Resistance to Targeted Anti-Cancer Therapeutics 2

Benjamin Bonavida
Editor

Resistance to Immunotherapeutic Antibodies in Cancer

Strategies to Overcome Resistance

Springer

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Volume 2

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ISSN 2196-5501 ISSN 2196-551X (electronic)
ISBN 978-1-4614-7653-5 ISBN 978-1-4614-7654-2 (eBook)
DOI 10.1007/978-1-4614-7654-2
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013943719

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

A new era of anti-cancer therapeutics has emerged with significant objective clinical responses, prolongation of survivals, and even cures. These have been the result of the successful introduction of monoclonal antibodies (mAbs) directed against surface-bound membrane antigens on cancer cells. More than 20 mAbs have been approved for human use targeting a range of different cancers. The observed successes achieved by such antibodies against cancers stem from their high levels of specificity, long biological half lives, ability to recruit host effector cells, synergy with conventional drugs, and minimal toxicity. However, a major drawback of mAbs therapeutics, like any other therapeutics, is that a subset of patients does not initially respond and another initially responding subset develops resistance to further treatments. At the present time, there are no effective therapies for these subsets of cancer patients. Clearly, it is imperative that analyses of underlying mechanisms responsible for resistance will be required to develop and generate new targeted therapies that overcome the resistance. In addition, it will be possible to determine a priori whether a patient will be susceptible to response or not and which will allow oncologists to make proper decisions for treatment of the cancer patient at the individual level.

This volume titled, *Resistance to Immunotherapeutic Antibodies in Cancer: Strategies to Overcome Resistance* has been developed with the objective of highlighting up-to-date information on several investigations that deal with various mechanisms of resistance to anti-cancer mAbs therapeutics as well as those that deal with novel approaches to overcome resistance. The reviews in this volume are written by highly qualified, established, and experienced leaders in the field of resistance to anti-cancer mAbs.

This volume consists of 10 reviews that cover a wide range of topics on resistance. A summary highlighting each chapter is briefly presented. Dr. Dumontet's review titled, *Resistance to Anticancer Antibodies: From Mechanism to Solutions* discusses the importance of finding closed links between preclinical observations and the clinic. These links are imperative to unravel various mechanisms of resistance for the benefit of the patients. He raises the important point that not a simple mechanism of resistance will be found in a cancer patient type but multiple mechanisms will work in concert, due primarily to the heterogeneity of the cancer in question. As an example, he discusses $HER2^{+}$ breast cancer response to

trastuzumab and how to identify both biomarkers to predict an optimal response and gene products that regulate resistance for novel targeted therapies. Dr. Ferrone's review titled, *Tumor Antigen-Specific Monoclonal Antibody-Based Immunotherapy, Cancer Initiating Cells and Disease Recurrence* discusses an important facet of resistance, namely, the intrinsic resistance of a small subgroup of cancer initiating cells (CICs) that are primarily responsible for mAb resistance, relapse, and metastasis. He presents several examples of mAbs that do not affect CICs, however, the use of combination therapies with drugs/radiation and inhibitors of the CIC signaling pathways resulted in significant killing of CICs but not all. He suggests the development of additional targeted therapies and combination to completely eliminate CICs. Dr. Cragg's review titled, *Overcoming Resistance to Therapeutic Antibodies by Targeting Fc Receptors* discusses the clinical finding demonstrating the important role of Fc gamma receptors (FcγRs) polymorphism and response to mAb therapeutics. Due to the number of various FcRs with activating or inhibitory functions, their biology is very complex indeed. Noteworthy, while the role of FcγRs expression by cytotoxic effector cells was primarily reported for the observed polymorphism, however, new findings show that FcRs on both the cancer cell and the effector cell participate in determining the therapeutic efficacy of the monoclonal antibody in question. Several novel strategies are provided to circumvent the unresponsiveness of resistance with the aim to develop more successful mAb therapeutics. Dr. Hernandez-Ilizaliturri and Dr. Czuczman's review titled, *Understanding the Mechanisms of Resistance to Rituximab: Paving the Road for the Development of Therapeutic Strategies to Overcome Rituximab Resistance* discusses the clinical problem of cancer patients resistance to rituximab (antiCD20 mAb) therapy. They discuss several reported mechanisms of resistance that have been observed in cancer patients, including surface receptors and intracellular hyperactivated survival pathways. Their approach to determine potential underlying mechanisms of rituximab-resistance has been to develop preclinically rituximab-resistance lymphoma cell lines. These have been analyzed for their therapeutic phenotypes and molecular properties compared to the parental wild-type cells. Such approaches are clearly important to identify new biomarkers of resistance for both prognostic and novel therapeutics. Dr. Bonavida's review titled, *Tumor Resistance to Antibody-Mediated Immunotherapy and Reversal of Resistance: Rituximab as Prototype* discusses several studies that investigated cell-mediated signaling by rituximab on B-Non-Hodgkin's lymphoma cell lines and which demonstrated the inhibition of several intracellular pathways (example NFκB, p38 MAPK, Raf/ERK/MEK, and PI3K/Akt) leading to inhibition of cell growth and inhibition of anti-apoptotic gene products. In addition, this review discusses the chemo-immunosensitization-mediated by rituximab when used in combination with chemo-immunotherapeutic drugs and various mechanisms of sensitization. Like the above studies by Dr. Hernandez-Ilizaliturri and Dr. Czuczman, the potential mechanism of rituximab resistance has been analyzed by generating rituximab-resistant clones in vitro and their general molecular profiles were compared to wild-type cells. While the resistant clones were unresponsive to rituximab treatment alone or in combination with drugs, however,

