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Postulated Mechanisms of Resistance of B-NHL to Rituximab Treatment Regimens: Strategies to Overcome Resistance

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Abstract

Antibody-mediated immunotherapy has gained significant momentum since the first FDA-approved monoclonal antibody (mAb) in 1997, namely, Rituximab (chimeric anti-CD20 mAb) for the treatment of B-NHL cells. Subsequently, over 20 approved mAbs have been in use clinically for the treatment of various cancers and several non-cancer related diseases. Further, the combination treatment of mAbs with chemotherapy, immunotherapy, proteaosome inhibitors and other inhibitors have resulted in synergistic anti-tumor activity with significant objective clinical responses. Despite their successful clinical use, the underlying mechanisms of rituximab *in vivo* activities remain elusive. Further, it is not clear why a subset of patients is initially unresponsive and many responding patients become refractory and resistant to further treatments; hence, the underlying mechanisms of resistance are not known, Attempts have been made to develop model systems to investigate resistance to mAb therapy with the hope to apply the findings in both the generation of new therapeutics as well as their use as new prognostic biomarkers. This review focuses on the development of resistance to Rituximab treatments and discusses possible underlying mechanisms of action, postulated mechanisms of resistance in model systems and suggested means to overcome resistance. Several prior reviews on the subject of Rituximab resistance have been published and the present review both complements as well as adds new topics of relevance.

I. Introduction

During the last decade, we have witnessed the emergence of anti-cancer targeted therapies, namely, of the use of monoclonal antibodies (mAbs) directed against surface tumor associated antigens. A major limitation of both conventional and targeted therapies is that a subset of patients does not initially respond to such therapies and another responding subset

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develops resistance to further treatments. Hence, many malignant cancers exhibit both intrinsic and acquired resistance.[1] Nevertheless, the introduction of antibody-mediated therapy has resulted in significant clinical objective responses and, in many cases, responses in cancers that did not respond to conventional chemotherapies.

Historically, several decades ago, antibody-mediated therapy originated by the use of polyclonal antibodies derived from mice, rabbits or rats. Treatment of cancer patients with such foreign antibodies (antigenic) resulted in the development of a humoral antibody response against these foreign antibodies. Hence, the therapeutic antibodies were blocked and cleared and, therefore, limited their ability to be effective against the cancer. Immunotherapy by antibodies became practical following the milestone discovery of the generation of antigen-specific mAbs by Kohler and Milstein in 1975.[2]

In order to overcome the obstacle of the host response to the administered xenogenic antibodies, engineering of chimeric humanized and privatized antibodies were developed by linking mouse or primate antibody recognition regions with human back bone fragments.[3,4] For example, humanized antibody is a human antibody consisting of the complementarity-determining regions (CDR) of non-human origin and human constant regions. The earliest clinically approved mAb was in Europe 1994 and consisted of Edrecolomab (Panorex®) for the treatment of patients with clororectal cancer. Subsequently, the first mAb approved in the USA for cancer therapy was in 1997 by the chimeric anti-CD20 mAb, Rituximab, Rituxan® for the treatment of low grade and follicular NHL.[5,6] Subsequently, over 20 mAbs have been approved for the treatment of various cancers and non-cancer diseases.[7]

Rituximab is a chimeric anti-CD20 mAb. It is directed against cell surface membrane receptors, CD20, expressed on mature B cells but not on pre-B cells or plasma cells. The receptor CD20 is a tetramembrane spanning molecule of molecular weight 33–37 kDa and the gene is located on chromosome 11q12-q13.1. CD20 is resident in lipid raft domains of the plasma membrane.[8] In this review, I'll briefly summarize the findings reported on Rituximab treatment regimens both *in vivo* and *in vitro*, with emphasis on postulated mechanisms of actions, postulated mechanisms of resistance and suggested means to overcome resistance. Rituximab has been chosen as a putative prototype for other anti-cancer mAbs.

