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Treatment of B-cell non-Hodgkin's lymphoma with anti CD 20 monoclonal antibody Rituximab

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Abstract

Rituximab is a chimeric anti CD-20 monoclonal antibody containing human IgG1 kappa constant regions, with murine variable regions. The anti-lymphoma effects of Rituximab are probably due to complement and antibody-dependent cell-mediated cytotoxicity, and induction of apoptosis. Phase II trials have demonstrated a strong activity of rituximab alone in indolent B non-Hodgkin lymphoma, especially in patients with follicular lymphoma. The most utilized dose-schedule is 375 mg/m^2 weekly $\times$ 4. The association with chemotherapy or with interferon-alpha increases Rituximab efficacy. More recently, Rituximab have showed activity also in diffuse large cell lymphoma, mantle cell lymphoma and in other B-malignancies. Good results have also been obtained utilizing Rituximab for in vivo purging. However, we are still far from having found a definite position for Rituximab in the treatment of lymphoproliferative disorders. The aim of future studies should be to develop new strategies that will hopefully produce the most effective Rituximab-based regimens in order to find the Rituximab key position in the treatment of B-malignancies

Keywords: Anti-CD20; MAbs; Rituximab; NHL; B-Lymphoproliferative disorders

1. Introduction

The broad category of non-Hodgkin lymphomas (NHLs) includes a large number of distinct lymphoid neoplasm. The majority of NHLs, are derived from B-cells, with a sizeable minority of about 15%, arising from T cells. The most frequently utilized classifications of NHL are the International Working Formulation [1], the Kiel Classification [2], and the more recently introduced REAL Classification [3], based on morphologic, immunophenotipic and genetic features. As classification of NHLs are numerous, thus it could be sometimes difficult to refer and compare results obtained by different authors, who have utilized different classification systems. Further, in clinical practice, the lymphoma subgroup, which have an indolent clinical course are often grouped together and defined 'low grade' lymphoma, and these with a more aggressive clinical course are termed 'intermediate or high grade' lymphoma. This review describes recent results obtained with Rituximab, a chimeric anti-CD20 monoclonal antibody (MAb) in the treatment of different B-NHL entities classified, when possible, according to REAL classification.

2. Monoclonal antibodies

2.1. Monoclonal antibody production

Murine MAbs are produced by fusing an immortalized plasmacell with a normal antibody producing cell. The resulting hybridoma cell produces homogenous antibody and can be expanded [4] (Figs. 1 and 2).

Murine MAbs, in early clinical trials did not demonstrate the expected efficacy in humans. It happened because they were murine and targeted to antigens that appeared on normal and tumor cells. Many technical difficulties hampered their evolution from laboratory to clinical success, including a low affinity for target antigen, the inability of murine MAbs to effectively recruit human immune cells, the presence of circulating antigen, the shedding or internalization of the targeted antigen after administration of antibody, poor tumor vascularitation, the appearance of antigen-negative tumor cells and the development of human anti-mouse antibodies (HAMA).

Once this occurs, the patient antibodies attack the MAbs, resulting in MAbs complexation, which reduces the circulating time of MAbs and their binding to target tumor, thereby limiting the antibody anticancer activity.

HAMA may also cause allergic reactions upon second exposure to the antibody, and these reactions range from the uncomfortable to the potentially life threatening. Even monoclonal antibodies derived partly or entirely from human cell lines can evoke antibody responses. Chimeric antibodies can elicit human antichimeric antibody (HACA) responses and even human MAbs can evoke human antihuman antibody (HAHA) responses.

2.2. Modification of monoclonal antibody

Techniques to humanize murine MAbs have been developed to ameliorate the host humoral response [5–10].

Using a human antibody rather than a mouse antibody would eliminate the HAMA response. The tech-

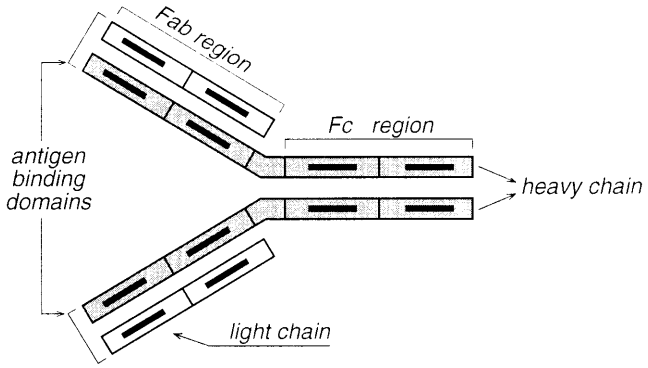


Fig. 1. Antibody structure.

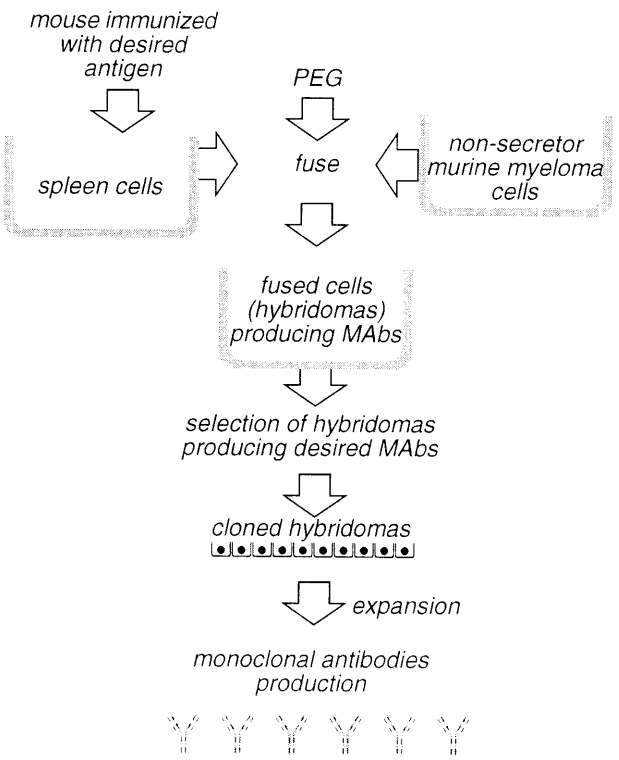


Fig. 2. Hybridoma technique for monoclonal antibody production.

nique for developing human MAbs involves fusing human immune B cell with a human myeloma cell line or a human lymphoblastoid cell line. Several problems have been encountered with the development of human MAbs, including the scarcity of human myeloma cell lines and ethical issues related to the necessity to immunize the human subject with tumor cell or extract.

More commonly, MAbs are partly 'humanized' through genetic engineering. The most common method of antibody humanization involves replacement of the constant region of the mouse MAb with a human constant region, resulting in a mouse/human chimera. Chimeric antibodies are created by cloning the murine gene that codes for the antibody variable region and the human gene that codes for the antibody constant region. This type of genetic engineering enables scientists to produce antibodies with a murine variable region combined with a human constant region.

