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Mechanisms of Resistance to Monoclonal Antibodies (mAbs) in Lymphoid Malignancies

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Abstract

Purpose of Review Passive immunotherapy with therapeutic monoclonal antibodies (mAbs) has revolutionized the treatment of cancer, especially hematological malignancies over the last 20 years. While use of mAbs has improved outcomes, development of resistance is inevitable in most cases, hindering the long-term survival of cancer patients. This review focuses on the available data on mechanisms of resistance to rituximab and includes some additional information for other mAbs currently in use in hematological malignancies.

Recent Findings Mechanisms of resistance have been identified that target all described mechanisms of mAb activity including altered antigen expression or binding, impaired complement-mediated cytotoxicity (CMC) or antibody-dependent cellular cytotoxicity (ADCC), altered intracellular signaling effects, and inhibition of direct induction of cell death. Numerous approaches to circumvent identified mechanisms of resistance continue to be investigated, but a thorough understanding of which resistance mechanisms are most clinically relevant is still elusive. In recent years, a deeper understanding of the tumor microenvironment and targeting the apoptotic pathway has led to promising breakthroughs.

Summary Resistance may be driven by unique patient-, disease-, and antibody-related factors. Understanding the mechanisms of resistance to mAbs will guide the development of strategies to overcome resistance and re-sensitize cancer cells to these biological agents.

Keywords CMC · ADCC · Apoptosis · Rituximab · Anti-CD20

Introduction

Over the past 20 years, several monoclonal antibodies (mAbs) have been adopted for clinical use, and many others are under development (Table 1) [1]. With the widespread use of therapeutic mAbs, resistance to these agents has become a major clinical issue. Resistance is broadly defined as "a lack of response to, progression during, or within 6 months post treatment with a monoclonal antibody-containing regimen". Resistance could be either innate (primary) or acquired (secondary) with differing mechanisms in each scenario. Preclinical models of

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Table 1 Monoclonal antibodies approved by FDA in hematological malignancies

Name	Target	Year of approval	Indication
Naked antibodies			
Rituximab	CD20	1997	B-NHL
Alemtuzumab	CD52	2001	CLL
Ofatumumab	CD20	2009	CLL
Obinutuzumab	CD20	2013	CLL
Daratumumab	CD38	2015	Myeloma
Elotuzumab	SLAMF7	2015	Myeloma
Nivolumab	PD-1	2016	HL
Pembrolizumab	PD-1	2017	HL
Mogamulizumab-kpkc	CCR4	2018	Mycosis fungoides/Sezary's syndrome
Polatuzumab vedotin-piiq	CD79b	2019	DLBCL
Antibody-drug conjugates			
Inotuzumab ozogamicin	CD22	2017	B cell precursor ALL
Gemtuzumab ozogamicin	CD33	2000	AML
Brentuximab Vedotin	CD30, MMAE	2011	HL
Moxetumomab pasudotox-tdfk	CD22, PE38	2018	Hairy cell leukemia
Radioimmunoconjugates			
Ibritumomab	CD20 with Y-90	2002	B-NHL
Tositumomab	CD20 with I-131	2014	ALL
Bispecific antibodies			
Blinatumomab	CD3-CD19	2017	B cell precursor ALL

B-NHL B cell non-Hodgkin lymphoma, *AML* acute myeloid leukemia, *CLL* chronic lymphocytic leukemia, *NHL* non-Hodgkin's lymphoma, *HL* Hodgkin lymphoma, *ALL* acute lymphoblastic leukemia

mAb action and/or resistance have contributed to unravel a myriad of mechanisms of resistance; however, their relative clinical importance, predictability, and timing in any particular patient remain unclear. Most mAbs share common mechanisms of action (Fig. 1): (a) antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP), (b) complement-mediated cytotoxicity (CMC), and (c) direct induction of apoptosis. In addition, mAbs can synergize with chemotherapeutic agents by sensitizing tumor cells to their cytotoxic effects when used in combination [2]. Alternatives to naked mAb therapeutics have included antibody constructs that enhance immune effector cell-tumor interaction leading to an accentuated adaptive immune response, or conjugation of antibodies to compounds that are toxic to tumor cells such as mitotic toxins, chemotherapeutic agents, or radiotherapeutic agents. These effects are dependent on several factors, many of which are common to all mAbs and are likely to be involved in development of resistance: (1) antigen density expression; (2) cellular pathways driving the underlying malignancy; (3) pharmacokinetics/pharmacodynamics; (4) tumor burden; and (5) status of the host's innate (i.e., complement system proteins, neutrophils, natural killer [NK] cells, and macrophages) and adaptive (i.e., antigen-presenting cells [APCs] and T cells) immune systems. In this review, we will discuss available data regarding preclinical models of resistance to mAbs focusing on rituximab and suggest possible strategies to circumvent these resistance phenomena (Table 2). While a detailed discussion of mechanisms of resistance pertaining to every mAb is beyond the scope of this review, we will discuss common themes and highlight differences between the various antibodies.