II. Rituximab

Rituximab-containing regimens have emerged as current therapeutics for NHLs and other lymphomas. Rituximab is routinely incorporated into the conventional treatment of follicular NHL, namely, in first line therapy, maintenance and salvage therapy.[9–11] The treatment of patients with FL and diffuse large B-cell lymphoma (DLBCL), alone or in combination with chemotherapy, resulted in significant clinical responses and prolongation of survival.[7] Rituximab off label uses in other malignant and non-malignant diseases were reported.[12]

Postulated mechanisms of action mediated by rituximab

Several mechanisms of action have been reported including ADCC, CDC, and apoptotic activities as well as its cell signaling-mediated effects that are responsible, in part, for its

chemo and immuno-sensitizing activities. The combination of Rituximab and chemo-immuno-therapeutic drugs resulted in reversal of resistance and synergy. Below, a brief description of the various mechanisms of action by rituximab

A. Antibody-dependent cellular cytotoxicity (ADCC)—ADCC has been attributed a significant role on the *in vivo* mechanism of action of rituximab. ADCC consists of the ligation of the human Fc portion of rituximab in antibody-coated tumor cells to the Fc receptors expressed on the surface of NK cells, macrophages and neutrophils and resulting in triggering the cytotoxic cells for killing of the bound target cells. For instance, the reported depletion of B-CLL in patients-derived PBMCs (which contain circulating effector cells) was significantly augmented following treatment with rituximab (even more by rituximab combination with GMCSF.[13] The treatment of patients with rituximab and low dose IL-2 resulted in clinical responses of 55% in patients with a relapsed and refractory FL.[14]

B. Complement-dependent cellular cytotoxicity (CDC)—It has been reported that rituximab-coated tumor cells bind C1q and activate the complement cascade for cytotoxicity.[3] Sensitivity to CDC is dependent on the origin of lymphoma cells. Rituximab induces significant CDC killing of FL cells whereas it has only moderate cytotoxicity in MCL, DLBCL, and small lymphocytic leukemia (SLC) cells.[15] Various agents have been shown to induce CDC activity *in vitro*. For example, dexamethasone enhances rituximab-mediated CDC but it has no effect on ADCC.[16]

C. Apoptosis—In certain NHL cell lines, rituximab has been reported to exert moderate apoptosis *in vitro*.[17] However, cross linking of rituximab with a secondary anti-human IgG results in significant induction of apoptosis in resistant cells. Cross-linking is accompanied by the activation of tyrosine-kinases, Plc-2 phosphorylation, calcium influx, and caspase 3 activation. These various manifestations were inhibited by PP2, a selective inhibitor of the Src family kinases.[18] Freshly isolated B-CLL coated with rituximab and cross-linked with anti-human IgG (Fab'2) resulted in a concentration and time-dependent apoptosis, independent of ADCC and CDC.[19] The mechanisms of cross-linking induced apoptosis and were reported by Pedersen et al.[19] and Mathias et al..[20] In addition, the role of activation of the apoptotic mitochondrial pathway by cross linking was reported.[21] These findings show the ability of rituximab to kill cells directly if cross-linking takes place.

D. CD20 redistribution to lipid rafts—Lipid rafts are heterogeneous lipid microdomains enriched in sphingomyelin, glycosphingo lipids and cholesterol. Lipid rafts serve as a platform for cell signaling. The binding of anti-CD20 antibody to B cells results in the rapid redistribution of CD20 molecules (up to 98%) to low density detergent insoluble lipid rafts.[22] It appears that the redistribution of CD20 into membrane lipid rafts regulates the efficacy of anti-CD20 antibodies to induce CDC in lymphoma cells.[23] The co-existence of CD20 and Src family kinases in the lipid rafts following rituximab treatment suggests a role of CD20-mediated cell signaling.

