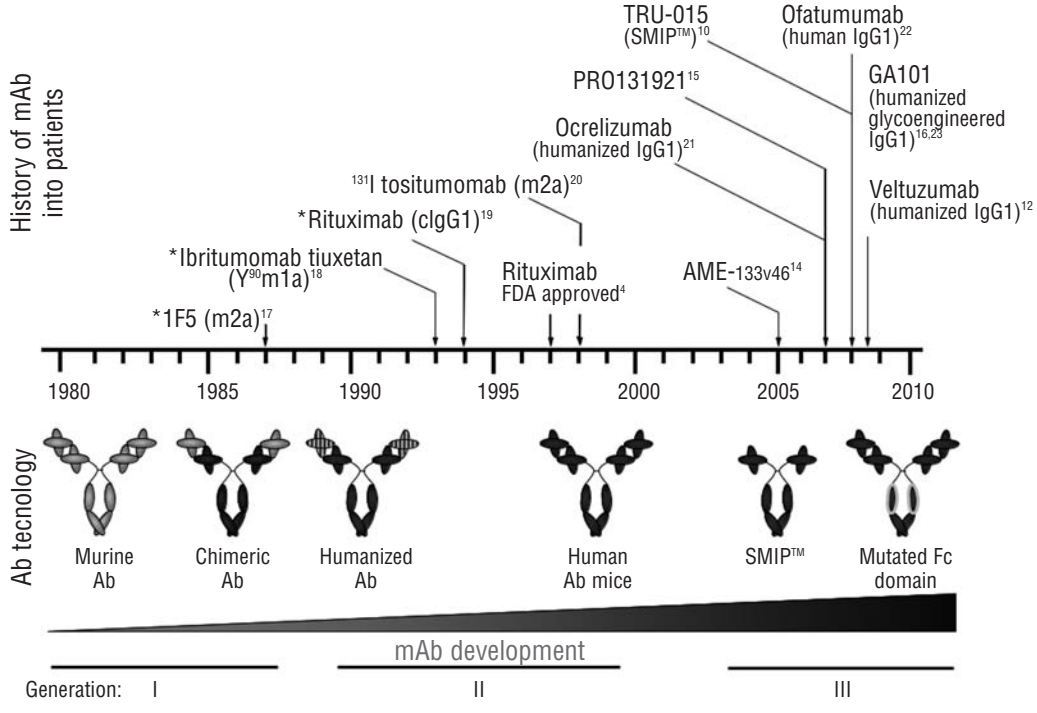

Figure 1. History of CD20 mAb in clinical translation. The timeline describes the chronological introduction over the last three decades of respective anti-CD20 mAb in human trials, as well as the corresponding progress in Ab technology from 1st through to 3rd generation reagents: generation Ab are murine 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 clodronate[69] 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 Küpffer 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 GMCSF 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 GCSF 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 Kroemer[81] 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, veltuzumab 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 I^{131} 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 macrophages[39] and eliciting ADCC[84], it might be expected that both mAb Types would perform equivalently *in vivo*. However, in xenograft tumor studies[32] and more recently syngeneic models of normal B-cell depletion[39] (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 NHL[91-93] 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 overexpressed in lymphoid malignancies[97] 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 mAb[59] 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 ^{131}I-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 proteosome 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-κB 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.

References

1. Fisher RI, Gaynor ER, Dahlberg S, Oken MM, Grogan TM, Mize EM, et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med. 1993; 328(14): 1002-6.
2. Sehn LH, Donaldson J, Chhanabhai M, Fitzgerald C, Gill K, Klasa R, et al. Introduction of combined CHOP plus rituximab therapy dramatically improved outcome of diffuse large B-cell lymphoma in British Columbia. J Clin Oncol. 2005; 23(22):5027-33.
3. Salles GA. Clinical features, prognosis and treatment of follicular lymphoma. Hematology Am Soc Hematol Educ Program. 2007;216-25.
4. Leget GA, Czuczman MS. Use of rituximab, the new FDA-approved antibody. Curr Opin Oncol. 1998;10(6):548-51.
5. Emmanouilides C. Radioimmunotherapy for non-Hodgkin's lymphoma. Semin Oncol. 2003;30(4):531-44.
6. Hagenbeek A, Gadeberg O, Johnson P, Pedersen LM, Walewski J, Hellmann A, et al. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: results of a phase 1/2 trial. Blood. 2008;111(12):5486-95.