intracellular intervention inhibiting the hyper-activated survival pathways by various inhibitors resulted in the reversal resistance to cytotoxic drugs. The analysis with the resistance clones yielded several candidate targets of potential prognostic and therapeutic values. Doctors Saridaki and Souglakos's review titled, *Resistance to the Anti-EGFR Therapy, Beyond KRAS, in Patients with Metastatic Colorectal Cancer* discuss the role of mutation profiles in the treatment decision in patients with metastatic colorectal cancer. They critically reviewed the underlying mechanisms of resistance to anti-EGFR mAbs and their relationship to various mutations. The reported studies are aimed to identify novel biomarkers that may be useful to select cancer patients who will respond favorably to anti-EGFR mAbs. Dr. Hersey and colleagues' review titled, *Overcoming Resistance of Melanoma to Immunotherapy with Monoclonal Antibodies Against Checkpoint Inhibitors* discusses the poor clinical response in melanoma patients following treatment with monoclonal antibodies against checkpoint inhibitors on T cells such as Ipilimumab (anti-CTLA-4) and PD1 (programmed death receptor-1). They discuss various mechanisms of resistance to immunotherapy including changes in the microenvironment, regulation of T-cells infiltration into melanoma tumors and suggest mechanisms to augment T-cell infiltration into the tumors. They also discuss the important role of NF-κB activation as a key regulator of anti-tumor immune resistance. Dr. Fulda's review titled, *Strategies to Overcome TRAIL Resistance in Cancer* discusses the mechanism that underlies the resistance of cancer cells to TRAIL/agonist antibodies directed against TRAIL receptors DR4 or DR5 currently under clinical investigation. She discusses several mechanisms conferring resistance to TRAIL such as the impairment of various members of the TRAIL signaling apoptotic pathways. These include signaling by death and decoy receptors that result in both the activation and the inhibition of apoptosis, the aberrant expression of anti-apoptotic gene products and the regulation of caspases. She implies that a better understanding of the mechanisms that regulate the sensitivity to resistance to TRAIL-apoptosis should lead to the successful application of TRAIL and agonist monoclonal antibodies as new therapeutics in the treatment of cancer.

The above chapters discuss several limitations by the use of therapeutic monoclonal antibodies. The next two chapters discuss the new engineered monoclonal antibody-conjugates as the new generation of antibody therapy. Dr. Smider's review titled, *Unnatural Amino Acid Antibody Conjugates as Next Generation Biologics* discusses the first approved monoclonal antibody against solid tumors, namely, trastuzumab (anti-HERT2$^+$ mAb; herceptin) in 1998 for the treatment of HERT2$^+$ overexpressing metastastatic breast cancer. He reviews several mechanisms of resistance to trastuzumab. He discusses the use of novel antibody-conjugates as novel therapies to overcome resistance. For example, unnatural amino acids were used to create the site specifically linking protein–protein dimers, such as antibody-toxin conjugates and bispecific antibodies. The antibody-drug conjugate, trastuzumab-DM1, has shown biological activity and clinical efficacy in HERT2$^+$ breast cancer and other applications have also been discussed. Dr. Rabuka's review titled, *Antibody-Drug Conjugates: Can Coupling Cytotoxicity and Specificity Overcome Therapeutic Resistance?* discusses the