E. Cell signaling mediated by Rituximab—The findings that Rituximab inhibits cell proliferation and induces apoptosis suggested that it may trigger signal transduction. This

was supported by the findings that anti-CD20 antibodies redistribute CD20 into the lipid drafts, a cell signaling site.[24] The cytotplasmic domain of CD20 is not involved in transmembrane signaling and, thus, CD20 may be interacting with other molecules associated with signaling. Indeed, Rituximab treatment inhibits a Src kinase present in lipid drafts, namely, Lyn, and decreases both phospho-Lyn and phospho-Cbp/PAG.[24] Bezombes et al.[26] reported that treatment with Rituximab resulted in rapid, although transient, increase in acid sphingomyelinase activity with concomitant accumulation of cellular ceramide in the raft microdomains. Further, Dean et al.[27] reported that inhibition of cell growth by Rituximab is mediated through a ceramide-triggered pathway in a MAPK-dependent mechanism. These findings were corroborated by us with the demonstration that treatment of B-NHL cells with Rituximab inhibited several intracellular/anti-apoptotic signaling pathways.[28]

We have initially reported that treatment of AIDS-related lymphoma (ARC) cell lines with Rituximab resulted in inhibition of the JAK/STAT pathway and inhibition of the targeted gene product IL10 which was, in part, responsible for resistance.[29] Vega et al.[30] and subsequently Jazirehi et al.[31] reported that treatment of B-NHL cells with Rituximab inhibited the Raf/MEK/ERK pathway and downstream downregulated AP-1-dependent gene target transcripts. In addition, Rituximab treatment inhibited the NF-κB pathway.[28] The PI3K/AKT pathway was also inhibited by Rituximab in B-NHL cells.[32]

The above findings demonstrated the Rituximab can signal the cells via the CD20 receptor. However, the role of the Fc fragment in participating in cell signaling via interaction with FcRs on the target membrane was not examined. To address this issue, Rituximab (Fab'2) was generated and examined for cell signaling in comparison to wild type Rituximab. The findings revealed that treatment with (Fab'2) Rituximab resulted in inhibiting cell signaling pathways similar to Rituximab, both qualitatively and quantitatively.[33]

III. Rituximab-mediated chemo-immuno-sensitization of resistant B-NHL cells to apoptosis by chemo-immuno-therapeutic drugs

The above findings on cell signaling inhibition of intracellular survival/anti-apoptotic pathways by Rituximab treatment suggested that the treated cells threshold of resistance must have been significantly compromised and, thus, may be more sensitive to cytotoxic stimuli. This hypothesis was tested by delineating for each of the inhibited pathways by Rituximab of its involvement in the regulation of resistance as well as underlying molecular mechanisms involved. Rituximab-mediated chemo-sensitization and immuno-sensitization are briefly summarized below.

A. Rituximab-mediated chemosensitization

1. Role of p38 MAPK—The role of phospho-STAT3 inhibition by Rituximab in sensitization to drugs was examined.[29] Inhibition of p-STAT3 resulted in downstream inhibition of the IL-10 transcription factor SP-1 and inhibition of the transcription of its targeted gene product Bcl-2. The combination of treatment of Rituximab and CDDP resulted in the reversal of drug resistance of B-NHL cells and synergy in apoptosis was achieved.

The synergistic activity was the result of activation of the type II mitochondrial apoptotic pathway. The direct role of Rituximab-mediated inhibition of p38 MAPK activity in sensitization was corroborated by the use of specific chemical inhibitors.[30,34]

2. Role of the Raf/MEK/ERK pathway—The inhibition of the Raf/MEK/ERK pathway in B-NHL cells by Rituximab was paralleled by inhibition downstream of the transcription factor AP-1 and AP-1 transcription gene products including the anti-apoptotic gene product Bcl-xl.[35] The direct role each of the Raf/MEK/ERK pathway factors and Bcl-xl in chemosensitization was corroborated by the use of specific chemical inhibitors.