Rituximab

Rituximab is an IgG1 chimeric mAb directed against the CD20 antigen. It is composed of murine variable regions (Fab region) from the anti-CD20 antibody 2B8 that are linked to human constant regions (Fc). Rituximab was the first mAb to be approved by the Federal Drug Administration (FDA) for therapeutic use in lymphoid malignancies in 1997 [3]. Several biological effects have been attributed to rituximab's mechanism(s) of action as mentioned above. A "vaccinal" effect has also been described, especially in the re-treatment setting [4]. It has been demonstrated that rituximab facilitates the uptake and presentation of antigens by dendritic cells to T cells and elucidates an adaptive immune response against B cell antigens [5]. Having been in use for over two decades, rituximab is thus the therapeutic mAb for which there are currently the most data in terms of mechanisms of action, parameters

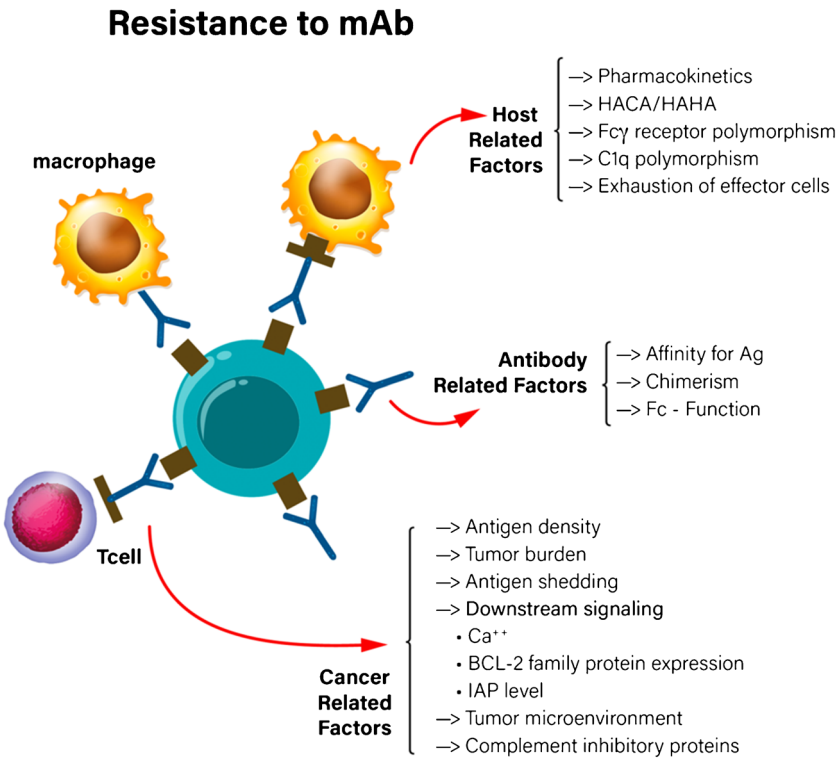

Fig. 1 Factors that influence the anti-tumor activity of monoclonal antibodies. Mab: monoclonal antibodies; HACA: human anti-chimeric antibodies; HAHA: human anti-human antibodies

associated with sensitivity or resistance, and strategies to enhance its antitumor effect (Figs. 2 and 3). Numerous approaches to circumvent identified mechanisms of resistance continue to be investigated, but a thorough understanding of which mechanisms of action are most relevant to rituximab's efficacy and which resistance mechanisms are most clinically relevant is still elusive. Since it has the most extensive body of literature regarding mechanisms of resistance, rituximab will be used as a prototype for our discussion.

