Interindividual Variability of Response to Rituximab: From Biological Origins to Individualized Therapies

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Abstract

Rituximab has markedly changed the treatment of B-cell malignancies. Despite its widespread use, however, its precise mode of action and the impact of host- and tumor-related factors on rituximab-activated biological pathways were only recently clarified. Biological mechanisms resulting in complete resistance to rituximab may exist at both the cellular and subcellular level; however, their frequency and their impact on clinical response are unclear. The identification of Fcγ receptor polymorphisms that can influence anti-CD20 antibody activity has resulted in the development of third-generation anti-CD20 antibodies. However, it is also now appreciated that pharmacokinetic variability is a major factor affecting clinical response to anti-CD20 antibodies. The concept of antigenic mass, which takes into account the total tumor load and the expression levels of the target antigen CD20, is able to explain the correlation between rituximab plasma concentrations and treatment responses. Thus, it can be hypothesized that dosing regimens that take this information into account will help to improve response rates. Clin Cancer Res; 17(1); 19–30. © 2011 AACR.

Rituximab has become the standard of care for treatment of aggressive and indolent non-Hodgkin's lymphomas (NHLs) and chronic lymphocytic leukemia (CLL). In these indications, the addition of rituximab to standard chemotherapy has resulted in a significant improvement in patient survival. Rituximab has been widely used in clinical practice for a number of years, but it is only recently that we have started to better understand its precise biological modes of action in humans. Recent findings on the role of host- and tumor-related factors in pathways activated by rituximab have provided insight into the interindividual variability of response to rituximab treatment for CD20-positive lymphoma (1), and recently published studies have described different biological mechanisms whereby lymphoma cell lines can gain resistance to rituximab (2, 3).

In this review we summarize the recent clinical literature that suggests considerable interindividual variability in treatment response resulting from host-related parameters, tumor characteristics, or rituximab itself. We review the current understanding of the biological mechanisms of action of rituximab and describe emerging strategies to increase the efficacy of anti-CD20 treatment. These strategies include new rituximab dosing concepts and the use of new generations of anti-CD20 antibodies. Because most of the causes of response variability for rituximab can be applied to other humanized immunoglobulin (Ig) G1 antibodies, the issues of response variability versus complete resistance to the antibody are likely to be critical for optimizing many antibody-mediated treatment strategies.

Mechanisms of Rituximab Action

The mechanisms of action for rituximab include apoptosis, complement-dependent cytotoxicity (CDC), and Fcγ receptor (FcγR)-mediated mechanisms, including antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (Fig. 1). Other mechanisms, such as complement-enhanced ADCC (CR3-ADCC) and a vaccinal effect whereby cell kill by rituximab results in elicitation of a lymphoma-specific T-cell response, have also been suggested (4, 5). Most studies evaluating rituximab mechanisms have been performed in vitro on CD20-positive lymphoma cell lines and fresh lymphoma cells or in vivo in murine models. It is therefore difficult to evaluate the relative contribution of each of these mechanisms in patients, especially as such contributions may vary according to lymphoma subtype.

Binding of type I anti-CD20 antibodies such as rituximab to CD20 induces rapid translocation of the molecule to lipid rafts (6, 7). Lipid rafts are signal transduction zones that enable the colocalization of receptors and signal effectors. CD20-induced apoptosis is significantly reduced by C-terminal Src kinase (Csk) inhibitors (8), caspase inhibitors, and calcium chelation (8–10), supporting a model in which CD20 ligand binding induces
Translational Relevance

We present an overview of the current knowledge about the mechanism of action for anti-CD20 monoclonal antibodies in the treatment of B-cell malignancies. We discuss the impact of host- and tumor-related factors on the interindividual variability of response, as well as the role of Fcγ receptor polymorphisms, which led to the development of third-generation anti-CD20 antibodies. Because differential pharmacokinetics has been shown to be a major factor in the variability of response to rituximab, and treatment response appears to be directly correlated with rituximab plasma concentration, which in turn varies depending on the lymphoma type and tumor burden, a recently established pharmacokinetic/pharmacodynamic model for dosing of rituximab is discussed. It is essential to understand the reasons behind the interindividual variability of clinical response, and the effects of that variability, to increase therapeutic efficacy and hence clinical benefits.

Csk activity, leading to phospholipase C-γ phosphorylation, increased cytoplasmic Ca2+, and subsequent caspase-3 activation (8, 11). However, the final step of caspase-3 activation has been questioned because apoptosis is not blocked by caspase-specific inhibitors or Bcl-2 overexpression (12, 13).