3. Role of the NF-κB pathway—The inhibition by Rituximab of the anti-apoptotic gene product Bcl-xl expression and the finding that the inhibition of Bcl-xl sensitizes the drug resistance B-NHL cells to apoptosis by various chemotherapeutic drugs[28] suggested that the NF-κB pathway, which also regulates Bcl-xl, might have been involved.[36,37] In fact, the NF-κB pathway was inhibited by Rituximab and its direct role in chemo-sensitization was corroborated by the use of various inhibitors of the pathway, which mimicked the Rituximab-mediated chemosensitization.[28]

4. Role of the PI3K/AKT pathway—In addition to the roles of the JAK/STAT3, Raf/MEK/ERK and NF-κB pathways, all of which regulate Bcl-xl, and all are inhibited by Rituximab, these findings suggested that, in addition, the PI3K/AKT pathway that also regulates Bcl-xl may be inhibited by Rituximab.[38] Rituximab treatment mediated inhibition of the PI3K/AKT pathway and resulted in the inhibition of p-PI3K, p-PDK1 and p-AKT with no inhibition of the unphosphorylated proteins. In addition, downstream of the PI3K pathway, Rituximab inhibited phospho-Bad leading to augmentation of the association of Bad with Bcl-xl and, thus, resulting in the inhibition of Bcl-xl activity on the mitochondria. The direct role of PI3K inhibition by Rituximab in chemosensitization was corroborated by the use of specific chemical inhibitors and by the use of small interference RNA (siRNA) AKT.[32]

B. Rituximab-mediated immunosensitization

Immune cells such as CTL and NK mediate their cytotoxic activity by both necrotic and apoptotic mechanisms.[39–41] Since rituximab treatment alone was shown to regulate the apoptotic pathways and leading to chemosensitization, we hypothesized that rituximab may also sensitize resistant B-NHL cells to immune-mediated apoptosis, namely, by the death ligands Fas-L and TRAIL.

1. Rituximab-mediated sensitization to Fas-L-mediated apoptosis—We have reported that treatment of B-NHL with rituximab sensitized the cells to recombinant Fas-L-induced apoptosis.[42] The mechanism underlying sensitization was examined. Previous findings demonstrated that the transcription factor, Yin Yang 1 (YY1), negatively regulates Fas transcription and expression and inhibition of YY1 resulted in the upregulation of Fas expression and sensitization to Fas-L apoptosis.[43] Since YY1 is a target gene product of NF-κB and we have shown that NF-κB is inhibited by rituximab, we expected that inhibition of NF-κB by rituximab will be accompanied by inhibition of YY1 and, therefore, sensitization

of B-NHL to Fas-L apoptosis. Indeed, the findings corroborated this hypothesis. The rituximab-mediated sensitization to Fas-L apoptosis was the result of the activation of type II mitochondrial apoptotic pathway.[42]

2. Rituximab-mediated sensitization to—TRAIL apoptosis Like Fas-L above, we have found that treatment of TRAIL-resistant B-NHL cells with rituximab sensitized the cells to recombinant TRAIL-induced apoptosis. Based on previous findings demonstrating that YY1 negatively regulates the TRAIL receptor DR5 and regulates resistance to TRAIL, its inhibition sensitized the cells to TRAIL apoptosis along with upregulation of DR5.[44] We have also found that rituximab sensitization to TRAIL apoptosis was accompanied by upregulation of DR5 along with inhibition of YY1. In addition, treatment of cells with YY1 siRNA sensitized the cells to TRAIL apoptosis and, thus, mimicking rituximab.[45]

IV. Resistance to Rituximab

Currently, the combination of Rituximab and chemotherapy (CHOP) is the approved protocol for the treatment of B-NHL. While the use of Rituximab for treatment has been successful, however, a subset of patients has an innate resistance. In follicular lymphoma only about 15% of patients respond to the initial treatment with Rituximab monotherapy.[46] Furthermore, the majority of responders becomes refractory to Rituximab.[47] The five year overall survival (OS) for patients with low grade follicular lymphoma who fail to respond to or develop resistance to Rituximab or Rituximab containing treatment regiments is 58%[48], which is markedly a decrease compared to survival of all lymphoma patients.[49]

The mechanisms of resistance *in vivo* are not clear. Several mechanisms have been reported including inhibition of ADCC by deposition of C3 activating fragments[50], polymorphism of the FcγRIIIa on cytotoxic cells,[51,52] inhibition of CDC,[53] loss of CD20 expression on the surface of subclones,[47,54] overexpression of anti-apoptotic gene products (eg Bcl2)[55], CD20 mutations,[56] shedding of CD20 Rituximab complexes,[57] the tumor micro-environment,[58] distribution of Rituximab *in vivo* and its pharmacokinetics and failure to respond to Rituximab-mediated cell signaling. Briefly (below each) the postulated mechanisms are presented.