Factors Influencing and/or Contributing to Naked mAb Activity/Resistance

Pharmacokinetics

Clinical studies have shown that there is significant inter-individual variability in rituximab exposure despite administering it at similar doses in patients [6]. Variability may be in part due to interethnic differences; pharmacokinetic studies conducted in Japanese patients with indolent B cell

Table 2 Potential strategies to overcome rituximab resistance

Mechanism of resistance	Potential strategy	Available drugs
↓ CD20	Restore epigenetic regulation of CD20	HDAC inhibitors
	Develop more potent mAb	ObinutuzumabOfatumumab
	Enhance MOA that are not dependent on CD20 antigen expression	G-CSFGM-CSFImiDs-NK cells
	Bi-specific mAb	Mosunetuzumab
	Antibody drug conjugates	
	Use alternate antigen	Polatuzumab (CD79b)MOR208 (CD19)
↓ BAX	UPS inhibition	Proteasome inhibitors
↑ BCL-XL, BCL2, MCL1	BH3 mimetics	Venetoclax, AMG 176
↑ IAP	Targeting IAPs	IAP inhibitors

HDAC histone deacetylase, *mAb* monoclonal antibody, *G-CSF* granulocyte colony-stimulating factor, *GM-CSF* granulocyte monocyte-colony-stimulating factor, *PMN* polymorphonuclear cells, *MOA* mechanism of action, ImiDs immunomodulatory agents, *NK cells* natural killer cells, *UPS* ubiquitin-proteasomal system

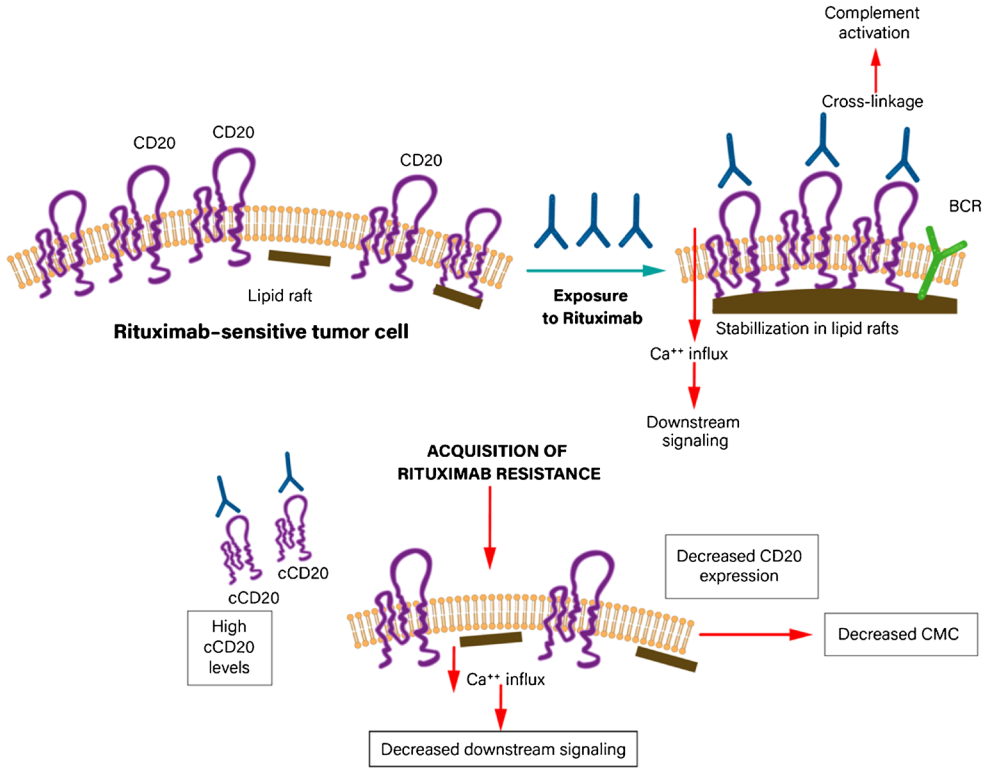

Fig. 2 CD20 changes observers in rituximab resistant lymphoma models. CMC: complement-mediated cytotoxicity; BCR: B-cell receptor; cCD20: circulating CD20

Non-Hodgkin's lymphoma (B-NHL) or mantle cell lymphoma (MCL) found lower median serum concentrations following rituximab dosing than those reported in patients from western countries [7]. Serum rituximab concentration has been associated with the likelihood of response in some studies. It has also been noted that patients with high disease burden, and thus high antigen load, have lower rituximab levels [8–11]. Since clinical response and progression-free survival were related to rituximab concentration [10], it is imperative to understand the factors influencing rituximab pharmacokinetics. The half-life of a mAb in human circulation is determined by the distribution, availability, and number of target binding sites [12]. Rapid metabolism of rituximab, due either to high numbers of accessible CD20 molecules and/or to alterations in host antibody metabolism, could contribute to rituximab resistance. Additionally, higher circulating CD20 (cCD20) has been inversely correlated with overall survival in chronic lymphocytic leukemia (CLL) [13]. It has been postulated that the efficacy of rituximab in CLL may be impaired due to high levels of cCD20 leading to preferential binding of rituximab with cCD20 with decreased binding to cell surface CD20 [14]. The formation of cCD20/rituximab complexes can lead to enhanced rituximab clearance. High cCD20 levels prior to receiving therapy and higher cCD20 after therapy have also been associated with lower probability of survival in B-NHL. These mechanisms of innate resistance may be overcome by administering higher rituximab dose density [15]. Indeed, a dose-response relationship has been established in CLL with higher doses leading to better responses [16]. A PK-PD-based model to optimize rituximab-based dosing in follicular lymphoma has also been suggested [17].