Potential advantages for chimeric antibodies include less immunogenicity, longer circulation of the antibody and better cell killing. Cell killing is enhanced because the chimeric antibody combines the antigenic specificity of a murine antibody with the fragment crystallizable (Fc) portion of a human antibody. The human Fc portion should, in theory, mediate complement activation and antibody-dependent cellular cytotoxicity more efficiently than murine monoclonals. The patient receiving a chimeric MAb is still at risk for developing HAHA response or HACA response. Rituximab is a chimeric antibody (Fig. 3).

A third technique to reduce the immunogenicity of the MAbs is to include even more human portions in the engineered antibody. In this approach, reshaped MAbs are produced by grafting murine complementarity-determining regions onto a human antibody framework. MAbs thus adapted are termed humanized and are differentiated from chimeric antibodies in that they contain a human constant region and a variable region that consist of both the murine complementary-determining regions and the human variable-region framework determinants (Fig. 3).

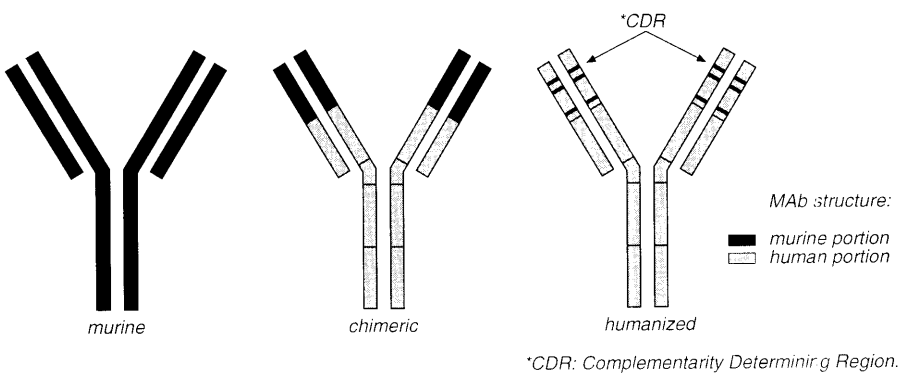


Fig. 3. MAbs modified through genetic engineering.

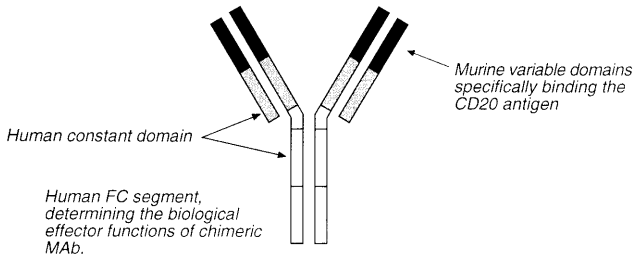


Fig. 4. Structure of Rituximab, the chimeric anti-CD20 MAb produced through genetic engineering.

Monoclonal antibodies used in the treatment of cancer may be unconjugated or conjugated to other agents, such as radionuclides, chemotherapeutic agents, or toxins. The aim of this review is to focus on Rituximab, that is an unconjugated MAbs.

3. Rituximab

3.1. Chemistry

The chimeric mouse/human anti-CD20 antibody Rituximab is an IgG1 kappa antibody containing murine light-and heavy-chain variable regions and human gamma 1 heavy-chain and kappa light-chain constant region ([11] Fig. 4). Rituximab is a glicosilated protein containing 1328 amino acids. Its molecular weight is approximately 144 kDa. The antibody reacts specifically with the CD20 antigens found on the surface of malignant and normal B-cells and on established B-cell lines. Rituximab recognizes CD20 antigen with an apparent affinity of approximately 5.2×10^{-9}M [11].

3.2. CD20 antigen as a target of immunotherapy

The human B lymphocyte-restricted differentiation antigen CD20 is a hydrophobic transmembrane protein with only a small portion of the protein exposed on its outer surface (Fig. 5). This non-glycosylated phosphoprotein of 35 000 Da is expressed on normal and malignant B cells, but not on other normal tissues [12,13]. The CD20 antigen is expressed on the majority of B-cell lymphomas; in 151 patients with B-cell non-Hodgkin's lymphoma, 93% of tumor cells expressed the CD20 antigen [14].

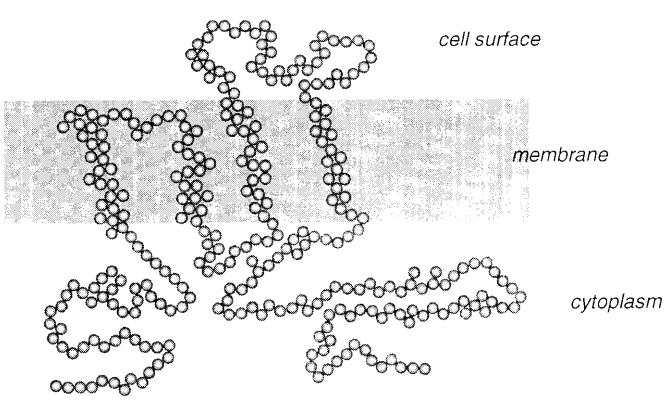


Fig. 5. CD20 antigen: a hydrophobic transmembrane protein.

It appears to play an important role in the process of B-cell differentiation. Many studies suggest that the CD20 antigen may regulate a step in the B-cell activation process required for cell cycle initiation and differentiation [15–18]. Thus, this antigen may act to control B lymphocyte progression through the cell cycle [16]. CD20 is a potential ion channel and may be involved in the regulation of signal transduction, the mean by which an exogenous signal is carried from the outside to the inside [19].

The CD20 antigen appears nearly an ideal target to treat B cell lymphomas with unconjugated MAbs for the following reasons:

- This molecule does not circulate in the plasma as free protein that could block targeting of antibody to lymphoma cell [14].
- The antigen does not shed from the surface of cells after binding of anti-CD20 monoclonal antibody [20].
- The antigen does not internalize upon antibody binding [21].

The CD20 antigen arises during the pre-B-cell stage of B-cell differentiation but it is not expressed in earlier and late step of B-cell differentiation (Fig. 6). The formation of stem cells is an earlier step in this process. Therefore, since Rituximab targets the CD20 antigen, treatment does not affect stem cell or pre-B-cell development. Thus, following depletion, a normal B-cell population can be reconstituted from the stem cells and pre-pre B-cells.

3.3. Mechanism of action

Rituximab may affect B-cell growth and differentiation because the CD20 antigen regulates a step in the activation process required for cell cycle initiation and differentiation [15,16]. The CD20 molecule is expressed during early pre-B-cell development [14]. It provides a suitable target for antibody-mediated treatment because hematopoietic tumors are sensitive to lysis through immune effector mechanisms. In vitro, Rituximab binds human C1q and affects both CDC and ADCC [10,11,22]. Also, in vitro experiments show [23,24] that the antibody induces apoptosis (Fig. 7). These experiments also demonstrate that synergy exists between Rituximab and chemotherapies and toxins, specifically that the antibody sensitizes drug-resistant human B-cell lymphoma lines to the cytotoxic effects of CDDP, VP-16 and ricin.

