CD20 monoclonal antibody therapy for B-cell lymphoma
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Citation for published version (APA):

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Chapter 7

Summary and General Discussion
Summary

Nowadays, chimeric CD20 monoclonal antibodies (rituximab) are widely used in the treatment of relapsed low-grade non-Hodgkin’s lymphoma (NHL). Although results with rituximab as single agent therapy are encouraging with 48% of patients responding for >13 months, response rates still might be improved.

Possible anti-tumor mechanisms of chimeric CD20 mAbs include the induction of antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and apoptosis. One possibility to augment the efficacy of chimeric CD20 mAbs in vivo might be to increase the efficacy of effector cells involved in ADCC. Since granulocyte colony-stimulating factor (G-CSF) is known to increase the cytotoxic capacity of neutrophils in ADCC, addition of G-CSF to rituximab treatment could theoretically enhance the efficacy of rituximab.

Chapter 2 describes the results of a phase I/II clinical trial evaluating the safety and efficacy of the combination of rituximab and G-CSF in relapsed low-grade NHL patients. The combination of rituximab and G-CSF appeared to be well tolerated. Side effects observed in the present study were comparable to side effects observed during rituximab monotherapy and consisted of fever, chills and allergic reactions, occurring mainly during the first infusion. The efficacy of rituximab and G-CSF was comparable to the efficacy of rituximab monotherapy as well. However, although the overall response rate was similar, the number of complete remissions was high and remission duration seemed to be longer than observed after rituximab monotherapy. These results might suggest that the combination of rituximab and G-CSF is more efficient in eradicating minimal residual disease than rituximab monotherapy. However, more patients and longer follow-up duration are required to substantiate this finding.

Still, the results on efficacy of the combination of rituximab and G-CSF were not as favorable as hoped for. Several possible explanations for these findings are discussed in chapter 2 as well.

Treatment with rituximab is generally well tolerated in patients with low numbers of circulating tumor cells. However, side effects can be severe and life threatening in patients with high numbers of circulating CD20-positive B-cells. Therefore, we investigated the mechanism underlying these side effects (chapter 3). At multiple early time points during the first infusion of rituximab, complement activation products (C3b/c and C4b/c) and cytokines (tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-8) were measured in five patients treated with the combination of rituximab and G-CSF.

During the first infusion of rituximab, we observed rapid complement activation, followed
by the release of TNF-α, IL-6 and IL-8. Although the 5 investigated patients had very low levels of circulating B-cells and only mild side effects, the level of complement activation was correlated both with the number of circulating B-cells prior to the infusion and with the severity of the side effects. We conclude that complement activation plays an important role in the side effects of rituximab treatment, and propose a mechanism explaining the pathogenesis of these side effects.

All measurements were performed in patients treated with rituximab and G-CSF. We did not see any influence of G-CSF administration on either complement activation products, cytokine levels or side effects. Therefore, a possible influence of G-CSF, although not excluded, is unlikely. Complement activation, in contrast to cytokine release, can not be prevented by corticosteroids. It has been described that patients with high levels of circulating tumor cells may develop severe side effects during the first infusion of rituximab, despite pretreatment with corticosteroids. This observation not only supports a role for complement activation products in the development of the side effects, but also forms a rationale for studies investigating the use of complement inhibitors during the first rituximab-infusion, especially in patients with high numbers of circulating tumor-cells who are at risk of severe first-dose side effects.

In chapter 4, the ability of G-CSF stimulated neutrophils to kill CD20-coated B-cells was measured in vitro, by using ADCC-assays. For this purpose, G-CSF-stimulated neutrophils were derived from patients treated in the clinical trial, after three injections of G-CSF, i.e. just prior to the infusion of rituximab.

G-CSF-primed neutrophils proved to be capable of functioning as effector cells in CD20-dependent ADCC. However, HLA class II mAbs were far more effective in inducing B-cell lysis. In chapter 4, possible explanations for the difference between CD20 mAbs and HLA class II mAbs in inducing neutrophil-mediated B-cell lysis are investigated and discussed. Although our data demonstrate that rituximab is capable of inducing ADCC with G-CSF-primed neutrophils as effector cells, NK cells are more potent inducers of CD20-dependent cytotoxicity. Thus, stimulating the effector function of NK cells might also form a possibility to enhance the efficacy of rituximab therapy. A recent clinical trial investigated the combination of rituximab with IFN-α, which is known to stimulate NK-cell function. Interestingly, results were similar to results of rituximab and G-CSF, i.e. the response rate after rituximab + IFN-α was comparable to the response rate of rituximab monotherapy whereas the response duration appeared to be longer. These results strengthen the suggestion, that responses induced by rituximab monotherapy might be improved by an adjuvant stimulating the anti-tumor mechanism of ADCC. However, randomized trials should be performed to validate these findings.