exquisite selected antibody-drug conjugates (ADCs) for the target antigen and that kill cells at very low concentrations with little effect on normal tissues. This review presents the general properties of ADCs and their mode of action and how they can revert resistance to antibody therapeutics. The development of Mylotarg, gemtuzumab ozogamicin, was approved in 2000 and consists of an anti-CD33 mAb conjugated with a DNA-damaging agent, calicheamicin, for the treatment of $CD33^{+}$ leukemia. There are currently 20 new ADCs in clinical studies that should provide information about their therapeutic efficacy and their ability to reverse resistance.

I wish to acknowledge the assistance of Daphne Liang, Melissa Cao, Kathy Nguyen, and Suzie Vardanyan for their valuable assistance in the preparation and editing the various contributions in this volume.

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Resistance to Anticancer Antibodies: From Mechanisms to Solutions

Lina Reslan and Charles Dumontet

Abstract Therapeutic monoclonal antibodies exert their antitumor effect through a variety of mechanisms, including apoptotic induction, extracellular mechanisms and the involvement of the innate and possibly the adaptative immune systems. Due to this complexity there are still few data regarding mechanisms of resistance to monoclonal antibody therapy. In this review, we discuss the available data for three of the best described antibodies, rituximab, trastuzumab and cetuximab. A variety of approaches and strategies has been suggested or are currently being tested to circumvent resistance to these antibodies.

Keywords Monoclonal antibodies · Rituximab · Trastuzumab · Cetuximab · Resistance

Abbreviations

ADCs	Antibody–drug conjugates
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCP	Antibody-dependent cellular phagocygtosis
CDC	Complement-dependent cytotoxicity
CLL	Chronic lymphocytic leukemia
CRP	Complement regulatory proteins

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B. Bonavida (ed.), *Resistance to Immunotherapeutic Antibodies in Cancer*,
Resistance to Targeted Anti-Cancer Therapeutics 2, DOI: 10.1007/978-1-4614-7654-2_1,

DLBCL	Diffuse large B cell lymphoma
EGFR	Epidermal growth factor receptor
FcR	Fc receptor
FL	Follicular lymphoma
HER2	Human epidermal growth factor
HGF	Hepatocyte growth factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IFN	Interferon
IL	Interleukin
IGF-1	Insulin-like growth factor 1
MAbs	Monoclonal antibodies
mTor	Mammalian target of rapamycin
MUC4	Mucin-4
NHL	Non-hodgkin lymphoma
NK	Natural killer
OS	Overall survival
PCD	Programmed cell death
PFS	Progression-free survival
RTK	Receptor tyrosine kinase
TKI	Tyrosine kinase inhibitor
VEGF	Vascular endothelial growth factor

Introduction

Anticancer immunotherapy in 2012 is still largely based on the production of monoclonal antibodies (MAbs) that bind with high specificity to secreted proteins or to the extracellular domain of membrane-bound proteins. The principle of the MAbs is to target molecules that are expressed at higher levels on neoplastic cells, with a lower expression on normal cells.

MAbs achieve their therapeutic effects through various mechanisms. The specific binding of the antibody to its target prevents the binding of ligand-receptor interaction, by blocking growth factor receptors, neutralizing the target antigen, disrupting or promoting receptor internalization, shedding of the extracellular portion of the receptor, or induction of apoptosis. In addition, evidence has shown that activation of the innate immune response against the targeted tumor cells, upon recognition of the bound antibody, can also account for their biological activity to induce complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). More recently, it has been shown that the full effect of *MAbs* may also involve an adaptative immune response.

Unfortunately, in most tumor types, tumors either possess an intrinsic resistance to targeted therapies or acquire resistance after having displayed an initial

response. In both cases, patients eventually succumb to disease progression. Therefore, understanding resistance mechanisms will benefit patients in several ways. Patients more likely to respond to a specific targeted therapy would be selected on the basis of markers of response and resistance, thus allowing oncologists to make earlier decisions for treatment with more effective therapies. Patients with highly refractory disease could be oriented early on to novel experimental therapies. Furthermore, understanding novel mechanisms of tumor growth and resistance will contribute to novel therapeutic strategies or agents.