A. Poor ADCC

ADCC neutralizes the Fc fragment of bound Rituximab to interact with the FcR on cytotoxic cells (e.g. NK, macrophages, neutrophils) to initiate the cytotoxic process. Some patients showed expression of a variant FcγRIIIa and receptor expressing 158 V and 158 F cell types. The homozygosity of the FcγRIIIaA-158 V allotype was the single parameter associated with the clinical response of Rituximab at 12 months post treatment.[51,59] Rituximab-resistant clones were also shown to be resistant to ADCC.[55] These findings established the importance of ADCC in the clinical response to Rituximab.

B. Inhibition of CDC

Rituximab is capable of binding to $C1q^3$ and thus activating the complement cascade. The major contributors to CDC resistance consists of CD46 (MCP), CD55 (DAF), and CD59. The levels of CD20 and complement inhibitors were determinants in the clinical response of

isolated BCLL, prophylactic leukemia (PLC) and MCL. Blocking of the inhibitors enhanced CDC.[60] However, a controversy exists on the relevance of CDC and inhibitors in response to Rituximab.[52] Rituximab resistance clones were also resistant to CDC.[55]

C. Resistance to apoptosis

Rituximab treatment alone poorly displays apoptosis on B-NHL cells. However, cross-linking with secondary anti-Ig triggers a significant apoptotic response. Treatment of Rituximab-resistant clones, however, with Rituximab and secondary anti-Ig did not trigger apoptosis.[55,61]

D. CD20 modulation

The role of CD20 expression levels and response to Rituximab is not clear *in vivo.* Terner, et al.[62] reported deletion at the C terminal region of the *CD20* gene in a subset of tumor samples from patients with NHL. However, it is not clear what was the clinical response in those patients. Also, Pederson, et al.[63] described a process “shaving” whereby complexes of CD20 and Rituximab are shed off from the cells by monocytes and, hence, the cells become unresponsive to Rituximab. We have also reported that the *in vitro* generation of Rituximab-resistant clones showed a reduction of cell membrane CD20 expression compared to wild type.[51,61] Treatment of patients (relapsed and refractory) with a combination of Rituximab and Bortezomib resulted in ORRs of 49% and 43% respectively, for two different regimens.[64] The loss of CD20 expression was observed following Rituximab treatment in a subset of patients. There were cases of CD20 deficient lymphoma relapses identified following treatment with Rituximab containing regimens in DLBCL.[54] In addition, Hiraga et al.[65] reported that 30% of patients with B-cell lymphoma and treated with Rituximab and chemotherapy, their tumor CD20 expression was lost. DNA demethylating agents restored CD20 expression suggesting an epigenetic mechanism responsible for loss of CD20.

E. Generation of Rituximab-resistant clones

A survey[66] of 92 immortalized normal lymphoblastoid cell lines and separated for sensitive and resistant lines. The findings were as follows: (1) The level of CD20 protein and surface expression was decreased in the resistant lines. (2) The susceptibility was not correlated with mRNA that is post-transcriptional. They also have selected resistant cell lines that were cultured with Rituximab in various LCL and lymphoma cell lines. They found that levels of CD20 expression were reduced in all resistant cell lines. Also, they found CD20 mRNA spliced variants associated with resistance. In addition, ofabumumab was more active as compared to Rituximab *in vitro*.