In chapter 5, the effect of rituximab treatment on the primary and secondary humoral
immune response was investigated. Treatment with rituximab leads to a rapid depletion of B cells from the peripheral blood, lasting for 9-12 months after completion of treatment. Clinically, this sustained B-cell depletion neither leads to a decrease in immunoglobulin levels, nor to an increase in the number of infectious complications. However, it is not unlikely that rituximab treatment influences the humoral immune responsiveness. Therefore we investigated the effect of rituximab treatment on the primary and secondary humoral immune response, by immunizing patients both before and after rituximab treatment with two primary antigens (keyhole limpet hemocyanin and hepatitis A) and two recall antigens (tetanus toxoid and poliomyelitis vaccine).

Somewhat unexpectedly, none of the patients mounted a response to either primary antigen, neither before, nor after rituximab treatment. Therefore, our study does not allow a conclusion as to the influence of rituximab on the primary immune response. Interestingly though, after rituximab treatment, the response to the recall antigens was decreased when compared to the response before treatment. This might be due to a decrease in the amount of memory B-cells after rituximab treatment. The possible implications of this finding for the use of rituximab as maintenance therapy and for the use of rituximab in the treatment of antibody-mediated autoimmune diseases are discussed in chapter 5.

In chapter 6, we investigated the intracellular pathways of CD20-induced apoptosis. We found that chimeric CD20 mAbs only induce apoptosis after crosslinking (CD20XL). Furthermore, we found that CD20XL-induced apoptosis is independent of the Fas/FasL apoptosis pathway, is not inhibited by overexpression of Bcl-2 and is only partially dependent on active caspases. Most chemotherapeutic drugs require the activation of caspases to exert their cytotoxicity, and chemoresistance may be caused by a defect in apoptosis-inducing pathways, leading to the inability of drugs to activate caspases. Therefore, these data imply that CD20XL-induced apoptosis may circumvent important causes of chemoresistance.

B-cell lymphomas are known to become increasingly chemoresistant after being treated with multiple chemotherapy regimens. However, in clinical trials evaluating the efficacy of rituximab in B-cell lymphomas, it was observed that the number of prior chemotherapy regimens did not influence the response to rituximab. This suggests that the chemoresistance of the tumor did not influence the response to rituximab, thus supporting our in vitro data on CD20-induced apoptosis.

As mentioned, chimeric CD20 mAbs require crosslinking in order to induce apoptosis. In vitro, goat-anti-human antibodies have been used as crosslinking antibodies. Furthermore, it has been demonstrated in vitro that FcγR-expressing cells were able to provide functional crosslinking of CD20 mAbs and thereby induce apoptosis in target cells. Thus, FcγR-expressing cells might play a role in vivo not only by inducing ADCC, but also by inducing apoptosis.
**General discussion**

In the present studies, several aspects of the combination of rituximab and G-CSF were evaluated. The main goal of this study was to increase the efficacy of rituximab treatment via the addition of G-CSF. Although the overall response rate of the combination of rituximab and G-CSF was comparable to rituximab monotherapy, we observed a high percentage of complete remissions and a remarkably long duration of response. A randomized trial, comparing rituximab monotherapy with the combination of rituximab and G-CSF should be conducted in order to validate this finding.

Several other strategies have been developed in order to increase the efficacy of rituximab treatment, not only by combining rituximab with an immunologic adjuvant (e.g. IFN-α, IL-12 or IL-2), but also by coupling CD20 mAbs to a radioisotope or toxin. In general, the data obtained in different studies combining rituximab with IL-2, IL-12 or IFN-α are too preliminary to allow conclusions as to their additional value. However, one study has a longer follow up. In this study, treatment of patients with the combination of rituximab and IFN-α resulted in a similar response rate as obtained with rituximab monotherapy. Here too, the response duration appeared to be longer than observed with rituximab monotherapy. Thus, these results resemble those obtained in our study.