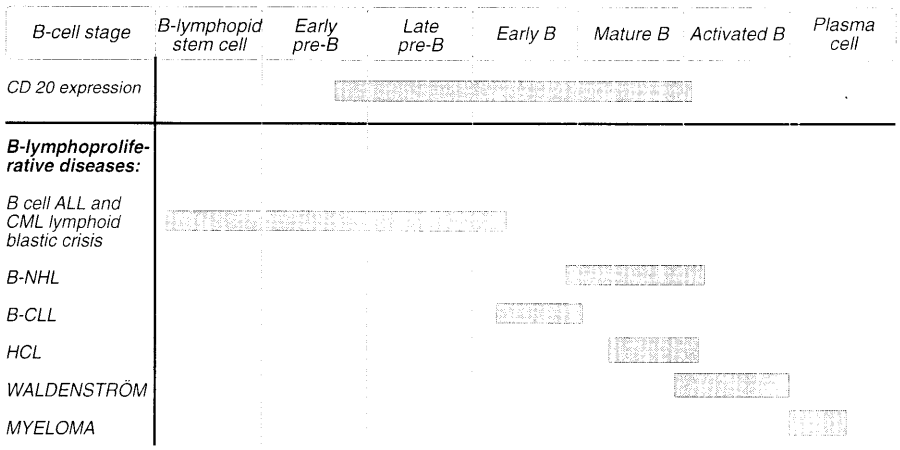
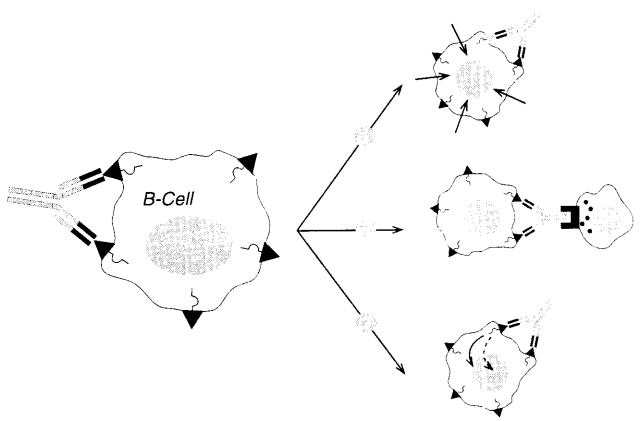


Fig. 6. CD20 antigen expression on human normal and neoplastic B-cells.

3.4. Clinical pharmacokinetic in human

After preliminary studies in animals, Rituximab was tested in human. Of the 47 patients enrolled in a phase I/II protocol, 16 were included in the pharmacokinetic analysis (three patients at 125 mg/m^2, four patients at 250 mg/m^2, and nine patients at 375 mg/m^2). Most patients analyzed showed an accumulation of antibody through the fourth infusion. Both AUC and C_{max} increased with increasing dose. At the 375 mg/m^2 dose, mean $t^{1/2}$ was 226.4 h (range of 12.7–441.1 h). Data analysis showed that a significant degree of variation exists in the values of the pharmacokinetic parameters, even when comparing patients within the same dose group. It is known that levels of circulating tumor and overall tumor burden varied significantly from patient to patient and this is very likely to have an effect on the pharmacokinetic of Rituximab [25]. Pharmacokinetic studies during pivotal trial [26] showed that attainable serum antibody concentrations correlated negatively with the number of circulating B-cells, with the size of the largest measurable tumor pretreatment and with the baseline sum of the products of the diameters of the six largest lesions. Serum levels were significantly lower in patients with small lymphocytic lymphoma than other histologic types.

For patients who had detailed pharmacokinetic monitoring, the mean serum half-life after the first infusion was 76.3 h, while after the fourth infusion, it was 205.8 h. The maximum observed concentration was higher after the fourth than after the first infusion. The clearance was slower, and the area under the curve was



1. **Complement dependent cytolysis**: Mabthera binding induce complement cascade that activates perforins. Ions move (⟶) through pores, determining cell lysis.
2. **Antibody-dependent cell mediated cytotoxicity:** Mabthera FC portion mediates recruitment of immune effector cells. Lysis of the target cell requires cell to cell contact.
3. **Other effects:** Mabthera binding can induce apoptosis (⤳) and block (⋯➚) growth and differentiation of target cells.

Fig. 7. Rituximab mechanisms of action.

greater. A significant correlation was found between median serum antibody levels and clinical response: Rituximab serum levels were higher for responders than non responders.

4. Clinical experience with rituximab alone

4.1. Rituximab for the treatment of low-grade/FL

A Phase I clinical trial in patients with NHL had been completed at Stanford University Medical Center and published in 1994 [27]. Fifteen heavily pretreated patients (three per dose level) with low-grade (11 follicular lymphomas) relapsed B-cell lymphoma received single doses (10, 50, 100, 250 or 500 mg/m^2) of Rituximab given intravenously.

Treatment-related symptoms correlated with the number of circulating CD20-positive cells and consisted of fever, skin rash, nausea, rigor, orthostatic hypotension and bronchospasm. No significant toxicities were observed during 3 months of post treatment follow-up.

Serum C3, IgG, IgA and IgM levels, platelets, neutrophils and T-cell counts were essentially unchanged. CD20-positive B cells were rapidly and specifically depleted in the peripheral blood at 24–72 h and remained depleted for 2–3 months in most patients. Two-week post-infusion tumor biopsies showed Rituximab antibody bound to tumor cells and a decrease in the percentage of B-cells. Tumor responses, occurred in six of 15 patients (two partial and four minor responses). Based on these results obtained with a single dose of chimeric anti-CD20 antibody, a multiple dose study in patients with relapsed B-cell lymphoma was initiated [27,28]. Treatment consisted of weekly × 4 infusions of Rituximab. Dose levels of 125, 250 and 375 mg/m^2 were explored. Using this dose schedule it was anticipated that antibody accumulation would occur in some patients and that they would experience prolonged exposure to Rituximab through the therapeutic course. Saturation of the tumor sites was also expected for those patients in whom Rituximab accumulation occurred.

Twenty patients were entered in the Phase I dose-finding portion of the study: ten males and ten females, median age 60, mostly low grade, 17/20 stage III/IV at diagnosis, with a median of two prior chemotherapy regimens. There was no dose-limiting toxicity and the maximum tolerated dose was not attained. The hypothesis of Rituximab accumulation and prolonged antibody exposure was confirmed. Infusional symptoms were brief, manageable, and primarily associated with the first infusion. Circulating B cells were rapidly depleted. There were no clinically significant changes in median serum immunoglobulin levels. No quantifiable anti-Rituximab reactivity (HAMA or HACA) was observed. Complement levels decreased in some patients without correlation to response. Clinical activity was documented in 11 of 18 valuable patients. There were seven partial and four minor responses. These occurred primarily in patients with low-grade lymphoma. In the Phase II portion of the study were enrolled 24 patients with advanced stage low-grade or follicular lymphoma (LG/F NHL) [27,28].