Information obtained from cellular models and relapsed patients have provided little knowledge on how cells adapt to the treatment, whether by reducing the expression or modifying the structure of the target protein or by activating alternative survival pathways. A better understanding of the functional interactions occurring within the target and the corresponding antibody is essential to efficiently target the individual tumor and to select appropriate patients for therapy, thereby maximizing drug efficacy and minimizing toxicity. Additionally, a deeper understanding of the role of the microenvironment will be decisive in improving the use of therapeutic MAbs.

Three MAbs, rituximab, trastuzumab and cetuximab, have been considered as pioneers in their abilities to change the landscape of different malignancies. In this chapter, we will provide the readers with recent mechanisms of resistance as well as novel potential therapies that will help in circumventing these mechanisms.

Rituximab

The introduction of rituximab in the management of B cell non-Hodgkin's lymphoma (NHL) as well as in Chronic Lymphocytic Leukemia (CLL) has improved the response rate, progression-free survival (PFS), and overall survival (OS) of patients [1]. Rituximab is routinely administered during all phases of conventional treatment, including first-line therapy, maintenance as well as salvage therapy. Although the clinical data testing its efficacy have shown variable response rates in different CD20+ malignancies [2], its effectiveness is sometimes unsatisfactory since a significant percentage of patients treated with rituximab-containing chemotherapy show relapse or progression [3]. The development of rituximab resistance is an emerging clinical problem. However, the mechanisms underlying the resistance to rituximab are mostly unresolved and the clinical significance of those mechanisms has remained obscure [4].

Rituximab-induced B cell lysis is thought to occur via a number of different mechanisms, including CDC, ADCC/ADCP and/or delivery of direct death signaling (apoptosis). Potential mechanisms of tumor resistance have been described in each of these major pathways of proposed rituximab action. While therapeutic MAbs share with small molecule anticancer agents possible resistance mechanisms such as altered pharmacokinetics or metabolism and reduced diffusion to the tumor site, monoclonals differ from the latter because of their much larger

molecular weight which accentuates these resistance mechanisms as well as the importance of extracellular mechanisms (complement system and/or effector cells). Overall reported resistance mechanisms include alterations in rituximab pharmacokinetics, loss of CD20 expression, either through downregulation or through "shaving" of rituximab/CD20 complexes, and antigenic modulation that has recently been reconsidered as a mechanism of resistance.

The acquisition of phenotypic changes in cancer cells or host immune cells over time may affect rituximab responsiveness and underscores the complexity of the potential mechanisms of resistance to anti-CD20 MAbs. Among these, reduced CD20 antigen expression has been among the most extensively studied. The deregulation of CD20 protein expression has been reported to be associated with a decrease in rituximab sensitivity in B cell NHL [5, 6]. There are several potential mechanisms leading to reduced functional CD20 expression, including transcriptional or translational regulation, altered membrane environment, CD20 mutations and altered membrane half-life.

Unraveling the clinical relevance behind the phenotypic changes or the reduced level of CD20 expression in B cell lymphoma has been initiated by the Rituximab Extended Schedule or Retreatment (RESORT) trial which is an ongoing study of the frequency of phenotypic changes occurring in rituximab-resistant lymphoma. This study is aiming to define the optimal duration of treatment with rituximab as a single agent in patients with indolent CD20+ B cell lymphoma and low tumor burden [7]. Phenotypic changes can occur as a consequence of repeated exposure to rituximab, especially over prolonged periods of time in B cell lymphoma (such as in maintenance programs). A 70 % reduction in CD20 expression was observed in cell lines induced to be rituximab-resistant in vitro, even at very early stages in the process of acquiring resistance to rituximab.