Of specific interest are the results obtained with iodine-131 iodine (131I) labeled murine CD20 mAbs (tositumomab, mlgG2a; Bexxar) and yttrium (89Y) labeled CD20 mAbs (ibritumomab tiuxetan, mlgG1; Zevalin). In 59 patients with either low-grade or transformed low-grade NHL (n=42) or de novo intermediate- or high-grade NHL (n=17) who were treated with 131I tositumomab (75 cGy), an overall response rate (ORR) of 71% with a complete remission (CR) rate of 34% was reported. Comparable results were obtained with Zevalin (0.4 mCi/kg, maximum 32 mCi): in 143 patients with either relapsed or refractory low-grade NHL or transformed low-grade NHL, who were randomized between treatment with Zevalin or with rituximab monotherapy, the ORR in the Zevalin group was 80% (30% CR), versus 56% (4% CR) in the rituximab group. Furthermore, in 54 follicular lymphoma patients refractory after rituximab therapy treatment with Zevalin resulted in an ORR of 74% (15% CR). Thus, response rates with radiolabeled CD20 mAbs exceed those obtained with rituximab monotherapy.

Toxicity was primarily hematologic and although grade IV thrombocytopenia and/or neutropenia were observed in approximately 20% of the patients, hematologic toxicity was always transient and reversible, not requiring stem cell support. 131I tositumomab has also been applied in conjunction with stem cell rescue. In this phase I/II study, 29 patients were treated with myeloablative doses of 131I tositumomab. The response rate was 86% (79% CR) and the time to treatment failure was 37 months.
Besides the expected severe myelosuppression after treatment with $^{131}$I tositumomab, cardiopulmonary toxicity was found to be the dose limiting toxicity. Thyroid dysfunction has been the most common late complication, occurring in 60% of patients. None of the 29 patients has developed myelodysplasia or acute leukemia during the follow up period of (median) 47 months.

Other applications of rituximab include its use in combination with chemotherapy, which is being investigated in indolent as well as in aggressive lymphomas. Because of their non-overlapping toxicities, these two treatment modalities may be safely combined. In elderly patients with diffuse large cell lymphoma ($n = 400$), addition of rituximab to CHOP chemotherapy resulted in a significantly higher response rate and, after a median follow up period of 12 months, a significant prolongation of event-free survival and overall survival, without significant additional toxicity.\textsuperscript{15} The combination of CHOP and rituximab in low-grade or follicular lymphoma was well tolerated and resulted in a response rate of 100% with a median duration of response of 39+ months after a median follow up period of 41 months.\textsuperscript{16} Conversion of Bcl-2 positivity to negativity in 7/8 patients tested.\textsuperscript{17} Although the results of these studies are promising, long-term follow up is required in order to determine the value of the addition of rituximab to CHOP chemotherapy.

The conversion of Bcl-2 from positive to negative in bone marrow and peripheral blood after treatment with the combination of CHOP and rituximab has not been observed after CHOP alone, suggesting that rituximab might effectively clear minimal residual disease (MRD). To investigate the application of rituximab in MRD, the efficacy of rituximab as maintenance therapy in B-cell malignancies is currently being investigated (no studies published yet). Furthermore, rituximab is currently been applied in combination with high dose chemotherapy before stem-cell harvest ('in vivo purging')\textsuperscript{18} and after stem-cell transplantation, in order to eradicate minimal residual disease.\textsuperscript{19} The complete molecular responses observed in these studies has lead to speculations that addition of rituximab to (high dose) chemotherapy might form a curative treatment option.

Although initial studies primarily focussed on the use of rituximab in low-grade NHL, rituximab has also been applied in various other CD20-positive B-cell malignancies (table 1). The efficacy of rituximab in aggressive lymphomas is less favorable than in low-grade lymphomas. Several groups have studied the efficacy of rituximab in mantle cell lymphoma, and response rates were ~35%, which was comparable in the different studies. The response rate of patients with diffuse large cell lymphoma treated with rituximab was 39%. In contrast, post-transplant lymphomas were found to be highly sensitive to rituximab treatment (table 1). In several reports on patients with post-transplant lymphoproliferative disease (PT-LPD) treated with rituximab, response rates were >80%.