Considering together the 24 patients entered in Phase II and the ten who entered Phase I at a dose of 375 mg/m^2, there were 20 males and 14 females with a median age of 56.5 years. All patients had relapsed with a median of two prior chemotherapy regimens. The mean half-life of free serum antibody was 1.8 days following the first dose and 6.2 days following the fourth infusion. Quantifiable immunoreactivity (HAMA or HACA) was not observed. Three complete responses (CR) and 14 partial responses (PR) were noted in these 34 assessable patients, with a median response duration of 8.6 months. Median time to progression (TTP) exceeded 20 months in five patients and 30+ months in two patients. Adverse experience were mostly grade 1 or 2 and consisted primarily of infusion-related events including fever, asthenia, chills and less commonly, bronchospasm, hypotension, and angioedema. Hematologic toxicity was usually mild and reversible. Based upon these results, Rituximab administration at a dose level of 375mg/m^2 once weekly has been selected for a single agent, multicenter pivotal Phase II clinical trial in patients with relapsed LG/F NHL [26]. From 31 centers, 166 patients (120 patients with follicular lymphoma) were entered. Of this intent-to-treat group, 48% responded with 6% CRs. With a median follow-up duration of 11.8 months, the projected TTP for responders is 13.0 months. Serum antibody levels were sustained longer after the fourth infusion than after the first, and were higher in responders and patients with lower tumor burden. The majority of adverse events occurred during the first infusion and were the most common events. Only 12% of patients had grade 3 and 3% grade 4 toxicity. A HACA was detected in only one patient. As expected the normal mature B-cell population rapidly declined after treatment and recovered over 3–9 months. There was no increase in the rate of infections, probably because Ig and T cell levels remained stable. Based on these data, the Food and Drug Administration approved the regimen of 375 mg/m^2 weekly for 4 weeks for the treatment of relapsed LG/F NHL. At the 1998 ASH meeting in Miami, the same author presented the long term follow-up of the study [29]. Twenty patients out of 80 respondants, were still in remission with a duration remission (DR) ranging from 19.3+ to 36.7+ months. In this study by a logistic regression analysis of the prognostic features previously studied by univariate analysis only three factors emerged as statistically sig-

nificant: histologic type (follicular histology), bcl-2 status at baseline, and prior ABMT. A 60 patient phase II study was conducted to determine the safety and efficacy of re-treatment with Rituximab in patients with LG/F NHL [30]. Upon progression of disease, patients received four infusions of Rituximab at 375mg/m^2. Patient characteristics were 54% males with medians of: age 56, three prior therapies, 14.2 months since last treatment, and 4.7 years since diagnosis. The safety profile was similar to that reported for initial treatment with Rituximab consisting primarily of infusion-related events usually occurring within the first few hours of the first infusion. The most common related adverse events (Aes) included leukopenia, nausea, transient bronchospasm, and mild hypotension. No HACA was reported. Fifty six of 60 patients were evaluable for efficacy. The overall response rate (ORR) was 41% with 12.5% CRs and 28.6% PRs. Medians for TTP and DR have not been reached after 10+ and 7.7+ months of observation, respectively. This study shows that patients with relapsed or refractory LG/F NHL can be safely and effectively treated with multiple courses of Rituximab without significant myelosuppression or induction of HACA. Rituximab has also been used in relapsed or refractory LG/F NHL patients with bulky disease [31]. In this phase II trial 31 patients received Rituximab at 375mg/m^2 weekly × 4 infusions. Patient characteristics included: 52% male, median 4 years from diagnosis, and median three prior therapies. Most frequent related AEs were mild to moderate, usually first infusion related: fever, chills, leukopenia, nausea, dizziness, and throat irritation. Tumor lysis syndrome was not reported. Four patients had grade 3 or 4 non-hematological AEs: pulmonary, chills, and hypotension. One patient died with bronchiolitis obliterans 10 months post-treatment. Seven patients had transient grade 3 or 4 hematologic AEs: hemoglobin, neutrophils, and neutrophils + platelets. None developed HACA. There were no grade 3 or 4 infections. In evaluable patients the ORR was 43% with 1 CR and 11 PR. The median TTP was 8.1 months with a median DR of 5.9 months. Thus, this outpatient treatment with Rituximab can be considered safe and effective in patients with bulky LG/F NHL and moreover, it does not limit subsequent treatment options.

4.2. Rituximab for the treatment of intermediate or high-grade NHL

Recently Rituximab has also been used to treat small number of patients with aggressive NHL. A randomized Phase II trial enrolled 54 patients with relapsed or refractory aggressive NHL, which included 13 patients with mantle cell lymphoma (MLC), 30 with diffuse large cell lymphoma (DLCL), one with follicular lymphoma and ten with not otherwise specified type of aggressive lymphoma. The patients received 8 weekly infusions of Rituximab at the dose of 375 mg/m^2 in arm A or one infusion of 375 mg/m^2 followed by 7 weekly infusions of 500 mg/m^2 in arm B. Patients were evaluated 2 months after the last Rituximab infusion. Fifty-four patients were randomized (28 in arm A and 26 in arm B). A total of five CR and 12 PR were observed among the 54 enrolled patients, with no difference between the two doses. In an intent-to-treat analysis, the CR rate was 9% and the PR rate was 22%, for an overall response rate of 31%. Three responses were in the 11 assessable MCL patients. An analysis of prognostic factors showed that response rates were lower in patients with refractory disease, patients with lymphoma not classified as DLCL, and patients with a tumor larger than 5 cm in diameter. DLCL and MCL patients had response rates of 37 and 33%, respectively. The TTP exceeded 246 days for the 17 responding patients. The most frequently reported AEs were related to an infusion syndrome and were mild: 19% of the patients had a grade 3 related AEs and only one had a grade 4 related AE in arm A. Two patients (3.7%) withdrew from treatment because of severe AEs, one patients in each arm [32]. In an another recently published study, ten patients with resistant or relapsed MCL were treated with the usual schedule of 375 mg/m^2 weekly × 4. Rituximab induced PR in two patients, showing some efficacy also in this poor prognosis group of patients [33]. A trial of CHOP and Rituximab has also been conducted in 33 patients (27% aged older than 65 years) with previously untreated intermediate- or high-grade NHL, 73% of whom had stage III or IV disease. This combination resulted in a response rate of 97%, with 73% CRs [34].

5. Toxicity

5.1. Safety profile

The safety profile of Rituximab has been impressive to date. The large majority of AEs were observed during the first infusion and were mild to moderate in intensity. After first infusion most patients had no toxicity for the remainder of the treatment. Adverse events were typically brief.

During pivotal study [26] the most frequent adverse side effects were: fever, chills, nausea, angioedema, headache, asthenia, pain, pruritus, hypotension, rash and bronchospasm. The number of grade 3 and 4 events observed was 18 and 2, respectively. In a few number of patients a decrease of hemoglobin, WBC and PLT counts was observed. As expected, the normal mature B-cell population rapidly declined after Rituximab and recovered over 3–9 months. There was no increase in the rate of infection, probably because Ig

and T-cell levels remained stable. No instances of HAMA and a $<1\%$ incidence of HACA development were reported, even on re-treatment with Rituximab.