7. Osterborg A, Kipps TJ, Mayer J, Stilgenbauer S, Williams CD, Hellmen A, et al. Ofatumumab (HuMax-CD20), a Novel CD20 Monoclonal Antibody, Is An Active Treatment for Patients with CLL Refractory to Both Fludarabine and Alemtuzumab or Bulky Fludarabine-Refractory Disease: Results from the Planned Interim Analysis of An International Pivotal Trial. Blood. 2008; 112 (abstr. 328).
8. Genovese MC, Kaine JL, Lowenstein MB, Giudice JD, Baldassare A, Schechtman J, et al. Ocrelizumab, a humanized anti-CD20 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I/II randomized, blinded, placebo-controlled, dose-ranging study. Arthritis Rheum. 2008;58(9):2652-61.
9. Hayden-Ledbetter MS, Cerveny CG, Espling E, Brady WA, Grosmaire LS, Tan P, et al. CD20-directed small modular immunopharmaceutical, TRU-015, depletes normal and malignant B cells. Clin Cancer Res. 2009;15(8):2739-46.
10. Burge DJ, Bookbinder SA, Kivitz AJ, Fleischmann RM, Shu C, Bannink J. Pharmacokinetic and pharmacodynamic properties of TRU-015, a CD20-directed small modular immunopharmaceutical protein therapeutic, in patients with rheumatoid arthritis: a Phase I, open-label, dose-escalation clinical study. Clin Ther. 2008; 30(10):1806-16.
11. Milani C, Castillo J. Veltuzumab, an anti-CD20 mAb for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and immune thrombocytopenic purpura. Curr Opin Mol Ther. 2009; 11(2):200-7.
12. Morschhauser F, Leonard JP, Fayad L, Coiffier B, Petillon MO, Coleman M, et al. Humanized Anti-CD20 Antibody, Veltuzumab, in Refractory/Recurrent Non-Hodgkin's Lymphoma: Phase I/II Results. J Clin Oncol. 2009; 27(20):3346-53.
13. Bowles JA, Wang SY, Link BK, Allan B, Beuerlein G, Campbell MA, et al. Anti-CD20 monoclonal antibody with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. Blood. 2006; 108(8):2648-54.
14. Weiner GJ, Bowles JA, Link BK, Campbell MA, Wooldridge JE, Breitmeyer BJ. Anti-CD20 monoclonal antibody (mAb) with enhanced affinity for CD16 activates NK cells at lower concentrations and more effectively than rituximab. Blood. 2005; 106 (abstr. 348).
15. A Trial of the Safety of Escalating Doses of PRO131921 in Patients With Relapsed or Refractory Indolent Non-Hodgkin's Lymphoma. 2009. (Accessed 20 May 2009, at http://clinicaltrials.gov/ct2/show/NCT00452127?term=PRO131921&rank=2.)
16. Salles GA, Morschhauser F, Cartron G, Lamy T, Milpied NL, Thieblemont C, et al. A Phase I/II study of RO5072759 (GA101) in Patients with Relapsed/Refractory CD20+ Malignant Disease. Blood. 2008; 112:93 (abstr. 234)
17. Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P, et al. Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood. 1987;69(2):584-91.
18. Kaminski MS, Zasadny KR, Francis IR, Milik AW, Ross CW, Moon SD, et al. Radioimmunotherapy of B-cell lymphoma with [131I]anti-B1 (anti-CD20) antibody. N Engl J Med. 1993;329(7):459-65.
19. Maloney DG, Liles TM, Czerwinski DK, Waldichuk C, Rosenberg J, Grillo-Lopez A, et al. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. Blood. 1994;84(8):2457-66.
20. Stagg R, Wahl RL, Estes J, Tidmarsh G, Kroll S, Shochat D, et al. Phase I/II study of iodine-131 anti-B1 antibody for non-Hodgkin's lymphoma (NHL): Final results. Proc Am Soc Clin Oncol. 1998;17:39a (abstr 150).