During the classical complement-activation pathway, the first step is the binding of C1q components to the Fc portions of IgG (Fig. 1). This binding triggers a proteolytic cascade, resulting in the generation of large portions of C3b. C3b molecules act as opsonins but also bind to C3 convertase to form a C5 convertase, leading to the generation of membrane attack complexes that kill by disrupting the cell membrane. Although C3 is abundant in plasma, C3b is rapidly inactivated unless it is bound to the C5 convertase or to complement receptors (CRs). CRs are expressed on effector cells such as granulocytes, macrophages, and natural killer (NK) cells, and can induce cell-mediated lysis (CR3-ADCC). Antibody-triggered tumor killing is achieved by the interaction of antibody Fc portions and FcγRs (Fig. 1) (14–18), resulting in the activation of immune effector cells such as monocytes/macrophages, neutrophils, and NK cells expressing FcγRs. The recruitment of NK cells and monocytes/macrophages by FcγRIIa induces the release of cytotoxic granules leading to cell death via ADCC, whereas the recruitment of FcγRIIa leads to phagocytosis of the target cell (19). In a study by Minar-Colin et al., (20), the role played by FcγRs in lymphoma B-cell depletion was revealed in an FcγRs KO murine model with the use of mouse CD20 antibody. Although there is a body of evidence showing that complement activation and ADCC occur in vivo (21), the apoptotic effects described for rituximab on cell lines in vitro still remain to be demonstrated in humans.

Origin of Interindividual Variability of Response to Rituximab

Most indolent lymphoma patients experience a variable decrease in the size of their tumor after rituximab monotherapy (Fig. 2); however, the origin of the interindividual variability of tumor response to rituximab can be related to the number and relative importance of different mechanisms of resistance affecting any of these modes of action. These biological mechanisms of resistance can be related to interindividual parameters, tumor characteristics, or the antibody itself.

Tumor-related origins

Histology subtype has been described as the main tumor parameter that influences rituximab efficacy. In a pivotal study by McLaughlin et al., (22), small lymphocytic lymphoma histology negatively influenced the objective response rate compared with other low-grade lymphomas. Several studies have described a low objective response rate in patients with CLL treated with the standard dose of 375 mg/m² rituximab monotherapy used in NHL (23–26). Furthermore, Ghieolini and colleagues (27) showed that patients with mantle cell lymphoma (MCL) had a reduced event-free survival versus patients with follicular lymphoma after treatment with single-agent rituximab. The level of CD20 expression, which can differ depending on tumor histology (19), was initially suggested to explain the variation in rituximab efficacy among different histologic subtypes; however, the in vitro data were conflicting (19, 28–31). More recently, van Meerten and colleagues (32) employed clonally related CD20-positive transgenic cells collectively covering a broad spectrum of CD20 expression levels to show that the CD20 expression level correlates with rituximab-mediated killing via CDC, but does not significantly affect killing via ADCC.

However, chronic exposure of cell lines to rituximab can lead to the acquisition of a phenotype resistant to rituximab-induced CDC and ADCC and downregulation of CD20. CD20 downregulation can result from both reduced mRNA levels and posttranscriptional modification, which in turn lead to a truncated CD20 protein (2). Hiraga and colleagues (33) recently presented evidence that epigenetic mechanisms may be partly related to the downregulation of CD20 expression after rituximab treatment, but to date there is no evidence that CD20 downregulation is an underlying cause of rituximab-refractory disease. In several case reports and small series, investigators have examined the development of CD20-negative phenotypes after treatment of CD20-positive lymphoma in different clinical settings, but the frequency with which this occurs still is unclear (33–39).

Similarly, in one study (3), repeated exposure of lymphoma cell lines to rituximab was associated with a rituximab-resistant phenotype due to modulation of Bax and Bak expression, resulting in resistance not only to rituximab-induced apoptosis but also to multiple chemotherapeutic agents. Rituximab-induced killing via CDC and
ADCC was not affected. Another study showed that exposure of lymphoma cell lines to rituximab may act on survival pathways, including p38MAPK, NK-kB, and PI3/akt, leading to chemosensitization (40). The observation that addition of rituximab to chemotherapy mainly benefits patients with diffuse large B-cell lymphoma (DLBCL) expressing bcl2 also argues for a chemosensitization activity of rituximab (41). Differences in active cell survival pathways may therefore underlie the differences in susceptibility to rituximab and chemotherapy among histological subtypes. Because CD20 translocation into lipid rafts is critical for rituximab and other type I anti-CD20 antibody binding, but not type II anti-CD20 antibody binding, membrane phospholipids or cholesterol content may have an impact on type I anti-CD20 antibody efficacy. Meyer zum Büschenfelde and colleagues (42) reported that expression of raft-associated sphingolipid GM1 is associated with the susceptibility of different lymphoma cell lines to rituximab, correlating with the recruitment of CD20 to lipid rafts. Furthermore, it was recently shown that statins induce conformational changes in the CD20 antigen in lymphoma cell lines, resulting in a decrease in rituximab-induced CDC and, to a lesser extent, ADCC (43). Binding of rituximab to CD20 and rituximab-induced CDC activity were restored by exogenous cholesterol supplementation. These data indicate that although the impact of histology subtype on rituximab efficacy is well demonstrated, the contribution of the CD20 expression level remains unclear as long as the lymphoma stays CD20 positive.