Rituximab has also been applied in patients with plasma cell dyscrasias, including multiple
### Table 1. Use of rituximab* in various CD20-positive B-cell malignancies

<table>
<thead>
<tr>
<th>histology**</th>
<th>number of patients</th>
<th>ORR (CR***</th>
<th>comments</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>follicular lymphoma (FL)</td>
<td>n=118</td>
<td>60%</td>
<td>assessable patients</td>
<td>29</td>
</tr>
<tr>
<td>small lymphocytic lymphoma (SLL)</td>
<td>n=30</td>
<td>13%</td>
<td></td>
<td></td>
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<tr>
<td>diffuse large cell lymphoma (DLCL)</td>
<td>n=30</td>
<td>37%</td>
<td>375 mg/m² x 8 or 375 mg/m² x 1 + 500 mg/m² x 7; no difference between treatment arms</td>
<td>30</td>
</tr>
<tr>
<td>mantle cell lymphoma (MCL)</td>
<td>n=13</td>
<td>33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCL1</td>
<td>n=34</td>
<td>38%</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>MCL2</td>
<td>n=40</td>
<td>37%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>immunocytoma</td>
<td>n=28</td>
<td>28%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>small lymphocytic lymphoma</td>
<td>n=29</td>
<td>14%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCL1</td>
<td>n=37</td>
<td>34% (14%)</td>
<td>no difference between MCL1 and MCL2</td>
<td>32</td>
</tr>
<tr>
<td>MCL2</td>
<td>n=50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT-LPD EBV positive (22/26 cases tested)</td>
<td>n=32</td>
<td>69% (63%)</td>
<td>solid organ transplantation n=26, RR 65%; bone marrow transplantation n=6, RR 83%</td>
<td>33</td>
</tr>
<tr>
<td>PT-LPD EBV negative</td>
<td>n=6</td>
<td>83% (83%)</td>
<td>CR n=4, SD n=2</td>
<td>34</td>
</tr>
<tr>
<td>PT-LPD EBV positive</td>
<td>n=13</td>
<td>85% (53%)</td>
<td>375 mg/m² x 7-11</td>
<td>35</td>
</tr>
</tbody>
</table>

*regimen: 375 mg/m² weekly x4, unless indicated otherwise; **relapsed lymphoma unless indicated otherwise; ***CR rate is given if known. In 30/32 patients and 6/6 patients, rituximab was used as first-line therapy (unknown). Abbreviations: ORR = overall response rate, on intent-to-treat basis; CR = complete remission; SD = stable disease; MCL1 = MCL, newly diagnosed; MCL2 = MCL, previously treated; PT-LPD = post-transplant lymphoproliferative disease; EBV = Epstein Barr virus.

myeloma (MM) and Waldenstrom’s macroglobulinemia (WM). Analysis of 7 (previously treated) WM patients treated with rituximab showed that 3 of them achieved a partial response upon rituximab treatment. Although CD20 is expressed on the monoclonal B-cell population of most WM patients, CD20 expression on plasma cells of most MM patients is either weak or absent. However, CD20-positive clonotypic B-cells have been detected in patients with MM, and these cells may function as myeloma precursor cells. Furthermore, experiments have been conducted to search for agents that might increase the CD20-
expression on plasma cells (e.g. IFN-γ). In initial clinical studies it was suggested that certain MM patients may benefit from rituximab therapy. Finally, promising results have been described on rituximab in antibody-mediated autoimmune diseases. Of 10 patients with chronic idiopathic thrombocytopenic purpura (ITP) treated with rituximab 375 mg/m² weekly x4, 6 patients responded. The duration of response varied from > 3 months to > 14 months. Evidently, these studies need to be extended. Furthermore, it will be interesting to determine the exact mechanism of action of rituximab in antibody-mediated autoimmune diseases.

In conclusion, chimeric CD20 mAbs are safe and well tolerated. The efficacy of rituximab in relapsed low-grade NHL has already been established. However, many other B-cell malignancies, especially the post-transplant lymphoproliferative diseases, are effectively treated by rituximab. Preliminary data on the combination of rituximab with (high-dose) chemotherapy even suggest that this combination might form a curative treatment option. Moreover, rituximab might be a valuable treatment option for antibody-mediated autoimmune diseases. Thus, although the optimal use of rituximab in the treatment of the various B-cell malignancies and other potential fields remains to be determined, many patients have already benefited of this safe and efficient treatment modality, and future prospects are highly encouraging.
Reference List