21. Morschhauser F, Marlton P, Vitolo U, Linden O, Seymour J, Crump M, et al. Interim Results of a Phase I/II Study of

Ocrelizumab, a New Humanised Anti-CD20 Antibody in Patients with Relapsed/Refractory Follicular Non-Hodgkin's Lymphoma. Blood. 2007;110 (11) (abstr. 645).
22. Coiffier B, Lepretre S, Pedersen LM, Gadeberg O, Fredriksen H, van Oers MH, et al. Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1-2 study. Blood. 2008;111(3):1094-100.
23. Dalle S, Reslan L, Manquat SB, Herting F, Klein C, Umana P, et al. Compared Antitumor Activity of GA101 and Rituximab against the Human RL Follicular Lymphoma Xenografts in SCID Beige Mice. Blood. 2008;112(11) (abstr. 1585)
24. Nimmerjahn F, Ravetch JV. Antibodies, Fc receptors and cancer. Curr Opin Immunol. 2007;19(2):239-45.
25. Glennie MJ, French RR, Cragg MS, Taylor RP. Mechanisms of killing by anti-CD20 monoclonal antibodies. Mol Immunol. 2007;44(16):3823-37.
26. Cragg MS, Walshe CA, Ivanov AO, Glennie MJ. The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun. 2005;8:140-74.
27. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood. 1994; 83(2):435-45.
28. Golay J, Zaffaroni L, Vaccari T, Lazzari M, Borleri GM, Bernasconi S, et al. Biologic response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab in vitro: CD55 and CD59 regulate complement-mediated cell lysis. Blood. 2000; 95(12):3900-8.
29. Cragg MS, Morgan SM, Chan HT, Morgan BP, Filatov AV, Johnson PW, et al. Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. Blood. 2003;101(3):1045-52.
30. Teeling JL, French RR, Cragg MS, van den Brakel J, Pluyter M, Huang H, et al. Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. Blood. 2004;104(6):1793-800.
31. Teeling JL, Mackus WJ, Wiegman LJ, van den Brakel JH, Beers SA, French RR, et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. J Immunol. 2006; 177(1):362-71.
32. Cragg MS, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. Blood. 2004;103(7): 2738-43.
33. Treon SP, Mitsiades C, Mitsiades N, Young G, Doss D, Schlossman R, et al. Tumor cell expression of CD59 is associated with resistance to CD20 serotherapy in patients with B-cell malignancies. J Immunother (1991). 2001;24(3):263-71.
34. Bannerji R, Kitada S, Flinn IW, Pearson M, Young D, Reed JC, et al. Apoptotic-regulatory and complement-protecting protein expression in chronic lymphocytic leukemia: relationship to in vivo rituximab resistance. J Clin Oncol. 2003;21(8):1466-71.
35. Kennedy AD, Beum PV, Solga MD, DiLillo DJ, Lindorfer MA, Hess CE, et al. Rituximab infusion promotes rapid complement depletion and acute CD20 loss in chronic lymphocytic leukemia. J Immunol. 2004;172(5):3280-8.
36. Klepfish A, Schattner A, Ghoti H, Rachmilewitz EA. Addition of fresh frozen plasma as a source of complement to rituximab in advanced chronic lymphocytic leukaemia. Lancet Oncol. 2007;8(4):361-2.
37. Golay J, Cittera E, Di Gaetano N, Manganini M, Mosca M, Nebuloni M, et al. The role of complement in the therapeutic activity of rituximab in a murine B lymphoma model homing in lymph nodes. Haematologica. 2006;91(2):176-83.
38. Gong Q, Ou Q, Ye S, Lee WP, Cornelius J, Diehl L, et al. Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. J Immunol. 2005;174(2):817-26.
39. Beers SA, Chan CH, James S, French RR, Attfield KE, Brennan CM, et al. Type II (tositumomab) anti-CD20 monoclonal antibody out performs type I (rituximab-like) reagents in B-cell depletion regardless of complement activation. Blood. 2008; 112(10):4170-7.
40. Weng WK, Levy R. Expression of complement inhibitors CD46, CD55, and CD59 on tumor cells does not predict clinical outcome after rituximab treatment in follicular non-Hodgkin lymphoma. Blood. 2001; 98(5):1352-7.