**Host-related origins**

Gender is a constitutive parameter that influences exposure to monoclonal antibodies and may potentially...
influence response and survival after treatment with rituximab. Ng and colleagues (44) reported a significant increase in rituximab clearance in men treated for rheumatoid arthritis compared with women, leading to a decrease in rituximab exposure of 30% in men. The influence of gender on antibody pharmacokinetics has also been described in studies with infliximab (45) and bevacizumab (46). The influence of gender on rituximab clearance rates or therapeutic response remains to be shown in the context of lymphoproliferative disorders.

Polymorphisms that are functionally relevant to the rituximab mode of action have been identified (Fig. 1). FCGR3A, which encodes the FcγRIIIa protein, displays a nucleotide polymorphism at position 559 that results in an amino acid change at position 158 of FcγRIIIa, which affects its affinity for human IgG1 (47, 48). Thus, human IgG1 binds more strongly to NK cells homozygous for FCGR3A-158V than to NK cells homozygous for FCGR3A-158F (48). In previously untreated follicular lymphoma, rates of response to rituximab monotherapy were significantly higher for FCGR3A-158V homozygous patients than for FCGR3A-158F carriers (49). Because FcγRIIIa is expressed only by monocytes/macrophages and NK cells (the main actors in ADCC), we have postulated that patients homozygous for FCGR3A-158V experience significantly better responses to rituximab because they have enhanced ADCC activity compared with FCGR3A-158F carriers. This was confirmed in other studies using rituximab (50, 51) and for other IgG1 monoclonal antibodies, including trastuzumab (52) and cetuximab (53). It is important to note that some FcγRIIIa-158V homozygous patients can experience progression after rituximab treatment, whereas FcγRIIIa-158F homozygous patients can maintain a complete response, indicating that other factors may affect rituximab efficacy (49). Therefore, FCGR3A polymorphism is one of the interindividual parameters that can influence response to monoclonal antibodies, which explains part of the variable response observed in clinical practice. The extent of the clinical effect of FCGR3A polymorphisms may depend on the varying importance of ADCC in different lymphoma subtypes (54), the presence of other biological mechanisms of resistance, or the racial origin of the patient. The FCGR3A-158V homozygous genotype is found in only 20% of the Caucasian population (47, 55) but in >40% of the Chinese population (56). The difference between these two populations with regard to the V allotype frequency may explain why the only study that showed the prognostic value of FCGR3A for rituximab combined with chemotherapy included Chinese patients (50). Weng and Levy (50) also showed that the FcγRIIa-131H/R polymorphism significantly affects response and time to progression after rituximab, with better results for FcγRIIa-131H homozygous patients. However, other authors found that this polymorphism affects the binding of FcγRIIa to human IgG2 but not to human IgG1 (57). The predictive value of the FCGR2A polymorphism was further called into doubt by studies that found no impact on response (52, 58), and by the suggestion that the effect results from linkage disequilibrium between FCGR3A and FCGR2A polymorphisms (59).

The genetic heterogeneity of CD11b, which plays a major role in rituximab’s CR3-ADCC, may also influence response to rituximab. We recently showed that a polymorphism located in the CD11b region interacting with C3bi influences progression-free survival (PFS) after rituximab treatment in follicular lymphoma (Cartron, G, Rossignol A, Selles G. et al. Unpublished).

Finally, NK cell target recognition essentially depends on the surveillance of human leukocyte antigen (HLA) class I molecules by killer Ig-like receptors (KIRs), modulating FcγRIIa- and CR3-dependent NK cell cytotoxicity (Fig. 1) (60). The effector functions of KIRs are thought to result from a balance between inhibition and activation signals to the cell. Although clinical and laboratory data on this issue are lacking, different HLA/KIR genotypes or different concentrations of KIR ligands (31) could impact different thresholds of activation to the NK cell repertoire, and thus might influence both the ADCC and CR3-ADCC response to rituximab.
To date, there is no evidence that inherited mutations or polymorphisms of the CD20 gene lead to a poor response to rituximab (39, 61).