There are currently few data regarding CD20 mutations in patients refractory to rituximab. Terui and colleagues identified CD20 gene mutations leading to C-terminus truncated forms of CD20 in a subset of patients with rituximab-relapsed/refractory lymphomas; thus, indicating that the C-terminal region of CD20 plays a critical role in presentation of the large loop in which the rituximab-binding site is located [8]. The same group demonstrated the possibility to predict the existence of lymphoma cells resistant to rituximab by using an antibody that recognizes N-terminal region of CD20 proteins including those having a mutation [9]. These authors suggested that the combination of antibodies that target two different epitopes could help identify the C-terminal CD20 mutations and thus help in deciding whether it is appropriate to switch to another treatment such as using a second-generation CD20 antibody that is effective against fewer CD20-expressing cells [10] or using an antibody targeting a different antigen such as CD22 [11].

Antigenic modulation of CD20 has been also demonstrated through two pathways, either internalization of CD20 into lysosomes in some types of B cell malignancies, leading to reduced macrophage recruitment, degradation of CD20/MAb complexes and shortening of MAb half life [12] or through "shaving" following rituximab exposure. The shaving mechanism occurred by removing

rituximab/CD20 complexes from the B cell surface by monocytes through the Fc receptor (FcR) pathway resulting in antigen loss and rituximab resistance [13].

In preclinical models, resistant cell lines showed decreased expression of CD20 at both the pre- and post-translational levels [14, 15]. This decreased CD20 expression was associated with changes in lipid rafts and downstream signaling, suggesting that the impact of CD20 expression on rituximab resistance is more complex than simple antibody-antigen ratios [14]. It has also been reported that combining rituximab with statins, inhibitors of the cholesterol synthesis, significantly decreased rituximab-induced CDC and ADCC [16]. Furthermore, the lipid raft-associated ganglioside GM1 was found to correlate with rituximab sensitivity of primary B-NHL and CLL B cells [17]. Conversely, attempts have been made to enhance the expression of CD20. The relatively low level of CD20 expression detected in B cells from patients suffering from CLL has been correlated with the hypermethylation of the transcription factor *PU.1*-encoding gene [18]. This is in line with the study by Hiraga et al. [19] showing that the incubation of CD20− negative primary B-NHL cells with the demethylating agent 5-aza-20-deoxycytidine in vitro restored CD20 mRNA expression in relapsed patients with downregulated CD20.

Epigenetic silencing of the CD20 promoter or CD20-regulating transcription factors could be involved in resistance mechanisms to rituximab in B-NHL. Clinical intervention strategies focusing on the combination of rituximab with demethylating agents, such as histone deacetylase inhibitors (HDAC-I) will help gain understanding concerning their impact on CD20 expression levels and treatment response. The immune modulator bryostatin-1 was also found to induce the expression of CD20 protein via Extracellular Signal-Regulated Kinases 1 and 2 (ERK1/2) and Protein Kinase C (PKC)-dependent mechanisms and to sensitization of CLL B cells to rituximab [18].

The role of resistance to ADCC and CDC-mediated toxicity remains controversial since the role of these mechanisms of antitumor activity remains itself controversial. During ADCC, immune effector cells such as natural killer (NK) cells, macrophages and neutrophils, recognize the Fc portion of rituximab via their FcR. The activation of FcR leads to the release of perforin, granzymes and tumor necrosis factor (TNF), which can induce target cell death [20–22]. In ADCP, the tumor cell is phagocytosed by the effector cell. The Fc of rituximab is also recognized by the complement component C1q, which activates the classical complement pathway leading to cell lysis via formation of the membrane attack complex (MAC) [23–25]. Genetic polymorphisms in the gene for C1q have been linked to variations in rituximab efficacy in humans, again supporting a key role for CDC in rituximab efficacy. The polymorphism G267A in the *C1qA* gene was reported to affect rituximab-induced CDC and the clinical response to rituximab therapy in Follicular lymphoma (FL) [26]. On the other hand, the complement regulatory proteins (CRP) CD46, CD55, and CD59 have been shown to inhibit rituximab mediated cell kill by interfering with complement activation [27, 28]. Therefore, blocking of the CRPs CD46, CD55 or CD59 may increase the sensitivity to complement [29].

Several groups have investigated the infusion of fresh frozen plasma to replete complement levels as a means of overcoming rituximab resistance in CLL. This approach resulted in a "rapid and dramatic clinical response in all patients", lending support to the hypothesis that complement depletion plays a clinically significant role in rituximab resistance [30]. Another group reported similarly positive results by combining fresh frozen plasma with rituximab to overcome complement depletion and rituximab resistance in CLL patients [31]. These results are promising but remain to be confirmed in controlled trials.