In an effort to recapitulate what might be responsible, in part, for resistance to Rituximab in patients, we have generated Rituximab-resistant clones (RR) from several B-NHL cell lines. These were developed by exposing wild type cells to increasing concentrations of Rituximab in culture and followed by multiple rounds of limiting dilution assays. The resulting RR clones were grown and analyzed for their properties compared to wild type cells. Several key findings were obtained. In comparison with the wild type cells, the RR clones expressed lower surface CD20 expression, resistance to both ADCC and CDC, and did not respond to Rituximab-mediated inhibition of cell proliferation, apoptosis by cross-linking and were not

chemo- or immuno-sensitized to drug apoptosis. The RR clones were not triggered by Rituximab to inhibit intracellular survival/anti-apoptotic pathways and, in contrast, the RR clones exhibited hyper activated survival pathways, such as the NF-κB and ERK1/2 pathways and overexpression of anti-apoptotic gene products, targets of above pathways such as Bcl2, Bclxl, and Mcl-1.[55,61] Czucman et al.[67] developed RR cell lines. They have found that the resistant cell lines have had changes in CD20 expression, decreased calcium mobilization and redistribution of CD20 in lipid grafts. There was a partial reversion of resistance by proteasome inhibitors (CCR 2008; 14156). In a subsequent study, Brem et al.[68] reported that treatment with the BH3 mimetic Obatoclax induced cell death in both Rituximab sensitive and Rituximab resistant cell lines and also in primary tumor cells. Synergy was achieved by a combination of Obatoclax and chemotherapeutic drugs. Using the more selective proteasome inhibitor Carfilzomib, in comparison with the predecessor Bortezomib, showed significant augmentation of cytotoxicity in the resistant lines and reversed resistance to chemotherapy. There was upregulation of the apoptotic gene product Bak.[69] Further, studies with the reversible proteasome inhibitor MLN2238 showed that it induces caspase independent cell death of the resistant cell lines and potentiated the cytotoxic activity of various chemotherapeutic drugs.[70]

V. Overcoming Rituximab resistance (Schematic diagram-Figure 1)

The development of RR clones and their unresponsiveness to Rituximab alone or in combination with drugs allowed the exploration of mechanisms underlying resistance and to examine means to overcome resistance. We have reported that the RR clones exhibited hyper-activated survival/anti-apoptotic pathways and we also reported that interference with the activity of such pathways in the Rituximab sensitive lines by various chemical inhibitors resulted in chemo-immuno-sensitization, mimicking Rituximab in combination with cytotoxic agents in wild type rituximab-sensitive B-NHL cells.[55,61] Therefore, we examined if inhibition of intracellular pathways can sensitize RR clones to cytotoxic agents.

The inhibition of NF-κB and downstream Bclxl expressed by Rituximab treatment in sensitive lines was corroborated by the use of inhibitors such as the inhibitors of NF-κB (DHMEQ and Bay-7805) and impairment of the function of Bclxl (by 2MAM-A3). Treatment with these inhibitors sensitized the B-NHL cells to various chemo therapeutic drugs. In the RR clones, treatment with the proteasome inhibitor Bortezomib in combination with chemotherapeutic drugs sensitized the RR cells to apoptosis. Further, inhibition of NF-κB by DHMEQ also sensitized the RR cells to apoptosis by various drugs.[33] Maiso, et al.[71] have reported that HDAC inhibitors reversed drug resistance in MM. Valproic acid and romidepsin were used on B-cell lines and resulted in augmentation of CD20 expression, enhancing CDC activity, and *in vivo* in mice bearing tumor xenografts resulted in response to combination of Rituximab and HDAC inhibitors.

The enhancement of CD20 expression in cases where resistance to Rituximab was observed, due in part, to downregulation of CD20 expression may be attempted to reverse resistance. Shimizu, et al..[72] reported that treatment of cells with HDAC inhibitors, valproic acid and romidepsin increased the expression of CD20 and enhanced Rituximab-mediated CDC cytotoxicity. This finding suggested that the epigenetic regulation of CD20 expression and

clinical application of HDAC inhibitors in combination with drugs or Rituximab may be effective.