**Antibody-related origins**

In a phase II trial in which patients with recurrent low-grade lymphoma were treated with four infusions of rituximab monotherapy, a correlation between rituximab concentrations and clinical response was identified, with responders having significantly higher rituximab serum levels than nonresponders (22). Further analysis revealed an inverse correlation between rituximab serum level and both tumor burden and lymphocyte count at baseline (62). These results suggest that the distribution, availability, and number of tumor antigens are factors that can influence rituximab efficacy (63). To test the hypothesis that antigenic mass can influence rituximab serum levels and treatment efficacy, we developed a murine model of disseminated lymphoma-expressing human CD20. A clear dose-response relationship was shown in this model, with increasing doses of rituximab leading to higher response rates and improved survival (64). With a fixed dose of rituximab, mice with a high tumor burden had lower rituximab serum levels and decreased survival compared with mice with a low tumor burden, and the rituximab serum level was associated with response to treatment (Fig. 3A). By applying pharmacokinetic/pharmacodynamic (PK/PD) models, we further showed that rituximab efficacy correlates directly with antigenic mass (65). An exploratory analysis of the prognostic significance of tumor bulk in young patients with good-prognosis DLBCL treated with chemotherapy with or without rituximab showed a linear prognostic effect of tumor bulk on event-free survival and overall survival (OS) (65). This effect was decreased (but not eliminated) by the addition of rituximab, also pointing to a negative effect of a high tumor mass that cannot be overcome by standard doses of rituximab (Fig. 3B). Thus, the evidence suggests that the variability of response to a fixed dose of rituximab can result from interindividual differences in tumor mass and CD20 antigen expression, with lower rituximab serum concentrations and a less favorable distribution of the antibody in patients with a high tumor burden, resulting in a poorer response to treatment that might be overcome by adjusting the dose of rituximab.

Epitope recognition by anti-CD20 antibodies can also influence treatment efficacy, and it has been shown that the ability of anti-CD20 antibodies to induce CDC is related to their ability to translocate CD20 into lipid rafts (66).

**Biological Resistance and Clinical Refractory Disease**

There are some limitations to extrapolating *in vitro* data on mechanisms of resistance to clinical practice. Most of the *in vitro* studies on this subject used B-lymphoma cell lines, which are distant from native tumor cells. It is also difficult to establish a system that can reproduce *in vitro* the complexity of rituximab activity involving both intra- and extracellular mechanisms. This technical point limits the conclusions of studies conducted with fresh lymphoma cells. Murine models do not completely overcome these limitations, mainly because of interspecies differences in expression and function of FcγRs (67). Although each murine FcγR recognizes human IgG1, any translation to the human situation must be done with caution. To accurately dissect rituximab resistance from rituximab-refractory disease, we need a precise definition. To evaluate the efficacy

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**Figure 3.** Correlations between tumor burden and rituximab treatment in a murine model and patients with NHL. A, survival after rituximab infusion was associated with tumor burden in a murine model of human-CD20 expressing lymphoma (65). B, survival was associated with tumor burden in a murine model and patients with NHL. A, survival after rituximab infusion was associated with tumor burden in a murine model of human-CD20 expressing lymphoma (65). B, survival was associated with tumor burden in a murine model and patients with NHL. A, survival after rituximab infusion was associated with tumor burden in a murine model of human-CD20 expressing lymphoma (65).
of therapies against B-cell malignancies, physicians and regulatory bodies use international response criteria (68). In these criteria, the term “refractory,” rather than “resistance,” is used to define failure to respond to treatment. According to the International Working Group criteria, refractory disease is defined as relapsed or progressive disease (PD) during or within 6 months after completing treatment. In contrast, resistance is a biological concept impacting clinical efficacy of a drug and results from a complex array of mechanisms, including delivery issues, intracellular metabolism, pharmacokinetics, targets, and challenged intracellular pathways (e.g., the apoptotic pathway). In clinical practice, patients may have or develop some kind of resistance resulting from one or more of these issues, which can result in a variable clinical response to a drug, and only those with a critical amount of resistance will not respond to therapy at all, resulting in PD or stable disease (SD).