Specific FcR profiles have been shown to correlate with reduced efficacy of rituximab-based immunotherapy. FcγRIIIa, a member of the leukocyte receptor family FcγRs, is known to be a major triggering receptor of ADCC in NK cells and may thus be one of the critical parameters determining antitumor activity [32]. FcγRIIIa on myeloid effectors appears critical in controlling antibody potency. Lymphoma patients bearing the higher affinity 158V allele in FcγRIIIa respond better to rituximab single agent therapy compared with those with the low affinity 158F allotype [33], leading many investigators to focus on augmenting the interaction of MAb with FcγRIIIa, for example via defucosylation [10].

Recently, a strong correlation between the internalization of rituximab and FcγRIIb expression on B cells was reported suggesting that FcγRIIb is a key participant in rituximab resistance mechanisms. Lim et al. [34] suggested that rituximab can crosslink CD20 and FcγRIIb predominantly on the same target B cell, resulting in phoshorylation of FcγRIIb, and internalization of CD20:rituximab:FcγRIIb complexes into lysosomes for degradation.

Response to anti-CD20 MAb therapy may be optimized using type II anti-CD20 MAbs such as obinutuzumab (GA101), which circumvent the limitations of internalization, regardless of FcγRIIb expression. It is similarly unclear, why type II anti-CD20 MAb tend neither to internalize nor to activate FcγRIIb, but this may relate to differences in orientation of type II MAb after binding or to the fact that type II antibodies do not lead to relocalization of CD20 in lipid rafts. A therapeutic approach could consist in the co-administration of an FcγRIIb inhibitor with rituximab. The evaluation of FcγRIIb expression by various types of effector cells and response to MAb immunotherapy in B cell neoplasms would be of particular interest.

A promising approach is to enhance ADCC by stimulating effector cells such as monocytes, granulocytes, and dendritic cell populations. This has been attempted with Interleukin-2 (IL-2), IL-12 or granulocyte-macrophage colony-stimulating factor (GM-CSF) [35]. GM-CSF plus rituximab results in high response rates, along with a tolerable safety profile in patients with relapsed or progressive FL [36]. However, the addition of GM-CSF to therapy with alemtuzumab and rituximab decreased the treatment efficacy and increased the rate of cytomegalovirus reactivation in high-risk CLL patients [37]. Alternatively, ADCC could indirectly be enhanced by changing the microenvironment of tumor cells, for example with CpG DNA sequences [38, 39]. These immunostimulatory sequences induce secretion of numerous cytokines (IL-12, IL-18, IFN-α, and IFN-h) by macrophages and dendritic cells [40].

In addition to dysregulations in CD20 protein expression, ADCC and CDC, alterations in the apoptotic pathway signaling have been described to induce resistance. Molecular mechanisms of acquired resistance to rituximab have been generated through repeated exposure to antibody using rituximab-resistant cell lines [41, 42]. These cell lines demonstrate resistance to apoptosis and lack sensitivity to multiple cytotoxic chemotherapeutic agents in addition to rituximab. These clones were found to exhibit upregulation of pro-proliferative and anti-apoptotic signaling pathways, such as hyperactivation of nuclear factor-κB (NF-κB), Phosphatidylinositol-3-Kinase (PI3 K)/protein kinase B (Akt) and ERK1/2. Moreover, up-regulation of the anti-apoptotic Bcl-2 protein family members Bcl-2, Bcl-xL and Mcl-1, as well as down-regulation of the essential pro-apoptotic Bak and Bax proteins have been observed [41, 43–45]. An in vivo model of resistance has also been reported by Dalle et al. [46]. This model was established by serial transplantion and rituximab exposure of a human FL cell line in immunodeficient mice. Analyses of these tumors revealed increased expression of the complement inhibitor CD59, the transcription factor Ying yang (YY1), and the anti-apoptotic protein Bcl-xL.

Many studies are ongoing based on combination therapies in order to modulate intracellular signal transduction, to inhibit proliferation and to induce apoptosis. Some of the most promising targets in intracellular signal transduction are within the PI3 K/Akt pathway. This can be partially blocked downstream of the serine/threonine kinase Akt using inhibitors of the mammalian target of Rapamycin (mTOR), such as temsirolimus and RAD00 [47]. Inhibitors of PI3 K or Akt are currently in clinical development for cancer therapy [48].