Rituximab PK/PD may also be impacted by the development of antibodies against rituximab leading to increased clearance. MAbs are large antigenic proteins, which can theoretically induce an immune response leading to the formation of anti-antibodies. The development of human anti-chimeric (HACA) or human anti-mouse (HAMA) antibodies was evaluated in early trials of rituximab use in patients with B-NHL. While some case reports have identified the presence of these inactivating antibodies, only one patient in early trials of rituximab in adults or children with B-NHL developed HACA or HAMA with the rate of HACA formation seeming to be more significant with the use of rituximab for auto-immune disease such as systemic lupus erythematosus [6, 11, 18, 19]. The lack of HACA antibody formation may be related to the humoral immunosuppressive effect of rituximab. Additionally, as most anti-cancer mAbs in clinical use are now human/humanized, reduced serum levels of antibody through anti-antibody formation is unlikely to play a role in resistance.

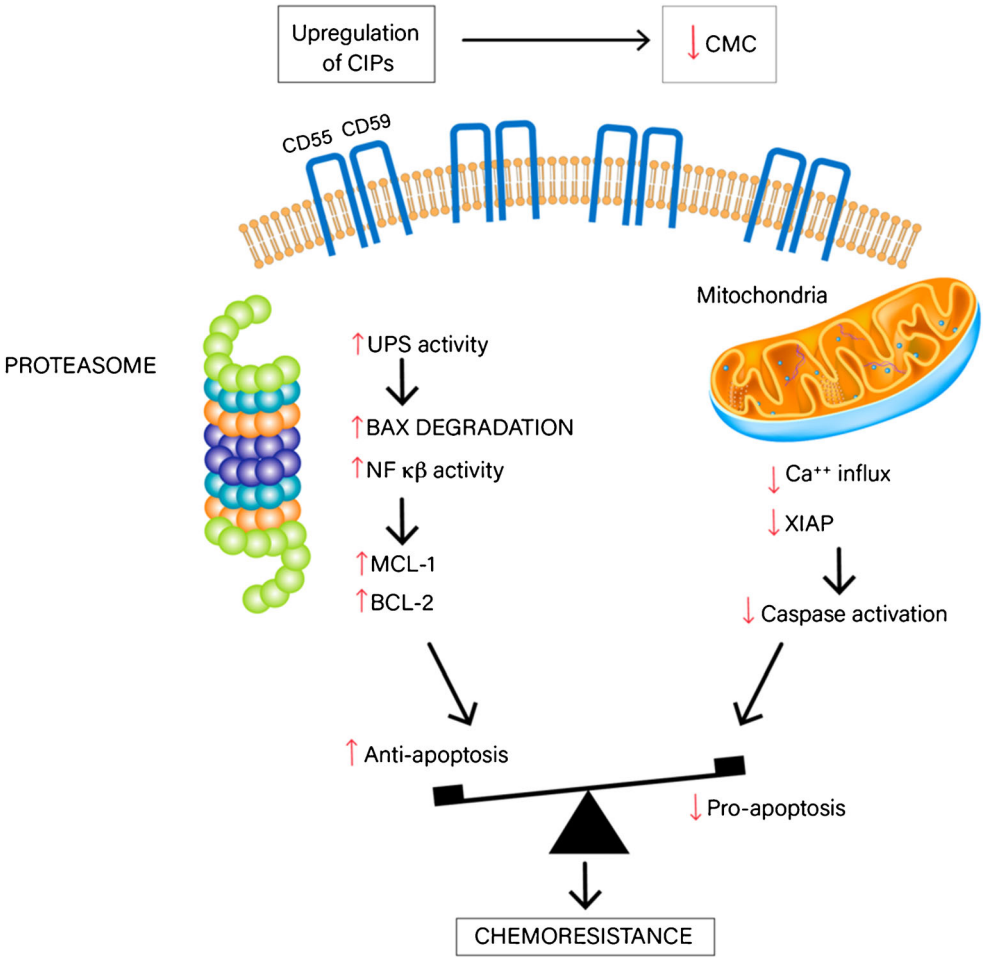

Fig. 3 CD20 independent mechanism(s) of resistance to rituximab observed in lymphoma laboratory models. CIP: complement inhibitory protein CMC: cell-mediated cytotoxicity, UPS: ubiquitin proteasome system