41. Taylor R. Fresh frozen plasma as a complement source. Lancet Oncol. 2007;8(5):370-1.
42. Li Y, Williams ME, Cousar JB, Pawluczkowycz AW, Lindorfer MA, Taylor RP. Rituximab-CD20 complexes are shaved from Z138 mantle cell lymphoma cells in intravenous and subcutaneous SCID mouse models. J Immunol. 2007; 179(6):4263-71.
43. Beum PV, Kennedy AD, Williams ME, Lindorfer MA, Taylor RP. The shaving reaction: rituximab/CD20 complexes are removed from mantle cell lymphoma and chronic lymphocytic leukemia cells by THP-1 monocytes. J Immunol. 2006;176 (4):2600-9.
44. Williams ME, Densmore JJ, Pawluczkowycz AW, Beum PV, Kennedy AD, Lindorfer MA, et al. Thrice-weekly low-dose rituximab decreases CD20 loss via shaving and promotes enhanced targeting in chronic lymphocytic leukemia. J Immunol. 2006;177(10):7435-43.
45. Wang SY, Racila E, Taylor RP, Weiner GJ. NK-cell activation and antibody-dependent cellular cytotoxicity induced by rituximab-coated target cells is inhibited by the C3b component of complement. Blood. 2008;111(3):1456-63.
46. Racila E, Link BK, Weng WK, Witzig TE, Ansell S, Maurer MJ, et al. A polymorphism in the complement component C1qA correlates with prolonged response following rituximab therapy of follicular lymphoma. Clin Cancer Res. 2008;14(20): 6697-703.
47. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med. 2001; 194(6):781-95.
48. Nauta AJ, Castellano G, Xu W, Woltman AM, Borrias MC, Daha MR, et al. Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. J Immunol. 2004;173(5): 3044-50.
49. Castellano G, Woltman AM, Schlagwein N, Xu W, Schena FP, Daha MR, et al. Immune modulation of human dendritic cells by complement. Eur J Immunol. 2007; 37(10):2803-11.
50. Tedder TF, Forsgren A, Boyd AW, Nadler LM, Schlossman SF. Antibodies reactive with the B1 molecule inhibit cell cycle progression but not activation of human B lymphocytes. Eur J Immunol. 1986;16(8): 881-7.
51. Shan D, Ledbetter JA, Press OW. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. Blood. 1998;91(5):1644-52.
52. Johnson PW, Glennie MJ. Rituximab: mechanisms and applications. Br J Cancer. 2001;85(11):1619-23.
53. Kennedy AD, Solga MD, Schuman TA, Chi AW, Lindorfer MA, Sutherland WM, et al. An anti-C3b(i) mAb enhances complement activation, C3b(i) deposition, and killing of CD20+ cells by rituximab. Blood. 2003; 101(3):1071-9.
54. Stolz C, Hess G, Hahnel PS, Grabellus F, Hoffarth S, Schmid KW, et al. Targeting Bcl-2 family proteins modulates the sensitivity of B-cell lymphoma to rituximab-induced apoptosis. Blood 2008;112(8): 3312-21.
55. Eeva J, Nuutinen U, Ropponen A, Mättö M, Eray M, Pellinen R. et al. The involvement of mitochondria and the caspase-9 activation pathway in rituximab-induced apoptosis in FL cells. Apoptosis. 2009; 14(5):687-98.
56. Chan HT, Hughes D, French RR, Tutt AL, Walshe CA, Teeling JL, et al. CD20-induced lymphoma cell death is independent of both caspases and its redistribution into triton X-100 insoluble membrane rafts. Cancer Res. 2003; 63(17):5480-9.
57. Byrd JC, Kitada S, Flinn IW, Aron JL, Pearson M, Lucas D, et al. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 2002;99(3):1038-43.
58. Egle A, Harris AW, Bath ML, O'Reilly L, Cory S. VavP-Bcl2 transgenic mice develop follicular lymphoma preceded by germinal center hyperplasia. Blood. 2004;103(6): 2276-83.
59. Ivanov A, Krysov S, Cragg MS, Illidge T. Radiation therapy with tositumomab (B1) anti-CD20 monoclonal antibody initiates extracellular signal-regulated kinase/mitogen-activated protein kinase-dependent cell death that overcomes resistance to apoptosis. Clin Cancer Res. 2008;14(15): 4925-34.