Frequency of Rituximab-Refractory Disease

To accurately evaluate the incidence of rituximab treatment failure, it is necessary to focus on results obtained in studies of rituximab monotherapy. In low-grade lymphomas, <10% of patients not previously exposed to rituximab progress after four once-weekly rituximab infusions (Table 1) and ~20% of patients experience SD (22, 69, 70). According to the International Working Group criteria, SD includes patients with a <50% decrease in tumor mass as well as patients with a <50% increase. In the pivotal rituximab trial, most of these patients experienced a decrease in their disease burden (Fig. 2) (21).

Rituximab monotherapy is not indicated for the treatment of DLBCL or MCL, and only a few studies have evaluated rituximab monotherapy for these indications in first- and salvage-line treatment, showing PD rates of up to 43% (27, 71, 72).

In posttransplant lymphoproliferative disease (PTLD), where DLBCL is related to immunosuppression, patients can achieve long-lasting remission with rituximab monotherapy and PD rarely occurs during treatment with rituximab, whereas patients who do not achieve a complete response (CR) have rapid disease progression shortly after treatment. This results in different PD and SD rates depending on the time of treatment evaluation, with PD rates of 51% in the trial reported by Choquet and colleagues (73).

Table 1. Frequency of PD in trials of rituximab monotherapy for indolent or aggressive CD20-positive B-cell NHL in patients who had not previously received rituximab

<table>
<thead>
<tr>
<th>Therapy and NHL</th>
<th>Indication</th>
<th>Study phase</th>
<th>No. of patients in R arm(s)</th>
<th>PD, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL and MCL</td>
<td>4× R-mono</td>
<td>Salvage-line FL and indolent lymphoma</td>
<td>II</td>
<td>166</td>
<td>&lt;13</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>First-line FL</td>
<td>II</td>
<td>49</td>
<td>6</td>
<td>(69)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>First-line FL</td>
<td>II</td>
<td>36</td>
<td>0</td>
<td>(70)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>First- and salvage-line FL and MCL</td>
<td>III</td>
<td>273</td>
<td>18</td>
<td>(27)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>First- and salvage-line MCL, IMC, SLL</td>
<td>II</td>
<td>120</td>
<td>19</td>
<td>(72)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>Salvage-line FL and SLL</td>
<td>II</td>
<td>114</td>
<td>21</td>
<td>(97)</td>
</tr>
<tr>
<td>B-PTLD</td>
<td>4× R-mono</td>
<td>First-line B-PTLD</td>
<td>II</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>First-line B-PTLD</td>
<td>II</td>
<td>43</td>
<td>51</td>
<td>(73)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>First-line B-PTLD</td>
<td>II</td>
<td>38</td>
<td>34</td>
<td>(93)</td>
</tr>
<tr>
<td>Aggressive NHL</td>
<td>8× R-mono</td>
<td>Salvage-line DLBCL, MCL, other aggressive lymphoma</td>
<td>II</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>CLL</td>
<td>4× R-mono</td>
<td>First-line CLL and SLL</td>
<td>II</td>
<td>44</td>
<td>32</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>Salvage-line CLL</td>
<td>I</td>
<td>40</td>
<td>NR</td>
<td>(26)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>Salvage-line CLL</td>
<td>II</td>
<td>28</td>
<td>32</td>
<td>(24)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>Salvage-line CLL</td>
<td>II</td>
<td>24</td>
<td>&lt;15</td>
<td>(99)</td>
</tr>
<tr>
<td>4× R-mono</td>
<td>Salvage-line CLL/SLL/FCC/MCL/DLCL</td>
<td>II</td>
<td>48</td>
<td>21</td>
<td>(24)</td>
</tr>
<tr>
<td>12× R-mono</td>
<td>First- and salvage-line CLL and SLL</td>
<td>I/II</td>
<td>29</td>
<td>10</td>
<td>(22)</td>
</tr>
</tbody>
</table>

Abbreviations: NHL, non-Hodgkin’s lymphoma; R, rituximab; PD, progressive disease; FL, follicular lymphoma; MCL, mantle cell lymphoma; mono, monotherapy; IMC, immunocytoma; SLL, small B-cell lymphocytic lymphoma; PTLD, posttransplant lymphoproliferative disease; DLBCL, diffuse large B-cell lymphoma; CLL, chronic lymphocytic leukemia; NR, not reported.
tumor burden (Tables 1 and 2; Fig. 2). Some patients did not respond to rituximab monotherapy at doses of 500–825 mg/m², but the response rate was improved at higher dose levels (1000–2250 mg/m²). Although this was a nonrandomized study, the authors reported complete response rates of 50% versus 19% and minimal residual disease-negative rates of 33% versus 0% which patients received only four doses of rituximab (22).