Alterations in Target Antigen

In order to exert their effect, mAbs must first bind their target antigen. This step can be impaired by alterations in the expression level of the target or mutations in the target that hinder binding. For example, CD20 surface expression is a limiting step for rituximab activity in pre-clinical models and clinical studies. Van Meerten et al. demonstrated a linear correlation between rituximab-induced CMC and CD20 expression in CD20 transfected T cells and in malignant cells isolated from patients with CLL [20]. No correlation between CD20 expression and rituximab-associated ADCC was noted in the same pre-clinical model [4, 20].

Studies have demonstrated changes in surface CD20 antigen density and/or structure in rituximab-resistant cell lines (RRCLs) and in a limited number of patients with rituximab-resistant B-NHL [21–23]. While the incidence of rituximab associated loss of CD20 is believed to be low in the clinical setting, downregulation of CD20 has been observed in patients with relapsed/refractory B-NHL following prior rituximab therapy [24–26]. Several mechanisms behind changes in CD20 expression have been described in RRCLs. Following exposure to rituximab, RRCLs exhibit decreased CD20 expression due to transcriptional and post-transcriptional mechanisms [23, 24, 27–29]. Some of the described mechanisms include [4]"CD20 shaving" by effector cells that expressed FcγR [30, 31], internalization of CD20 into lysosomes after rituximab binding [32], point mutation in the CD20 gene (leading to the formation of truncated forms of CD20 lacking the C-terminal region) [23] and generation of aCD20 transcript variant coding for a truncated protein which can be associated with rituximab resistance [4, 33]. Our group has demonstrated that RRCLs have lower CD20 promoter activity and/or a defect impairing the transport of CD20 from the intracellular compartment to the cell surface membrane [29]. CD20 expression may also be altered via epigenetic regulation. For example, the Sin3A-HDAC1 co-repressor complex has been noted to downregulate expression of MS4A1, which encodes CD20, with HDAC inhibition being able to increase CD20 expression [34]. Other epigenetic agents targeting DNA methylation, acetylation, or transcription have also been noted to alter CD20 expression and rituximab activity [35–40].

Therapeutic strategies to overcome rituximab resistance associated with altered CD20 structure/expression levels can be divided in the following categories [4]: (1) generation of novel anti-CD20 mAbs with higher binding capacity and/or re-engineered Fc regions facilitating more efficient CMC/ADCC than rituximab (ofatumumab or obinutuzumab) [41, 42]; (2) re-expression of CD20 antigen by changing its epigenetic regulation using pharmacological inhibitors or through the use of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), IL-4, and IL-2 [2, 43–46]; (3) targeting of multiple antigens through the use of multivalent antibodies (e.g., combining an anti-CD20 and anti-CD22 into an antiCD20/CD22 bivalent antibody enhanced *in vitro* and *in vivo* cell killing compared with the combination of the individual antibodies [47]; (4) developing a bivalent antibody containing both a type 1 and a type 2 CD20 antibody-enhanced CMC and direct killing [31]; (5) developing novel antibodies that target other lymphoma-associated antigens in rituximab-resistant or CD20-negative lymphomas [48–55].

Changes in Other Surface Antigens: Complement-Mediated Resistance

Host complement components may affect rituximab activity as demonstrated in pre-clinical models and in B-NHL patients [16, 17]. The importance of complement was suggested by the loss of rituximab activity noted in mice deficient in C1q or following depletion of complement by treatment with cobra venom factor. This data, however, data is contradicted by other studies that show that complement has little impact on rituximab activity and that ADCC may be a more critical mechanism of *in vivo* activity [21, 56]. Clinically, one study showed that rapid depletion of complement proteins in CLL patients may limit single-agent activity of rituximab in this entity [57]. Further evidence towards this mechanism of resistance came from studies which showed enhanced rituximab activity when patients were infused with fresh frozen plasma containing complement [58, 59].