Data on long-term toxicity are not reported in literature. However, B. Cheson during the VII. International Conference on Malignant Lymphoma, Lugano 1999, referred on unusual and rare immune phenomena that occur weeks and months after completion of the Rituximab treatment, including muco-cutaneous reaction, vasculitis, lupus like syndrome, pleuritis, arthritis and ocular disturbances. Cytokine release and rapid tumor lysis syndrome are infusion-related phenomena. Cytokine release syndrome usually occurs during first infusion (30–90 min). Patients suffer from dyspnea, broncospasm, fever, chills, rigors, urticaria and an-gioedema. The physician has to interrupt Rituximab infusion, utilize diphenhydramine and broncodilators and monitor closely the patient, until the symptoms resolve. Usually, Rituximab can be restarted at lower rate of infusion and subsequent treatment can be administered without recurrence.

More severe cytokine release syndrome can occur in patients with high numbers of circulating tumor cells, as CLL, MCL or leukemic phase of NHLs. A rapid tumor lysis syndrome has been described in six patients affected by CLL, PLL, MCL and transformed B-cell lymphoma at the beginning (30–60 min.) of the first Rituximab infusion. Patients presented with a significant infusion related side-effects, laboratory finding consistent with mild tumor destruction and thrombocytopenia [35]. The above referred toxicity has been observed using Rituximab alone, mostly in relapsed patients. Different toxicity profile has been observed using Rituximab as a maintenance therapy after chemotherapy and when follicular NHL is at the level of minimal residual disease. In this subset of patients infact, percentage and grade of AEs are extremely reduced [36]. Also utilizing Rituximab as a purging in vivo treatment, side effects remarkably decrease [37]. In conclusion, by virtue of the favorable safety profile of Rituximab it is a candidate for combination therapy with other biological agents and with chemotherapy regimens.

6. Clinical experience with rituximab in combination

6.1. Rituximab plus CHOP

Because of the encouraging results obtained with Rituximab alone in the treatment of relapsed LG/F NHL, a Phase II open label, single arm, multicenter study was designed to evaluate the safety and clinical activity of this new monoclonal antibody in combination with cyclophosfamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy in the treatment of patients with low-grade B-cell lymphoma [38]. CHOP chemotherapy was chosen because this cytotoxic regimen is an effective first-line therapy for LG/F NHL. The rationale for the combination of Rituximab and CHOP includes single agent efficacy, non-cross-resistant mechanisms of action, non-overlapping toxicities, and in vitro synergy with certain cytotoxic drugs, including doxorubicin. Forty patients, with low-grade (76% B-C) NHL (31 previously untreated) received six infusions of Rituximab at standard dose in combination with six doses of CHOP chemotherapy. The ORR was 95%. Twenty-two patients experienced a CR (55%), 16 patients had a partial response (40%), and two patients, who received no treatment, were classified as non-responders. Medians for DR and TTP had not been reached after a median observation time of $29+$ months. Twenty-eight of 38 assessable patients (74%) continued in remission during this median follow-up period. The most frequent AEs attributable to CHOP were alopecia, neutropenia and fever. The most frequent AEs attributed to Rituximab were fever and chills, observed primarily with the first infusion. No quantifiable immune response to the chimeric antibody was detected. In a subset of 18 patients, the bcl-2 [t (14;18)] translocation was positive in eight patients; seven of these patients had complete remissions and converted to polymerase chain reaction (PCR) negativity by completion of therapy. These results suggest an additive therapeutic benefit for the combination with no significant added toxicity. In an ongoing Phase II Italian trial, bcl-2^+ follicular lymphoma patients were treated at diagnosis with CHOP at standard schedule. Patients achieving clinical response, but still bcl-2^+, were treated with Rituximab at 375 mg/m^2 weekly $\times 4$. Preliminary results showed that CHOP alone is able to induce clinical response and bcl-2^+ conversion in 33% of patients. After Rituximab treatment 50% of patients who achieved clinical response with CHOP with persistent bcl-2^+ converted onto bcl-2 negativity. Thus, in this study Rituximab showed efficacy also at the level of minimal residual disease [36].

6.2. Rituximab plus IFN

Rituximab mechanism of action includes CDC, ADCC and induction of apoptosis. The administration of IFN-α before and during Rituximab treatment could be effective in increasing antigen surface expression; further the immuno modulators effect of IFN-α, including stimulation of T-cell cytotoxicity and natural killer cell activity, might synergize with the mechanism of action of Rituximab in inducing neoplastic clone suppression. In a pilot study, patients with re-

lapsed or refractory LG/F NHL were treated with IFN-α 5 million units/day/$m^2 \times 3$/weekly for three months and Rituximab 375 mg/m^2 weekly at weeks 5–8. Interim analysis was performed on 31 patients. Patient characteristics included: median age: 53, 71% male, 100% Caucasian, 10% IWF A, 90% IWF B, C, and D. All patients had progressive disease requiring therapy. Forty per cent had bone marrow involvement, 10% splenomegaly, 7% B symptoms, 7% tumor-related pain and 3% hepathomegaly. AEs were primarily grade I or II events occurring with the first infusion. Seven patients required dose modification and two discontinuation of IFN-α. One patient was hospitalized with fever and neutropenia on study day 149 and one with pneumonia on study day 38. Both recovered. There were no deaths. No patient developed HACA. In the 26 included patients 8% CRs and 50% PRs were observed for an ORR of 58%. Two additional patients were reported with minor responses and five with stable disease. This interim analysis suggests that combination immunotherapy with IFN-α and Rituximab is safe and effective. Final conclusions await completion and final analysis of this trial [39]. In a recently completed, non-comparative, multicenter, Phase II Italian study [40], 64 relapsed LG/F NHL patients were treated with IFN + Rituximab. Treatment started with IFA-α 1.5 MU/day sc for the first week and then at 3 MU during second week. At day 15 patients received the first Rituximab injection at 375mg/m^2, that was repeated at day 22, 29 on 36. IFN-α dose was increased to 3 MU during the third week and than to 6 MU during the fourth and fifth week. Patient characteristics included: median age 54, male 33%, 11% IWF A, 89% B, C and D; 81% stage III and IV previous chemotherapy regimens: 53% one regimen, 47% two or three regimens. All patients had progressive disease, requiring therapy. The ORR rate for the intent to treat analysis of 59 evaluable patients was 71%, of which 30% were CRs and 41% PRs. Four patients who have obtained PR were subsequently treated with radiotherapy and/or chemotherapy and they were excluded from time to progression (TTP) analysis. With a median observation time of 12.1 months the project median TTP for the 38 evaluable responders is 16 months (95% C.I. 9–23 months). Twenty two out of 38 responders (58%) have not yet relapsed. Fifty patients were evaluable for toxicity. AEs were primarily grade I or II, occurring with first Rituximab infusion. Nineteen patients required dose modification of IFN. Seven patients presented grade III and IV AEs. This interim analysis suggest that combination immunotherapy with IFN-α and Rituximab is more effective than Rituximab alone (71% ORR versus 50%), but the AEs rate increases.