Pharmacologic mimetics of the BH3 domain, such as ABT-737 [49–51] or ABT-263 [52, 53] act by functional inhibition of antiapoptotic Bcl-2 and Bcl-xL. In particular, the latter agents bear high hopes for combination therapy of B-NHL and phase I studies have been reported in several entities. Agents including the pan-Bcl-2 inhibitor AT-101 or the Bcl-2 antisense oligodeoxynucleotide oblimersen which also targets Bcl-2 and sensitizes cells to induction of apoptosis [54, 55]. In addition to the intrinsic, 'mitochondrial' pathway of apoptotic caspase activation, the extrinsic, receptor-mediated pathway harbors therapeutic targets, such as Apo2L/TRAIL [56, 57], FasL [58] and their respective receptors, which can be stimulated by the corresponding recombinant ligands or agonistic antibodies. The combination of rituximab with such pro-apoptotic therapies appears very promising in the treatment of resistant B-NHL.

Besides these targetted therapies, broad acting pathway-unspecific inhibitors with anti-proliferative activities, such as the proteasome inhibitor bortezomib, have been combined with rituximab. Bortezomib and rituximab have shown additive activity in preclinical models of lymphoma, and have been shown to be active and generally well tolerated in a randomized phase II study in patients with follicular and marginal zone lymphomas. This regimen might represent a useful addition to the armamentarium, particularly for some subgroups of patients [59]. However, an unresolved question concerns the effect of these novel therapies on the microenvironment and the resulting consequences on tumor cell sensitivity to

treatment. As the notion of a protective tumor "niche" appears to be more and more relevant in the clinic, the potential to pharmacologically target not only the tumor cells themselves but also their surroundings is becoming a priority.

Little knowledge of how to circumvent rituximab resistance is currently available as both the mechanisms of resistance and the mechanisms of action of rituximab remain incompletely elucidated. Variations in B cell lines, animal model systems, and techniques used to generate rituximab-resistant clones contribute to the complexity of synthesizing preclinical results. The lack of pharmacokinetic-driven clinical trials and the heterogeneity of patients with NHL and CLL further complicate the understanding of these mechanisms in vivo. Despite these limitations, a number of promising approaches have been explored to enhance the effectiveness of rituximab and to overcome rituximab resistance. Currently, there are several new-generation anti-CD20 MAbs undergoing clinical investigation (Table 1) [60]. The first difference to note of the next generation of MAbs is that they are humanized or fully human MAbs, unlike the chimeric rituximab. Some of these MAbs have been designed to enhance the effector functions including the enhancement of FcγR binding, cell death and CDC (obinutuzumab, ofatumumab, PRO13192, AME133 V; Table 1).

Well-designed clinical trials will help define and refine efficacy and provide further insights of which activ-ity of modified next generation anti-CD20 MAb will prevail to further improve anti-CD20 MAb therapy beyond rituximab [1, 3, 60–69].

Trastuzumab

Human-epidermal-growth-factor-receptor-2 (HER2) overexpressing breast cancers account for 20–25 % of invasive breast cancers and are associated with an aggressive biological behavior translating into poorer clinical outcomes [70]. Trastuzumab, targeting the extracellular domain IV (ECD) of the HER2 protein, has dramatically altered the natural history of HER2-positive breast cancer and ranks among the most significant advances in breast cancer therapeutics. The mechanisms of action of trastuzumab are still incompletely determined. Trastuzumab has been proposed to trigger HER2 internalization and degradation by promoting the activity of tyrosine kinase—ubiquitin ligase c-Cbl [71]. An important proposed mechanism of action of trastuzumab is ADCC, which is triggered through the detection of Fc portion of trastuzumab by the FcγR on immune effector cells, particularly NK cells, resulting in cell lysis of HER2-positive target cells bound to the antibody [72]. These observations are confirmed by in vivo data from a pilot study of 11 patients with HER2-positive early breast cancer, where a positive correlation was observed between responses to neoadjuvant trastuzumab and ADCC activity [73].

Musilino et al. [74] showed that FcγR polymorphisms plays a role in trastuzumab-mediated ADCC and may be a predictive tool for clinical outcome in