Pre-clinical studies have shown that Surface expression of complement inhibitory proteins (CIPs) CD46, CD55, and CD59 by tumor cells can impact rituximab-mediated CMC [60, 61]. Upregulation of CD55 and CD59 has been described in RRCL [27]. Takei et al. observed increased expression of CD55 and CD59 in rituximab-resistant Ramos cells [19]. Treon et al. demonstrated that anti-CD59 mAbs sensitized cells to rituximab cytotoxicity [18]. Despite the *in vitro* evidence of the detrimental effect of high CIP expression on rituximab activity, clinical data is conflicting [62, 63].

C1q gene polymorphisms may also impact rituximab activity as described in patients with follicular lymphoma [64]. Racila et al. genotyped C1qA([276A/G]) polymorphism in 133 subjects with FL treated with single-agent rituximab and observed a significant difference in time to progression between homozygous G-allele (282 days) and A-allele carriers (708 days, $p = 0.02$). Homozygous A subjects achieved higher rates of complete response than heterozygous or homozygous G subjects [17].

To overcome rituximab resistance, novel mAbs have been developed that induce CMC more effectively than rituximab. [65]. The ability of a mAb to induce CMC has been correlated with the proximity of its binding to the cell membrane and the ability to induce redistribution of CD20 to lipid raft domains [65, 66]. Ofatumumab is an FDA-approved, anti-CD20 mAb that shows enhanced CMC in comparison with rituximab associated with a more membrane proximal antigen-binding domain, especially in the setting of rituximab resistance and high levels of CD55 and CD59 expression [65, 67–70]. While it led to high response rates in CLL, ofatumumab has had limited success in other B-NHL highlighting that CMC may play a larger role in certain B cell malignancies (like CLL) compared with others [71–75]. Antibody hexamers may activate complement effectively by increasing C1q binding [76], hence Introducing polymorphisms in the Fc portion of an antibody that can enhance hexamer formation is another potential approach to overcome resistance to CMC activity [76].

Fcγ Receptor Polymorphism-Mediated Resistance

ADCC is one of the most well-characterized mechanism-of-action of rituximab. Once rituximab binds to its epitope in CD20, effector cells (natural killer [NK] cells, monocytes/macrophages and neutrophils) recognize the rituximab constant region (Fc) portion via the Fc receptors (FcR). The binding of the Fc fragment of the mAb to FcR on surrounding immune effector cells, in particular FcγRIIIa and FcγRIIa receptors, triggers them to kill the tumor cells [56, 77, 78]. Depletion of neutrophils and NK cells impairs rituximab *in vivo* activity highlighting the importance of ADCC to rituximab efficacy [56, 79]. A murine model demonstrated that FcγRIII receptor expression is necessary for rituximab activity [77]. Genetic polymorphisms in the FCGR3A gene and the FCGR2A gene have been linked to variations in the anti-tumor activity of rituximab in patients with follicular lymphoma with those harboring the FCGR3A-158 V/V and/or FCGR2A-131 H/H alleles showing better responses [80, 81]. Interestingly, the correlation of FCGR3A polymorphism with the therapeutic response to rituximab was noted only with single-agent rituximab and not when rituximab was combined with chemotherapy [4, 82–84]. The high affinity FcγRIIIa 158 V polymorphism may also play a role in rituximab-associated toxicity with an increased rate of late onset neutropenia observed in patients with the 158 V variant [85–87]. The differences in rituximab response have been

correlated to the binding affinity to IgG1 and the absolute number of CD16 receptors per effector cell (macrophages and NK cells) which ultimately influences the ability of effector cells to induce ADCC [88].

While activating receptors may play a role in rituximab activity, inhibitory FcγR receptors, such as FcγRIIb, may also limit response to bound rituximab by macrophages [77] while also promoting internalization of rituximab-bound CD20 inhibiting Fc-dependent function [32, 89]. This inhibitory effect of FcγRIIb receptors has been reported in follicular lymphoma patients where those with high FcγRIIb were noted to have lower EFS [90].

In order to overcome variability in FcγR binding affinity, novel mAbs with enhanced Fc receptor affinity were developed. Obinutuzumab is a third-generation type II, anti-CD20 mAb with enhanced pre-clinical activity compared with rituximab due to augmented ADCC and enhanced direct cell killing [91–96]. While obinutuzumab is certainly more potent than rituximab and shows responses in a subset of patients with rituximab-resistant B-NHL as demonstrated in multiple clinical trials, the margin of benefit is less than optimal, especially in aggressive B-NHL [97–99].