In the treatment of low-grade lymphoma, Piro and colleagues (91) reported that eight once-weekly infusions of rituximab at 375 mg/m² were compared with nine doses of rituximab at 750 mg/m² over 12 weeks. Although this was a nonrandomized study, the authors reported complete response rates of 50% versus 19% and minimal residual disease-negative rates of 33% versus 0% for the higher and lower doses, respectively.

In the treatment of low-grade lymphoma, Piro and colleagues (91) reported that eight once-weekly infusions of rituximab at 375 mg/m² resulted in a response rate that may be higher than that observed in the pivotal study, in which patients received only four doses of rituximab (22). Ghielmini and colleagues (27, 92) reported significantly improved and sustained event-free survival rates for follicular and mantle cell lymphoma patients who received four doses of rituximab followed by four more doses at a 2-month interval as compared with patients who received only four doses of rituximab in total.

How to Increase Rituximab Response: From Biological Origins to Improved Therapies

The Fc region of rituximab plays a critical role in triggering the cellular events that lead to B-cell elimination in vivo. The importance of the interaction between the Fc portion of rituximab and FcyR and C1q suggests potential ways in which anti-CD20 antibody activity might be increased. Changing the Fc region of anti-CD20 antibodies by genetic engineering or by modifying Fc glycosylation, which is necessary for anti-CD20 effector functions and particularly for the interaction with FcyR, may considerably improve the affinity of new anti-CD20 antibodies for FcγRIIA, and thereby enhance ADCC (75–78). Antibodies that have been modified in this way, such as GA101 (79) and AME-133 (80), have already entered clinical trials. In addition, Idusogie et al. (81) have reported the creation of a mutant IgG1 that has increased C1q binding and CDC activity but reduced ADCC activity.

Concomitant treatment of patients with cytokines, such as interleukin (IL)-2, IL-12, interferon-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and the use of immunocytokines that couple antibody and cytokine (82) have also been suggested to increase rituximab-dependent ADCC and enhance the phagocytic capacity of immune cells (83–85). It has also been shown that an anti-C3bi monoclonal antibody (86) and β-glucans (87) can enhance rituximab efficacy. β-glucans specifically bind to the lectin site of CR3 and prime it to engage C3bi fragments deposited on cells by any of the complement-activating antibodies. It is thought that the addition of orally administered β-glucans to rituximab therapy overcomes the lack of CR3-binding polysaccharides on the tumor cell membrane surface. Finally, accessibility to CD20 may be restored, enhanced, or optimized by improving the cholesterol content of lipid rafts or by critically upregulating CD20 expression level using GM-CSF, IL-4, tumor-necrosis factor-α (88), interferon-α (89), or 5-aza-2′-deoxycytidine (33).

Despite the development of more-potent antibodies, optimized dosing of any anti-CD20 antibody is still likely to be crucial for all treatment strategies targeting the CD20 receptor. Because antigenic mass greatly influences anti-CD20 antibody exposure and response, improved anti-CD20 antibody dosing could relevantly optimize treatment response. In the case of rituximab, a dose-escalation trial of rituximab monotherapy in patients with CLL revealed a significant dose-response correlation (26). Similarly, James and colleagues (90) evaluated high-dose rituximab combined with high-dose methylprednisolone in 28 chemotherapy-naïve patients with CLL. Twelve once-weekly rituximab infusions at 375 mg/m² were compared with nine doses of rituximab at 750 mg/m² over 12 weeks. Although this was a nonrandomized study, the authors reported complete response rates of 50% versus 19% and minimal residual disease-negative rates of 33% versus 0% for the higher and lower doses, respectively.

Table 2. Frequency of PD in trials of rituximab monotherapy for CD20-positive B-NHL in patients who had previously received rituximab

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Indication</th>
<th>Study phase</th>
<th>No. of patients</th>
<th>PD, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indolent lymphoma</td>
<td>4 × R-mono after prior chemo ± R and/or prior R-mono B-PTLD</td>
<td>Salvage-line low-grade lymphoma</td>
<td>II</td>
<td>60</td>
<td>&lt;27</td>
</tr>
<tr>
<td></td>
<td>4 × R-mono after prior chemo ± R</td>
<td>Salvage-line B-PTLD</td>
<td>N/A</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

NHL, non-Hodgkin’s lymphoma; R, rituximab; PD, progressive disease; mono, monotherapy; chemo, chemotherapy; B-PTLD, B-cell post-transplant lymphoproliferative disease; N/A, not applicable.

and 5% in the trial reported by Oertel and colleagues (74), and with inversely correlated SD rates covering 5% and 35% of patients, respectively.