60. Ivanov A, Beers SA, Walshe CA, Honeychurch J, Alduaij W, Cox KL, et al. Monoclonal antibodies directed to CD20 and HLA-DR can elicit homotypic adhesion followed by lysososme-mediated cell death in human lymphoma and leukemia cells. J Clin Invest. 2009;119(8):2143-59.
61. Horning SJ, Younes A, Jain V, Kroll S, Lucas J, Podoloff D, et al. Efficacy and safety of tositumomab and iodine-131 tositumomab (Bexxar) in B-cell lymphoma, progressive after rituximab. J Clin Oncol. 2005; 23(4): 712-9.
62. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood. 2002;99(3):754-8.
63. Weng WK, Levy R. Two immunoglobulin

G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol. 2003;21(21):3940-7.
64. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med. 2000; 6(4):443-6.
65. Treon SP, Hansen M, Branagan AR, Verselis S, Emmanouilides C, Kimby E, et al. Polymorphisms in FcγRIIIA (CD16) receptor expression are associated with clinical response to rituximab in Waldenstrom's macroglobulinemia. J Clin Oncol. 2005;23(3):474-81.
66. Farag SS, Flinn IW, Modali R, Lehman TA, Young D, Byrd JC. Fc gamma RIIIa and Fc gamma RIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia. Blood. 2004;103(4): 1472-4.
67. Uchida J, Hamaguchi Y, Oliver JA, Ravetch JV, Poe JC, Haas KM, et al. The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med. 2004;199(12):1659-69.
68. Minard-Colin V, Xiu Y, Poe JC, Horikawa M, Magro CM, Hamaguchi Y, et al. Lymphoma depletion during CD20 immunotherapy in mice is mediated by macrophage FcgammaRI, FcγRIII, and FcgammaRIV. Blood. 2008;112(4):1205-13.
69. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J Immunol Methods. 1994;174(1-2):83-93.
70. Golay J, Manganini M, Facchinetti V, Gramigna R, Broady R, Borleri G, et al. Rituximab-mediated antibody-dependent cellular cytotoxicity against neoplastic B cells is stimulated strongly by interleukin-2. Haematologica. 2003;88(9):1002-12.
71. Cartron G, Ohresser M, Salles G, Solal-Celigny P, Colombat P, Watier H. Neutrophil role in in vivo anti-lymphoma activity of rituximab: FCGR3B-NA1/NA2 polymorphism does not influence response and survival after rituximab treatment. Ann Oncol. 2008;19(8):1485-7.
72. Cartron G, Zhao-Yang L, Baudard M, Kanouni T, Rouillé V, Quittet P, et al. Granulocyte-macrophage colony-stimulating factor potentiates rituximab in patients with relapsed follicular lymphoma: results of a phase II study. J Clin Oncol. 2008; 26(16):2725-31.
73. Shibata-Koyama M, Iida S, Misaka H, Mori K, Yano K, Shitara K, et al. Nonfucosylated rituximab potentiates human neutrophil phagocytosis through its high binding for FcgammaRIIIb and MHC class II expression on the phagocytotic neutrophils. Exp Hematol. 2009; 37(3):309-21.
74. Shibata-Koyama M, Iida S, Okazaki A, Mori K, Kitajima-Miyama K, Saitou S, et al. The N-linked oligosaccharide at Fc gamma RIIIa Asn-45: an inhibitory element for high Fc gamma RIIIa binding affinity to IgG glycoforms lacking core fucosylation. Glycobiology. 2009;19(2):126-34.
75. Cartron G, Watier H, Golay J, Solal-Celigny P. From the bench to the bedside: ways to improve rituximab efficacy. Blood. 2004;104(9):2635-42.
76. Selenko N, Majdic O, Jager U, Sillaber C, Stockl J, Knapp W. Cross-priming of cytotoxic T cells promoted by apoptosis-inducing tumor cell reactive antibodies? J Clin Immunol. 2002;22(3):124-30.
77. Rafiq K, Bergtold A, Clynes R. Immune complex-mediated antigen presentation induces tumor immunity. J Clin Invest. 2002;110(1):71-9.
78. Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. J Exp Med. 2002;195(12): 1653-9.