Tumor Microenvironment

There is growing evidence that rituximab-mediated B cell reduction is achieved by different mechanisms depending on the microenvironment. Gong et. al. showed that circulating B cells were destroyed predominately by ADCC whereas B cells in the marginal zone of lymph nodes were depleted by CDC in a human-CD20 transgenic mouse model [99]. It is reasonable to postulate that differences in microenvironment signaling play a role in mediating rituximab resistance and may help account for the variation in response rates noted among CD20-expressing malignancies; however, definitive knowledge in this area is currently lacking [100]. Mraz et. al. proposed that the microenvironment can protect malignant B cells from rituximab-induced apoptosis and demonstrated that natalizumab, an antibody targeting VLA-4 (integrin alfa-4-beta-1/CD49d), decreased B lymphocyte adherence to fibronectin by 75–95% and partially overcame stromal protection against rituximab and cytotoxic drugs [100]. Laursen et. al. demonstrated that high CXCR4 expression was associated with poor prognosis in DLBCL patients treated with R-CHOP. In mouse models, rituximab-induced response was hampered by CXCR4 on the surface of DLBCL cells, with inverse correlation between CXCR4 surface expression level and degree of rituximab sensitivity for rituximab-responsive but not for rituximab-resistant cell lines, implying that CXCR4 plays a role in rituximab sensitivity but not in intrinsic resistance [101]. This data needs to be further explored to evaluate whether plerixafor, a CXCR4 antagonist, can be used to augment rituximab activity. Recently, lymphoma Gal-1 expression in the local microenvironment was identified as a significant mediator of resistance to CD20 immunotherapy and mAb-dependent phagocytosis by impeding macrophage activation and/or function [102]. However, not all CD20 mAb-resistant lymphomas secreted substantial Gal-1, indicating that these lymphomas must employ diverse mechanisms of resistance. Another study demonstrated that enhancing macrophage-dependent type 1-interferon associated cross-priming for cytotoxic T lymphocytes induced by anti-CD20 is essential for response [103•]. CTLA-4 from T regs played an essential role in adaptive resistance and anti-CTLA-4 administered together with anti-CD20 had significant synergy compared with either treatment alone in murine models [103•]. These results raise the tantalizing potential of using checkpoint blockade to overcome rituximab resistance in the clinical setting. Preclinical studies have shown that host immune system activation by colony-stimulating factors enhances the biologic activity of rituximab [104]. Various mechanisms of this synergy have been described such as increased trafficking of neutrophils into the tumor bed and enhanced ADCC by upregulation of CD11b/18 expression. Other contributing factors such as production of free oxygen radicals, complement activation by alternate pathways, cytokine "storms", and/or increased CD20 expression on tumor cells have also been proposed. In a small phase 2 study, our group demonstrated that combining pegfilgrastim with rituximab in patients with indolent B-NHL led to a response rate of 60% which was higher than that of historical cohorts [105].

Resistance to Apoptosis

Rituximab binding to CD20 results in several signaling events that eventually culminate in programed cell death (PCD, apoptosis) of the lymphoma cells via both caspase-dependent and caspase-independent mechanisms [106–109]. Following rituximab exposure, stabilization of CD20 into lipid rafts within the cell membrane is important to trigger downstream signal transduction events leading to PCD [110–112]. CD20 may also be important in calcium transport necessary for apoptosis induction ;an increase in intracellular calcium has been noted following rituximab exposure, and reduction in apoptosis was demonstrated following exposure to calcium chelators [107, 108]. The role of L-type calcium channel in rituximab-induced apoptosis was recently described with loss of CACNA1C expression being associated with lower CD20 stability and reduced rituximab-induced cell death in DLBCL cell lines and in xenograft mouse models [113]. Chronic exposure of B-NHL cell lines to rituximab resulted in deregulation of several members of the Bcl-2 family of proteins leading not only to rituximab resistance but also concomitant resistance to various chemotherapy agents [27, 114, 115]. In addition to deregulation of the Bcl-2 family members, gene expression profiling showed that RRCL upregulated

inhibitor of apoptosis protein (IAP) genes [27]. Since IAPs block apoptosis by negative regulation of caspases, IAP inhibitors have the potential to overcome rituximab resistance and show encouraging results in pre-clinical models [116]. Another important mechanism of disruption of the apoptotic balance in RRCL is increase in the activity of the ubiquitin-proteasome system which has been associated with increased degradation of pro-apoptotic Bcl-2 family members such as Bax and increased NFκB activity which then leads to upregulation of anti-apoptotic Bcl-2 proteins [114, 115]. As a result, pharmacological inhibition of the proteasome results in [4]: (1) decrease in NFκB activity and (2) accumulation of Bax, which then decreases the apoptotic threshold to chemotherapeutic drugs in rituximab-chemotherapy-resistant cell lines [117•]. Ongoing clinical studies are testing if proteasomal inhibitors such as bortezomib and carfilzomib can re-sensitize resistant lymphomas to the cytotoxic effects of rituximab-chemotherapy regimens in the relapsed/refractory setting [4, 118, 126]. Upregulation of Mcl-1 has also been described in RRCL; hence, Mcl-1 inhibitors could potentially be used in this setting [119].