Resistance to Rituximab appears to be due, in part, as a result of the overexpression of anti-apoptotic gene products and as a consequence overactivation of survival pathways that regulate these gene products as has been reported in RR clones.[55, 61] Bortezomib[50] and Temsirolimus[73] treatment of lymphoma cells sensitized the cells to Rituximab. Clinical trials have been run for the combination of Rituximab and Bortezomib for salvaged treatment for B-NHL.[64]

The Bcl2 pan inhibitor, Oblimersem, was clinically tested in a phase I and demonstrated inhibition of Bcl2 levels and an objective clinical response in heavily treated patients.[24] In a phase II combination of Rituximab and Oblimersem, there was a modest activity in patients with residual disease and in patients with DLBCL. A higher RR resistance was eliminated in Fl. The combination of Rituximab and Oblimersem was effective in patients refractory to Rituximab.[75] ADCC has been shown to play an important role *in vivo* in Rituximab. Thus, augmenting ADCC may improve Rituximab effects and may override resistance in a subset of resistant patients. In clinical trials, G-CSF and Rituximab produced a responsive rate of 42% in patients with indolent NHL,[76] with a possible longer duration of response compared to Rituximab alone. Combination of IL2 and Rituximab in Rituximab-resistant indolent NHL did not produce a clinical response.[77] Lenalidomide, an immuno-modulating drug, enhances ADCC and reverses immune suppression. Single agents have therapeutic activity in relapsed/refractory B-cell lymphoma. In a phase 2 trial, in patients resistant to rituximab, relapsed or refractory indolent B or mantle cell lymphoma. Lenalidomide in low dose with dexamethasone and rituximab were used. The combination achieved a high response rate with durable response. The over response rate increased from 29% after 2 cycles of lenalidomide and dexamethasone to 58% before the addition of rituximab.[78]

Combination of Rituximab and anti-CD22 mAb, epratuzumab

The fusion protein anti-CD20-hIFN-α, consists of anti-CD20 and hIFN-α, was engineered and shown to have a more potent activity and apoptosis on B-NHL cell lines *in vitro* and anti-tumor response *in vivo*.[79] Preliminary findings indicated that treatment of RR clones with anti-CD20-hIFNα, but not with Rituximab-hFNα. or combination, resulted in inhibition of cell recovery, induction of apoptosis, and sensitization to chemotherapeutic drugs.[80]

Novel anti-CD20 mAbs

1. Ofatumumab: patients with rituximab resistant follicular—NHL and treated with ofatumumab resulted in response rate of 20–22% and patients resistant to single agent rituximab with response rate of 9% and patient treated with combination treatment with rituximab and chemotherapy.[81] Ofatumumab is a fully humanized anti-CD20 mAb approved by the FDA.[82, 83] Ofatumumab was found to induce signaling for CDC, more potent than rituximab. It shows activity in rituximab-refractory lymphoma.[7]

2. GA-101 (Type 2) with Fd engineered for higher affinity for FcR.[83] There was no indication about the response in patients with rituximab resistance—The development of novel scaffolds of much smaller sequences and higher stability.[85–87] The size of the scaffolds (12–15 kDa) is an order of magnitude smaller than the size of IgG (160 kDa). These scaffolds leads to good penetration, they are more stable in the circulation and could be taken orally. Also, they can penetrate the blood brain barrier.

IX. Conclusions

This mini-review described briefly the current status of rituximab and suggested mechanisms of innate and developed resistance in patients to both rituximab monotherapy and rituximab-containing chemotherapy regimens. There are also suggestions of various means to reverse resistance to rituximab treatment based on analysis of rituximab-resistant clones investigated *in vitro*. Clearly, some of the mechanisms of resistance of the rituximab-resistant clones may or may not be applicable to the mechanisms *in vivo*; however, they will need to be validated. Future directions require the development of various clinical trials to address some of the postulated mechanisms and their validation. In addition, further studies from patient-derived tumor tissues from unresponsive patients may be useful to examine gene products that regulate resistance and validate their prognostic significance as novel biomarkers.