79. Hilchey SP, Hyrien O, Mosmann TR, Livingstone AM, Friedberg JW, Young F, et al. Rituximab immunotherapy results in the induction of a lymphoma idiotype-specific T-cell response in patients with follicular lymphoma: support for a "vaccinal effect" of rituximab. Blood. 2009;113(16): 3809-12.
80. Taylor C, Hershman D, Shah N, Suciu-Foca N, Petrylak DP, Taub R, et al. Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. Clin Cancer Res. 2007; 13(17):5133-43.
81. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008;8(1):59-73.
82. Haynes NM, van der Most RG, Lake RA, Smyth MJ. Immunogenic anti-cancer chemotherapy as an emerging concept. Curr Opin Immunol. 2008;20(5):545-57.
83. Umana P, Moessner E, Bruenker P, Unsin G, Puentener U, Suter T, et al. Novel 3rd Generation Humanized Type II CD20 Antibody with Glycoengineered Fc and Modified Elbow Hinge for Enhanced ADCC and Superior Apoptosis Induction. Blood. 2006;108:(abstr.229).
84. Cragg MS, Asidipour A, O'Brien L, Tutt A, Chan HTC, Anderson VA, et al. Opposing properties of CD20 mAb. In: Leukocyte Typing VII. Oxford: Oxford University Press; 2002:95-7.
85. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol. 1998;16(8):2825-33.
86. Foran JM, Gupta RK, Cunningham D, Popescu RA, Goldstone AH, Sweetenham JW, et al. A UK multicentre phase II study of rituximab (chimaeric anti-CD20 monoclonal antibody) in patients with follicular lymphoma, with PCR monitoring of molecular response. Br J Haematol. 2000; 109(1):81-8.
87. Feuring-Buske M, Kneba M, Unterhalt M, Engert A, Gramatzki M, Hiller E, et al. IDEC-C2B8 (Rituximab) anti-CD20 antibody treatment in relapsed advanced-stage follicular lymphomas: results of a phase-II study of the German Low-Grade Lymphoma Study Group. Ann Hematol. 2000;79(9):493-500.
88. Davis TA, White CA, Grillo-Lopez AJ, Velásquez WS, Link B, Maloney DG, et al. Single-agent monoclonal antibody efficacy in bulky non-Hodgkin's lymphoma: results of a phase II trial of rituximab. J Clin Oncol. 1999;17(6):1851-7.
89. Czuczman MS, Grillo-Lopez AJ, White CA, Saleh M, Gordon L, LoBuglio AF, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol. 1999;17(1):268-76.
90. Czuczman MS, Weaver R, Alkuzweny B, Berlfein J, Grillo-Lopez AJ. Prolonged clinical and molecular remission in patients with low-grade or follicular non-Hodgkin's lymphoma treated with rituximab plus CHOP chemotherapy: 9-year follow-up. J Clin Oncol. 2004;22(23):4711-6.
91. Marcus R, Imrie K, Belch A, Cunningham D, Flores E, Catalano J, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood. 2005;105(4):1417-23.
92. Hiddemann W, Kneba M, Dreyling M, Schmitz N, Lengfelder E, Schmits R, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood. 2005;106(12):3725-32.
93. Herold M, Haas A, Srock S, Neser S, Al-Ali KH, Neubauer A, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. J Clin Oncol. 2007;25(15):1986-92.
94. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(4):235-42.
95. Pfreundschuh M, Trumper L, Osterborg A, Pettengell R, Trneny M, Imrie K, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol. 2006;7(5):379-91.
96. Demidem A, Lam T, Alas S, Hariharan K, Hanna N, Bonavida B. Chimeric anti-CD20 (IDEC-C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs. Cancer Biother Radiopharm. 1997;12(3):177-86.
97. Yang E, Korsmeyer SJ. Molecular thanatopsis: a discourse on the BCL2 family and cell death. Blood. 1996;88(2):386-401.
98. Alas S, Emmanouilides C, Bonavida B. Inhibition of interleukin 10 by rituximab results in down-regulation of bcl-2 and sensitization of B-cell non-Hodgkin's lymphoma to apoptosis. Clin Cancer Res. 2001;7(3):709-23.
99. Alas S, Bonavida B. Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl-2 and sensitization to cytotoxic drugs. Cancer Res. 2001;61(13):5137-44.