Potential Mechanisms of Resistance to mAb-Drug Conjugates, Radio-Immunoconjugates, and Other Novel Antibodies Harnessing the Immune System Against Cancer

The development of antibody drug conjugates (ADCs), checkpoint inhibitors, bispecific T cell engager antibodies, and radioimmunoconjugates (RIC) provides alternatives to the use of naked mAbs. ADC and RIC agents improve drug or radioactive molecule delivery to malignant cells by using a covalently bound tumor antigen-targeting antibody. This differs from checkpoint inhibitors and bispecific mAbs which optimize host immune system killing of cancer cells by either clearing negative immune inhibitory signals in the tumor micro-environment or by bridging tumor and effector cells to enhance immune-mediated killing.

Resistance to ADCs can develop by changes to the target antigen or as a result of resistance to the cytotoxic molecules. ADC activity is largely dependent on the target antigen being present for effective delivery of cytotoxic agents; therefore, changes in antigen expression are likely to limit the activity of ADCs. There have been reports of CD30-negative relapses of ALCL after treatment with the anti-CD30 ADC brentuximab vedotin [120, 121]. Mechanisms pertaining to traditional chemotherapy agents can also drive resistance to ADCs. In brentuximab-resistant ALCL and Hodgkin lymphoma cell lines, both downregulation of CD30 expression and resistance to monomethyl auristatin E (MMAE) were noted [122•]. Resistant cells accumulated less MMAE intracellularly following exposure to either the ADC or free MMAE. Resistance to MMAE may have occurred due to increased expression of the multi-drug resistance gene MDR1. Inhibition of MDR1 with verapamil and cyclosporine reversed the MMAE resistance *in vitro* and *in vivo* [120]. Resistance to the conjugated molecule may also occur through impaired induction of apoptosis. When MMAE was conjugated to an anti-CD79b mAb, increased expression of Bcl-xL was associated with resistance, and inhibition of Bcl-2 with ABT-263 was able to enhance the activity of the ADC [121]. Potential mechanisms of bypassing these resistant mechanisms include alterations to antigen binding, including enhanced antigen affinity or targeting of alternative antigens, as well as improving the efficacy of the payload molecule through the use of agents that more effectively kill tumor cells or are not substrates for drug efflux.

Another alternative approach utilizing mAb technology is the use of bispecific antibodies targeting both the tumor and an immune effector cell. Blinatumomab is a bispecific T cell–engaging (BiTE) antibody targeting both CD19 and CD3 that has received regulatory approval for the treatment of acute lymphoblastic leukemia. Early trials in B-NHL report promising results with responses in 69% of 76 relapsed/refractory B-NHL patients [50] though the neurological toxicities were formidable. CD19-negative relapses following blinatumomab therapy have been reported and would impair antibody-antigen binding [123]. Additionally, increased expression of the checkpoint inhibitory PD-L1 molecule on tumor cells may lead to impaired T cell response to bound blinatumomab [124, 125]. Numerous bispecific antibodies targeting CD19 and alternative lymphoma cell surface antigens continue to be developed.

Conclusion

The approval of rituximab for treatment of B-NHL paved the way for development of monoclonal antibodies in hematological malignancies and constitutes a major advance in cancer therapeutics. Despite investigation for over two decades, the pathways that contribute to rituximab resistance are yet to be fully elucidated. Mechanisms of resistance have been identified that target all described mechanisms of monoclonal antibody activity including altered antigen expression or binding, impaired CDC or ADCC, altered intracellular signaling effects, and inhibition of direct induction of cell death. Alternative monoclonal antibody-based immunotherapeutic approaches such as ADCs and bispecific or multivalent antibody constructs have more recently been developed with success and many mAbs are in the pipeline. It is essential to preemptively study and understand their mechanisms of resistance to design optimal dosing schedules and rational drug combinations and employ strategies to mitigate resistance.

Compliance with Ethical Standards

Conflict of Interest Pallawi Torka, Mathew Barth, Robert Ferdman, and Francisco J Hernandez-Ilizaliturri declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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