7. Experimental use of rituximab

7.1. Enhancing response

A number of strategies to enhance the efficacy of Rituximab therapy are currently being investigated including biological agents. Two potential ways are currently under investigation: up-regulation of CD20 antigen expression, and increasing the activity of effector cells involved in ADCC. A recent research examined the effects of in vitro exposure to IFN on CD20 antigen expression in CLL. The study showed a significant up-regulation of the antigen expression after 24 h exposure to IFN [41].

Another recent study examined the effect of different cytokines on CD20 antigen expression in vitro, using CLL cells taken from the peripheral blood of 14 patients. The study showed a significant increase in CD20 expression with IL-4 and GM-CSF. This suggests that cytokines could potentially be used in combination with Rituximab to increase targeting of antibody to tumor cells by upregulating CD20 expression[42]. Cytokines could also increase Rituximab activity by raising the number of effector cells, such as neutrophils, that are involved in the cytotoxicity process. Examining the blood cell count of patients taking part in the pivotal Rituximab study, it appeared that T cell counts were unaffected by Rituximab treatment, and suggests that the response to Rituximab correlated with a higher absolute NK cell count at baseline [28]. Thus, agents such as G-CSF and GM-CSF that increase effector cell number could potentially boost Rituximab activity by increasing ADCC. [43].

A clinical trial is currently underway to test the efficacy of combination. The study combined a weekly infusion of Rituximab, 375 mg/m^2 with three daily infusions of G-CSF, 5 μ/Kg/day starting 2 days before each Rituximab infusions. So far nine patients have been evaluated for efficacy. The initial results revealed an overall response rate of 67% (four partial and two complete responses), and the median duration of response had not been reached after a median follow-up of 5 months. This suggests that combination therapy with G-CSF is at least as effective as Rituximab monotherapy [44].

7.2. In vivo purging

Looking for the use of Rituximab in a more curative setting, several research groups are now investigating this monoclonal antibody as an in vivo purging agent prior to autologous peripheral blood stem cell transplantation (PBSCT). High-dose chemotherapy (HDCT) supported by PBSCT offers the possibility of clearing the tumor cells from the

Table 1
Other possible therapeutic indications for Rituximab in malignant diseases

Chronic lymphocytic leukemia [47]
Prolymphocytic leukemia [48]
Hair cell leukemia
Hodgkin's disease
Multiple mieloma
Waldenström's macroglobulinaemia [45]
Post-transplantation lymphoproliferative disorders [49–51]
HIV-related lymphoma

Table 2
Possible therapeutic indications for Rituximab in non-malignant disorders

Immune thrombocytopenic purpura
Cold agglutinin disease [52]
Rheumatoid arthritis
Systemic lupus eriythematosus

body to a greater extent than can be achieved with standard chemotherapy or other first-line interventions. Theoretically, the benefits of autologous transplantation may be diminished if tumor cells are present in the stem cells returned to the body secondary to their potential contribution to post-transplant relapse of disease. Various approach to purging the stem cells have previously been investigated, including ex vivo procedures employing other monoclonal antibodies, but have been largely unsuccessful at completely purging the stem cells. More promising results have, however, been reported for in vivo purging with Rituximab [37,45]. Gianni et al. showed that Rituximab, in combination with one or two courses of an effective high-dose anti-lymphoma therapy, allowed the harvesting of large amounts of tumor-free progenitor cells in 13 out of 14 NHL evaluable patients, notably including all seven patients with MCL. The role of Rituximab clearly emerged from comparison with the control group. In fact, only four of the ten patients receiving chemotherapy alone, yielded a PCR-negative harvest, while the remaining six required ex vivo purging that was successful in four. In addition, the total amount of PCR-negative progenitors harvested from the Rituximab-treated patients was significantly superior. In conclusion, this in vivo purging strategy compares very favorable with ex vivo purging in terms of feasibility, cost, and overall success rate in harvesting an amount of uncontaminated CD34 + cells, fully adequate to support more than one cycle of subsequent myeloablative chemotherapy [37].

8. Rituximab in other indications

Because of the efficacy of Rituximab in low-grade NHL, this drug is currently being investigated in other therapeutic areas. Preliminary data from seven patients with Waldenström's macroglobulinaemia (WM) were presented at the 1998 ASH meeting [46]. WM tumor cells express high levels of the CD20 antigen, making the disease a potential target for Rituximab treatment. The results showed a partial response to treatment in three patients (43%), suggesting that Rituximab has activity against this tumor and warrants further studies. Initial results from a dose-escalation ongoing study of Rituximab treatment in CLL patients [47] showed a partial response in one of the 8 evaluable patients. Increasing doses of Rituximab, raging from 500–1500 mg/m^2 weekly, were used in the study following the initial 375 mg/m^2 infusion, with a view to countering the higher levels of circulating B cells seen in CLL compared with NHL. Other possible therapeutic indications for Rituximab in malignant and non malignant diseases are reported in Tables 1 and 2.

9. Conclusions

Biological therapies are now playing an important role in the treatment of many hematological malignancies. They range from differentiation-inducing molecules such as all-trans retinoic acid to immunotherapies such as IFN-α and interleukin-2, and to the more recently developed therapeutic MAbs. Studies conducted over the last three decades first allowed the supply of murine MAbs, through hybridoma technology and then the technique to humanize MAbs, through genetic engineering. These successes have permitted researchers to move from the laboratory to clinical application, transforming a dream into reality. The availability of effective MAbs has recently revolutionized the management of patients with indolent B malignancies. The results of several studies have established a role for Rituximab in the treatment of indolent B NHL and more recently in the treatment of more aggressive B-lymphomas. However, we are still far from having found a definite position for Rituximab in the treatment of B-malignancies. Although several clinical trials have been completed (Table 3), especially in patients with relapsed lymphoma, it is still not absolutely clear which strategy is the best with Rituximab. While we are learning which, among B malignancies, are good targets for Rituximab, we still ignore the best timing of Rituximab infusions when utilized in combination with chemotherapy. We still do not know which chemotherapeutic regimens are the best in synergizing with Rituximab. Indolent B-NHLs appear to be a good target for Rituximab, but relevant differences in overall response rate are reported in different entities, follicular lymphoma showing the best rate of objective responses. The activity of Rituximab in DLCL and in MCL is less known. Further, the impact of Rituximab therapy on survival is still unknown. Some