In a dose-escalation study conducted in patients with CLL, O’Brien and colleagues (26) found that 80% of patients did not respond to rituximab monotherapy at dose levels of 500–825 mg/m², but the response rate was improved at higher dose levels (1000–2250 mg/m²).

Together, these results suggest that only a few patients are truly refractory to rituximab. Most patients experience some response to rituximab monotherapy, ranging from complete response to SD, but with a significant decrease in tumor burden (Tables 1 and 2; Fig. 2).

The importance of the interaction between the Fc portion of rituximab and FcyR and C1q suggests potential ways in which anti-CD20 antibody activity might be increased. Changing the Fc region of anti-CD20 antibodies by genetic engineering or by modifying Fc glycosylation, which is necessary for anti-CD20 effector functions and particularly for the interaction with FcyR, may considerably improve the affinity of new anti-CD20 antibodies for FcγRIIA, and thereby enhance ADCC (75–78). Antibodies that have been modified in this way, such as GA101 (79) and AME-133 (80), have already entered clinical trials. In addition, Idusogie et al. (81) have reported the creation of a mutant IgG1 that has increased C1q binding and CDC activity but reduced ADCC activity.

Concomitant treatment of patients with cytokines, such as interleukin (IL)-2, IL-12, interferon-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and the use of immunocytokines that couple antibody and cytokine (82) have also been suggested to increase rituximab-dependent ADCC and enhance the phagocytic capacity of immune cells (83–85). It has also been shown that an anti-C3bi monoclonal antibody (86) and β-glucans (87) can enhance rituximab efficacy. β-glucans specifically bind to the lectin site of CR3 and prime it to engage C3bi fragments deposited on cells by any of the complement-activating antibodies. It is thought that the addition of orally administered β-glucans to rituximab therapy overcomes the lack of CR3-binding polysaccharides on the tumor cell membrane surface. Finally, accessibility to CD20 may be restored, enhanced, or optimized by improving the cholesterol content of lipid rafts or by critically upregulating CD20 expression level using GM-CSF, IL-4, tumor-necrosis factor-α (88), interferon-α (89), or 5-aza-2′-deoxycytidine (33).

Despite the development of more-potent antibodies, optimized dosing of any anti-CD20 antibody is still likely to be crucial for all treatment strategies targeting the CD20 receptor. Because antigenic mass greatly influences anti-CD20 antibody exposure and response, improved anti-CD20 antibody dosing could relevantly optimize treatment response. In the case of rituximab, a dose-escalation trial of rituximab monotherapy in patients with CLL revealed a significant dose-response correlation (26). Similarly, James and colleagues (90) evaluated high-dose rituximab combined with high-dose methylprednisolone in 28 chemotherapy-naïve patients with CLL. Twelve once-weekly rituximab infusions at 375 mg/m² were compared with nine doses of rituximab at 750 mg/m² over 12 weeks. Although this was a nonrandomized study, the authors reported complete response rates of 50% versus 19% and minimal residual disease-negative rates of 33% versus 0% for the higher and lower doses, respectively.

In the treatment of low-grade lymphoma, Piro and colleagues (91) reported that eight once-weekly infusions of rituximab at 375 mg/m² resulted in a response rate that may be higher than that observed in the pivotal study, in which patients received only four doses of rituximab (22). Ghielmini and colleagues (27, 92) reported significantly improved and sustained event-free survival rates for follicular and mantle cell lymphoma patients who received four doses of rituximab followed by four more doses at a 2-month interval as compared with patients who received only four doses of rituximab in total.
PK/PD models can accurately predict observed PFS after treatment with rituximab. A mathematical PK/PD model was designed on the basis of pharmacokinetic and efficacy data extracted from the literature (42). A, predicted and observed PFS using data from the McLaughlin et al (22) and Bernstein et al (63). The solid line indicates observed PFS, and the dashed lines are 5th, 50th, and 95th percentiles of predicted PFS. B, predicted and observed PFS based on the data reported by Weng and Levy (50). Patients homozygous for FCGR2A-158V (VV) and FCGR3A-158F carriers (Fx) are displayed as blue and pink lines, respectively. The solid lines indicate observed PFS, and the dashed lines are 5th, 50th, and 95th percentiles of predicted PFS. Reproduced with permission from Ternant et al (45).