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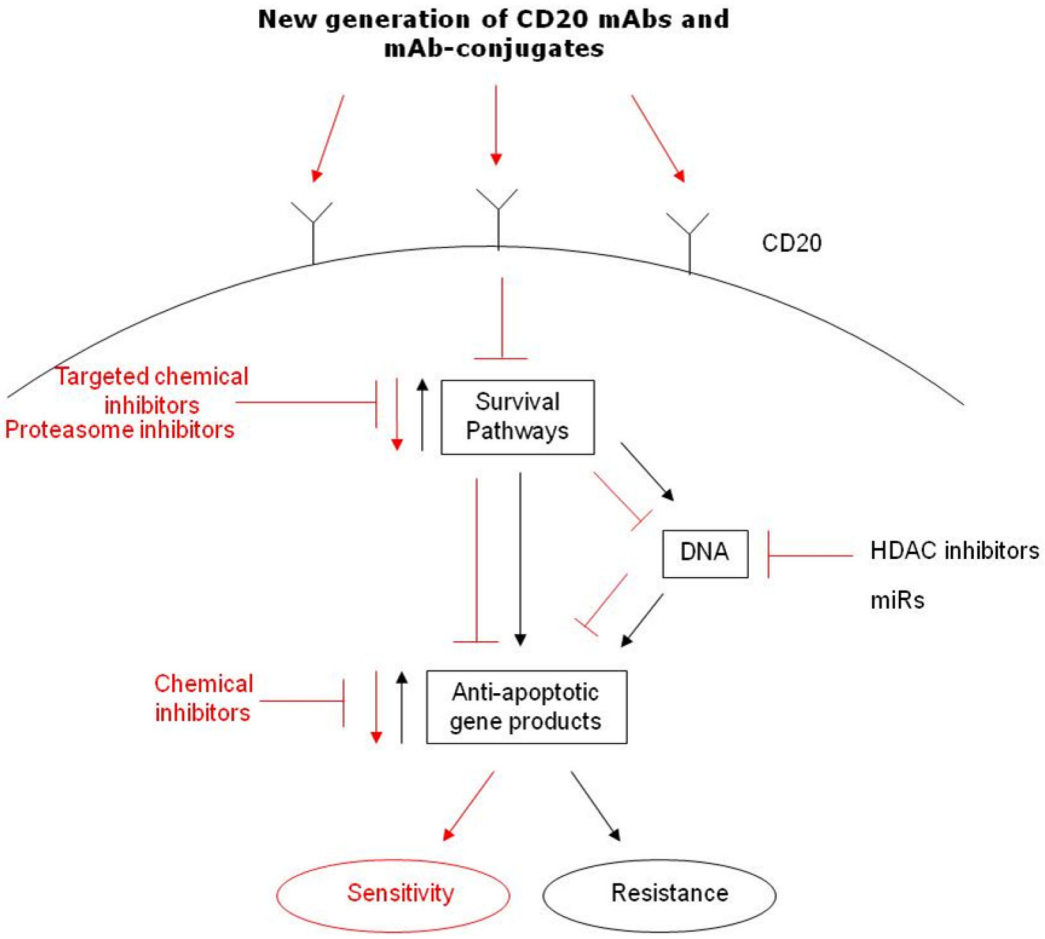

Figure 1.

Schematic diagram of the molecular mechanisms that regulate resistance to rituximab and approaches to overcome resistance. Briefly, the unresponsive B-NHL cells exhibit hyperactivated survival/anti-apoptotic pathways which regulate downstream anti-apoptotic gene products that result in the development of resistance to both rituximab, chemotherapy and combination. The intervention to inhibit the survival pathways may be achieved by targeted chemical inhibitors, proteasome inhibitors, selective chemical inhibitors for the anti-apoptotic gene products, as well as HDAC inhibitors and microRNAs. In addition, the potential application for the development of a new generation of CD20 mABs, alone or conjugated with various agents to enhance their activities.