trials of Rituximab in association with chemotherapy have been completed and others are ongoing, but it is still unclear whether the best results will be obtained utilizing Rituximab before, during or after chemotherapy. Looking at Rituximab mechanism of action it would seem better to utilize Rituximab before chemotherapy, when patient immunity, although impaired by lymphoma, is not further suppressed by chemotherapy. On the other hand, with widely spread disease, side effects could be more relevant, because of a higher cytokine release, during or immediately after Rituximab infusion. In particular with leukemic cells highly expressing CD20 in blood stream, a rapid tumor-lysis is described. A synergism between chemotherapeutic agents and Rituximab in inducing tumor cell apoptosis can be obtained by infusing chemotherapy and immunotherapy on the same day, but the risk of tumor-lysis could be further increased in some subsets of patients. Considering the experience with other biological drugs, such as inter-feron, the use of Rituximab could be more effective when the tumor load has already been reduced by chemotherapy to the level of minimal residual disease. Rituximab can transform clinical CR and PR to molecular remission, as shown in some studies in which patients in clinical CR after chemotherapy, but still BCL-2 positive, became negative after Rituximab treatment. The choice of chemotherapeutic regimens could also be crucial, with drugs which impair patient effector cells less or better synergize with Rituximab in inducing apoptosis, could be more effective. The association with interferon improves overall response rate, probably by either up regulating CD 20 expression or by increasing patient effector cell activity thus increasing ADCC. However toxicity increases mainly due to IFN-related side effects. Meanwhile, studies of Rituximab plus G-CSF indicate this combination is feasible and yields promising results. Rituximab related adverse events are common, but they are mainly grade 1 or 2. Thus the safety profile is favorable when Rituximab is utilized in combination with chemotherapy. The low toxicity of Rituximab makes it an attractive option for patients with HIV-related NHL. Other possible malignancies, which may respond to Rituximab and are thus worth investigating, include post-transplantation lymphoproliferative disorders, multiple myeloma, hairy cell leukemia and chronic lymphocytic leukemia. Rituximab could also be of value as maintenance therapy following autologous stem cell transplantation for NHL in order to eradicate or suppress minimal residual disease. Another attractive application of Rituximab especially in MCL patients is in vivo purging prior to the harvesting of CD34+ cells for PBPC transplantation. The aim of future studies should be to develop new strategies that

Table 3
Completed Clinical Trials with Rituximab in B-NHL[a]

Authors and reference	Phase and dose schedule	B-NHL	Patient no	Response (%)	TTP (months)
Maloney DG [28]	I/II 375 mg/m^2 × 4	Relapsed LG/F and intermediate grade NHL	37	ORR: 48 CR: 6	13
McLaughlin P [26]	II 375 mg/m^2 × 4	Relapsed LG/F NHL	166	ORR 46 CR:8	10.2
Davis T [30]	II 375 mg/m^2 × 4	Re-treatment of relapsed LG/F	60	ORR: 41 CR: 12.5	Not reached
Davis T [31]	II 375 mg/m^2 × 4	LG/F NHL with bulky disease	31	ORR: 43 CR: 3	8.1
Coiffier B [32]	(A) 375 mg/m^2 × 1 + 500 × 7 (B) 500 mg/m^2 × 8	Relapsed or older then 60 years intermediate and high-grade	28 26	ORR: 31 CR: 9	8.2
Czuczman MS [38]	II 375 mg/m^2 × 6 + CHOP × 6	New diagnosed LG/F NHL	31	ORR: 95 CR 55	Not reached
Davis T [39]	II 375 mg/m^2 × 4 + IFN	Relapsed LG/F NHL	26	ORR: 58 CR: 8	Not done
Sacchi S [40]	II 375 mg/m^2 × 4 + IFN	Relapsed LG/F NHL	64	ORR 71 CR: 30	16+

[a] NHL, non Hodgkin lymphoma; ORR, overall response rate; CR, complete response; TTP, time to progression; LG/F, low grade or follicular NHL.

will hopefully produce the most effective Rituximab-based regimens, in order to find the Rituximab key position in the treatment of B-malignancies.

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References

[1] The non-Hodgkin's lymphoma pathologic classification project, National Cancer Institute Sponsored Study of Classifications of Non-Hodgkin's Lymphoma. Summary and description of work formulation for clinical usage, Cancer 1982;49:2112–35.
[2] Lennert K, Feller A, editors, Hystopatology of Non-Hodgkin's Lymphomas. New York: Springer Verlag, 1992.
[3] Harris LH, Jaffe ES, Stein H, et al. A revised European American classification of lymphoid neoplasm: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361–92.
[4] Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495–7.
[5] Pratik Multani S, Grossbard ML. Monoclonal antibody-based therapies for hematologic malignancies. J Clin Oncol 1998;16:3691–710.
[6] Yarnold S, Fell HP. Chimerization of antitumor antibodies via homologous recombination conversion vectors. Cancer Res 1994;54:506–12.
[7] LoBuglio AF, Wheeler RH, Trang J, et al. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. Proc Natl Acad Sci 1989;86:4220–4.
[8] Caron PC, Jurcic JG, Scott AM, et al. A phase 1 B trial of humanized monoclonal antibody M 195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity. Blood 1994;83:1760–8.
[9] Caron PC, Co MS, Bull MK, et al. Biological and immunological features of humanized M195 (anti-CD33) monoclonal antibodies. Cancer Res 1992;52:6761–7.
[10] Liu AY, Robinson RR, Murray ED, Jr, et al. Production of a mouse-human chimeric monoclonal antibody to CD20 with potent Fc-dependent biologic activity. J Immunol 1987;139:3521–6.
[11] Reff ME, Carner K, Chambers KS, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody CD20. Blood 1994;83:435–45.
[12] Mueller BM, Romerdahl CA, Gillies SD, et al. Enhancement of antibody-dependent cytotoxicity with a chimeric anti-GD2 antibody. J Immunol 1990;144:1382–6.
[13] Stashenko P, Nadler LM, Hardy R, Schlossman SF. Characterization of a human B lymphocyte specific antigen. J Immunol 1980;125:1678–85.
[14] Anderson KC, Bates MP, Slaughenhoupt BL, Pinkus GS, Schlossman SF, Nadler LM. Expression of human B cell-associated antigens on leukemias and lymphomas: a model of human B cell differentiation. Blood 1984;63:1424–33.
[15] Tedder TF, Boyd AW, Freedman AS, Nadler LM, Shlossman SF. The B cell surface molecule B1 is functionally linked with B cell activation and differentiation. J Immunol 1985;135:973–9.
[16] Tedder TF, Forsgren A, Boyd AW, Nadler LM, Shlossman SF. Antibodies reactive with the B1 molecule inhibit cell cycle progression but not activation of human B lymphocytes. Eur J Immunol 1987;16:881–7.
[17] Golay JT, Clark EA, Beverley PCL. The CD20 (Bp35) antigen is involved in activation of B cells from the G0 to the G1 phase of the cell cycle. J Immunol 1985;135:3795–801.
[18] Clark EA, Shu G, Ledbetter JA. Role of the Bp35 cell surface polypeptide in human B-cell activation. Proc Natl Acad Sci 1985;82:1766–70.
[19] LoBuglio AF, Wheeler RH, Trang J, et al. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. Proc Natl Acad Sci 1989;86:4220–4.
[20] Einfeld DA, Brown JP, Valentine MA, Clark EA, Ledbetter JA. Molecular cloning of the human B cell CD20 receptors predicts a hydrophobic protein with multiple transmembrane domains. EMBO J 1988;7:711–7.
[21] Press OW, Appelbaum F, Ledbetter JA, et al. Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood 1987;69:584–91.
[22] Golay J, Zaffaroni L, Borleri GM, et al. Rituximab and complement induced lysis of neoplastic B cells is regulated by CD55. Haematologica 1999; 84 (EHA 4 Abstract Book): Abs 0324.
[23] Demidem A, Hanna N, Hariharan H, Bonavida B. Chimeric anti-CD20 antibody (IDEC-C2B8) is apoptotic and sensitizes drug-resistant human B-cell lymphomas and AIDS-related lymphomas to the cytotoxic effect of CDDP, VP-16 and toxins. FASEB J 1995;9:A206.
[24] Maloney DG, Smith B, Appelbaum FR. The anti-tumor effect of monoclonal anti-CD20 antibody (mAB) therapy includes direct antiproliferative activity and induction of apoptosis in CD20 positive non-Hodgkin's lymphoma (NHL) cell lines. Blood 1996; 83: Abs A 2535.
[25] Maloney DG, Leles TM, Czerwinski DK, et al. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B cell lymphoma. Blood 1994;84:2457–66.
[26] McLaughlin P, Grillo-Lopez A, Link BK, et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998;16:2825–33.
[27] Maloney DG, Bodkin D, Grillo-Lopez AJ, et al. IDEC-C2B8: Final report on a phase II trial in relapsed non-Hodgkin's lymphoma. Blood 1994; 84 (Suppl. 1), Abs 661.
[28] Maloney DG, White C, Bodkin D, et al. Initial report on a phase I/II multiple dose clinical trial of (IDEC-C2B8) (chimeric anti CD20) in relapsed B-cell lymphoma. Blood 1997;90:2188–95.
[29] McLaughlin P, Grillo-Lopez AJ, Maloney DG, et al. Efficacy controls and long-term follow-up of patients (pts) treated with Rituximab for relapsed or refractory, low-grade or follicular (R-LG/F) NHL. Blood 1998; 92: (Suppl 1), Abs 1712.
[30] Davis T, Levy R, White CA, et al. Rituximab: Phase II (PII) retreatment (ReRx) study in patients (pts) with low-grade or follicular (LG/F) NHL. Blood 1998; 92 (Suppl 1): Abs 1710.
[31] Davis TA, White CA, Grillo-Lopez AJ, et al. Rituximab: first report of a phase II (PII) trial in NHL patients (pts) with bulky disease. Blood 1998; 92: (Suppl 1), Abs 1711.