Accordingly, it was also reported that an extended course of eight infusions of rituximab in the first-line treatment of aggressive lymphoma in the context of solid organ transplantation (PTLD) resulted in an increased complete response rate compared with four courses of rituximab (93).

In a phase II trial including patients with DLBCL, Preudshchuh and colleagues (94) increased the number of rituximab infusions to achieve high rituximab levels early during treatment. Patients received six cycles of CHOP every 2 weeks (CHOP-14) combined with a total of 12 rituximab infusions. Patients who were treated with six cycles of CHOP-14 and eight applications of rituximab during the RICOVER-60 trial served as a control. This early, intense rituximab regimen resulted in a plateau of high rituximab serum concentrations that was achieved by day 1 of the first chemotherapy cycle and was maintained throughout treatment. Compared with the control group, patients who received the intense rituximab regimen, especially those patients with high-risk disease [International Prognostic Index (IPI) score 3–5], achieved a higher complete response rate and a lower rate of PD under therapy (94).

The optimal concentration of an antibody can also vary according to the antibody’s primary mechanism of action. In an in vitro study, it was shown that FcγRIIa-158V homozygous NK cells and FcγRIIa-158F homozygous NK cells had the same ability to induce ADCC at a high rituximab concentration, but the rituximab concentration required for lysis of 50% of cells was four times lower for the homozygous FcγRIIa-158V NK cells (48).

Using the known pharmacokinetic parameters of rituximab, we can simulate the mean plasma concentrations that will be obtained with different rituximab dosing regimens. Such simulations demonstrate that the various schedules currently used for rituximab induction or rituximab maintenance therapy lead to different levels of rituximab exposure (63). By combining these results with an understanding of factors that affect antibody exposure, such as tumor mass and FCGR3A polymorphism, we can calculate the concentration-effect relationship of monoclonal anti-CD20 antibodies. The resulting PK/PD models can be used in a mathematical simulation to determine the optimal dosing regimen in a given population. We have shown that PK/PD modeling for rituximab is possible in a syngeneic bioluminescent murine model expressing human CD20. The mathematical model accurately predicted the observed results, with a significant correlation between tumor burden and a constant quantifying rituximab efficacy (65). In a recent study, Ternant and colleagues (45) used PK/PD models for rituximab to predict PFS in relapsed human follicular lymphoma. In this model, pharmacokinetic and efficacy data were extracted from the literature, and in both studies that were used to design the model (22, 50, 62), the reported PFS was described satisfactorily by the model. The PFS of patients at 24 months from two independent data sets (49, 55) was also satisfactorily predicted, taking into account the FCGR3A polymorphism (Fig. 4) (45). Using these equations to simulate different dosing regimens of rituximab in follicular lymphoma, they showed that improved PFS could be predicted if higher doses of rituximab were given. This suggests that more patients may...
respond to treatment when appropriate dosing regimens are applied (96). Thus, PK/PD modeling should be a useful method to optimize rituximab dosage according to known parameters affecting response variability.

Conclusions

From the available clinical data on treatment efficacy, it is evident that complete biological resistance and rituximab-refractory disease is a rare condition in most of the clinical settings in which the antibody has been explored. Nonetheless, response to rituximab treatment is variable, depending on tumor histology, tumor burden, patient gender, Fcγ- and CR3-receptor polymorphisms, and the accessibility of CD20, which itself can be affected by concomitant treatment. Our current understanding of the rituximab mode of action has led to the development of subsequent generations of antibodies in which the Fc portion of the antibody has been genetically engineered to improve ADCC activity. Furthermore, improved dosing regimens may overcome some of the factors that account for a variable response to rituximab. Indeed, optimal rituximab dosing requires an even deeper understanding of the PK/PD relationship and how this is influenced by tumor mass and receptor polymorphisms. Such an understanding may result in different rituximab doses being employed for different lymphoma histologies, for men and women, for patients with high and low tumor burdens, and for patients with the FCGRA3-158F or FCGRA3-158V phenotype. Because of its fundamental role, PK/PD modeling is an important tool whose usefulness is not restricted to optimization of the response to rituximab, and one that should be included as part of any new antibody strategy that uses CD20 as a target.

Disclosure of Potential Conflicts of Interest

The authors have received research grants and honoraria from the speakers bureau of different companies, including Hoffmann La Roche, and have participated in scientific advisory boards and/or acted as a consultant for Hoffmann La Roche.

Grant Support


Received May 19, 2010; revised August 13, 2010; accepted September 3, 2010; published online January 5, 2011.

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Received May 19, 2010; revised August 13, 2010; accepted September 3, 2010; published online January 5, 2011.


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