100. Vega MI, Huerta-Yepaz S, Garban H, Jazirehi A, Emmanouilides C, Bonavida B. Rituximab inhibits p38 MAPK activity in 2F7 B NHL and decreases IL-10 transcription: pivotal role of p38 MAPK in drug resistance. Oncogene. 2004;23(20):3530-40.
101. Jazirehi AR, Gan XH, De Vos S, Emmanouilides C, Bonavida B. Rituximab (anti-CD20) selectively modifies Bcl-xL and apoptosis protease activating factor-1

(Apaf-1) expression and sensitizes human non-Hodgkin's lymphoma B cell lines to paclitaxel-induced apoptosis. Mol Cancer Ther. 2003;2(11):1183-93.
102. Jazirehi AR, Bonavida B. Cellular and molecular signal transduction pathways modulated by rituximab (rituxan, anti-CD20 mAb) in non-Hodgkin's lymphoma: implications in chemosensitization and therapeutic intervention. Oncogene. 2005; 24(13):2121-43.
103. Suzuki E, Umezawa K, Bonavida B. Rituximab inhibits the constitutively activated PI3K-Akt pathway in B-NHL cell lines: involvement in chemosensitization to drug-induced apoptosis. Oncogene. 2007;26(42):6184-93.
104. Vega MI, Martinez-Paniagua M, Jazirehi AR, Huerta-Yepez S, Umezawa K, Martinez-Maza O, et al. The NF-kappaB inhibitors (bortezomib and DHMEQ) sensitise rituximab-resistant AIDS-B-non-Hodgkin lymphoma to apoptosis by various chemotherapeutic drugs. Leuk Lymphoma. 2008;49(10):1982-94.
105. Paoluzzi L, Gonen M, Gardner JR, Mastrella J, Yang D, Holmlund J, et al. Targeting Bcl-2 family members with the BH3 mimetic AT-101 markedly enhances the therapeutic effects of chemotherapeutic agents in in vitro and in vivo models of B-cell lymphoma. Blood. 2008;111(11): 5350-8.
106. Cragg MS, Harris C, Strasser A, Scott CL. Unleashing the power of inhibitors of oncogenic kinases through BH3 mimetics. Nat Rev Cancer. 2009;9(5):321-6.
107. Dredge K, Marriott JB, Dalgleish AG. Immunological effects of thalidomide and its chemical and functional analogs. Crit Rev Immunol. 2002;22(5-6):425-37.
108. Habermann TM, Lossos IS, Justice G, Vose JM, Wiernik PH, McBride K, et al. Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma. Br J Haematol. 2009; 145(3):344-9.
109. Wiernik PH, Lossos IS, Tuscano JM, Justice G, Vose JM, Cole CE, et al. Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2008;26(30):4952-7.
110. Hernandez-Ilizaliturri FJ, Reddy N, Holkova B, Ottman E, Czuczman MS. Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model. Clin Cancer Res. 2005;11(16):5984-92.
111. Lapalombella R, Yu B, Triantafillou G, Liu Q, Butchar JP, Lozanski G, et al. Lenalidomide down-regulates the CD20 antigen and antagonizes direct and antibody-dependent cellular cytotoxicity of rituximab on primary chronic lymphocytic leukemia cells. Blood. 2008;112(13):5180-9.
112. Zhao WL, Wang L, Liu YH, Yan JS, Leboeuf C, Liu YY, et al. Combined effects of histone deacetylase inhibitor and rituximab on non-Hodgkin's B-lymphoma cells apoptosis. Exp Hematol. 2007;35(12):1801-11.
113. Nolan L, Johnson PW, Ganesan A, Packham G, Crabb SJ. Will histone deacetylase inhibitors require combination with other agents to fulfil their therapeutic potential? Br J Cancer. 2008;99(5):689-94.
114. Kirschbaum M, Popplewell L, Nademanee AP, Pullarkat V, Delioukina M, Zain JM, et al. A Phase 2 Study of Vorinostat (Suberoylanilide Hydroxamic Acid, SAHA) in Relapsed or Refractory Indolent Non-Hodgkin's Lymphoma. A California Cancer Consortium Study. Blood. 2008; 112 (abstr. 1564).