[32] Coiffier B, Haioun C, Ketterer N, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood 1998;92:1927–32.
[33] Nguyen DT, Amess JA, Doughty H, Hendry L, Diamond LW. IDEC-C2B8 anti-CD20 (rituximab) immunotherapy in patients with low-grade non-Hodgkin's lymphoma and lymphoproliferative disorders: evaluation of response on 48 patients. Eur J Haematol 1999;62:76–86.
[34] Link BK, Grossbard ML, Fisher RI, et al. Phase II pilot study of the safety and efficacy of rituximab in combination with CHOP chemotherapy in patients with previously untreated intermediate-or high-grade NHL. Proc Am Soc Clin Oncol 1998; 17: Abs A7.
[35] Byrd JC, Waselenko JK, Maneatis TA, et al. Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid tumor lysis. Blood 1998; 92 (Suppl. 1): Abs 432.
[36] Rambaldi A, Manzoni C, Baccarani M, et al. t(14;18) positive follicular lymphoma: front-line treatment with CHOP and anti-CD20 (Rituximab) monoclonal antibody. Haematologica 1999; 84 (EHA 4 Abstract Book): Abs p 142.
[37] Gianni AM, Magni M, Di Nicola M, et al. In vivo purging of circulating CD34+ progenitor cells in low-grade lymphoma with Rituximab and high-dose chemotherapy. Blood 1998; 92 (Suppl. 1): Abs 481.
[38] Czuczman MS, Grillo-Lopez AJ, White CA, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol 1999;17:268–76.
[39] Davis T, Maloney D, White CA, et al. Combination Immunotherapy in low grade or follicular (LG/F) non-Hodgkin's lymphoma (NHL) with Rituximab and alpha interferon: Interim analysis. Proc ASCO 1998; 17: Abs 39.
[40] Sacchi S, Federico M, Vinci G, et al. Phase II study of Rituximab (IDEC-C2B8) after priming with IFN-(2a in patients with relapsed low-grade or follicular lymphoma. Blood 1999;94 (Suppl. 1):91a.
[41] Sivaraman S, Venugopal P, Huang X, et al. Effect of in vitro exposure to interferon alpha (IFNα) on CD20 expression in chronic lymphocytic leukemia cells (CLL). Ann Oncol 1999;10: Abs 222.
[42] Venugopal P, Sivaraman S, Huang X, et al. Upregulation of CD20 expression in chronic lymphocytic leukemia (CLL) cells by in vitro exposure to cytokines. Blood 1998; 92 (Suppl. 1): Abs 1009.
[43] Janakiraman N, McLaughlin P, White CA, et al. Rituximab: Correlation between effector cells and clinical activity in NHL. Blood 1998; 92: Abs 1384.
[44] van der Kolk LE, Grillo-Lopez AJ, Gerritsen W, et al. Chimeric anti-CD20 monoclonal antibody (Rituximab) plus G-CSF in relapsed B-cell lymphoma: A phase I/II clinical trial. Blood 1998; 92 (Suppl. 1): Abs 4037.
[45] Flinn IW, O'Donnell SJ, Noga SJ, et al. In vivo purging and adjuvant immunotherapy with Rituximab during PBSC transplant for NHL. Blood 1998; 92 (Suppl. 1): Abs 2673.
[46] Byrd JC, White CA, Link B, et al. Rituximab therapy in previously treated Waldenstrom's macroglobulinaemia: preliminary evidence of activity. Blood 1998; 92 (Suppl. 1): Abs 433.
[47] O'Brien S, Freireich E, Andreeff M, Lerner S, Keating M: phase I/II study of Rituxan in chronic lymphocytic leukemia (CLL). Blood 1998; 92 (Suppl. 1): Abs 431.
[48] Tsiara SN, Vartholomatos G, Kapsali H, et al. Monoclonal antibody, Rituximab, administration for the treatment of resistant B-prolymphocytic leukemia. Ann Oncol 1999; 10: Abs 467.
[49] Milpied N, Antoine C, Garnier JL, et al. Humanized anti CD-20 monoclonal antibody (Rituximab) in B postrasplant lymphoproliferative disorders (B PTLDs): a retrospective analysis of 32 patients. Ann Oncol 1999; 10: Abs 11.
[50] Pavlick A, Markowitz J, Goldstein M, et al. Treatment of post-transplant lymphoproliferative disorder (PTLD) with IDEC Anti-CD20 antibody (Rituximab). Ann Oncol 1999; 10: Abs 648.
[51] Grillo-Lopez AJ, Lynch J, Coiffier B, et al. Rituximab therapy of lymphoproliferative disorders in immunosuppressed patients (pts). Ann Oncol 1999; 10: Abs 662.
[52] Lee EJ, Kueck B. Rituxan in the treatment of cold agglutinin disease. Blood 1999;92:3490–1.

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