Current status and perspective of antibody therapy in follicular lymphoma

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The development of antibody – based therapeutic strategies has clearly changed the standard clinical approach to patients with advanced stage follicular lymphoma. The chimeric monoclonal anti-CD20 antibody rituximab has shown high efficacy in previously untreated and relapsed or refractory patients. Rituximab combined with conventional chemotherapy is a highly attractive approach with proven synergism *in vitro* and *in vivo* and is widely accepted as standard treatment for advanced stage follicular lymphoma. Furthermore, rituximab maintenance has been shown to improve disease control after successful cytoreduction with rituximab as a single agent therapy or polychemotherapy. Additional antibodies, different target molecules and modified schedules are currently being evaluated in preclinical and clinical trials. Strategies to enhance the efficacy of antibody – based therapies include stimulation of innate immunity and the generation of immunotoxins and radioimmunoconjugates (radioimmunotherapy). Ongoing studies are evaluating the role of monoclonal antibodies in multimodal therapeutic approaches to further improve response rates and duration with the final aim of prolonging overall survival of patients with advanced stage follicular lymphoma.

Key words: follicular lymphoma, monoclonal antibody, rituximab, immunochemotherapy, radioimmunotherapy

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Follicular lymphoma (FL) is one the most frequent subtypes of lymphoma worldwide and its incidence is rapidly increasing in western countries.[1] The vast majority of patients with follicular lymphoma present with advanced stage disease (Ann Arbor stage III or IV) at initial diagnosis, which is considered incurable by conventional therapeutic approaches. Until recently, decades of intense clinical research and exploration of different therapeutic strategies seemed not to have had a major impact on overall survival of these patients.[2] However, a recent analysis using a large population-based registry challenged this belief and reported better survival over the last 25 years, attributed most likely to improved supportive care and sequential application of effective therapies.[3] In this rapidly evolving field of established and innovative therapeutic options, physicians will have to face the challenge of deciding on appropriate treatment algorithms in patients with FL. It is generally accepted that patients with advanced stage FL do not have an overall survival benefit from immediate treatment at diagnosis rather than to a watch and wait strategy until the disease becomes symptomatic.[4-7] When FL becomes symptomatic chemotherapy induces remissions in the majority of patients but does not prevent recurrent relapses resulting finally in refractory disease or transformation to aggressive lymphoma.[8] In recent years various novel treatment options have been developed for this group of patients, including the promising concept of myeloablative chemo- or radiochemotherapy supported by autologous bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT). Dose intensification has the potential to eradicate disease more completely, but is limited by its treatment-related short-term and long-term toxicity including a considerably increased risk of secondary leukemias and myelodysplastic syndromes.[9-12] Allogeneic stem cell transplantation, representing mainly a cellular immuno-therapeutic approach, is currently considered the only curative treatment option in appropriate patients with advanced stage FL. However, even with dose-reduced conditioning this therapeutic approach is hampered by considerable treatment-related morbidity and mortality mainly caused by serious graft-versus-host disease (GvHD) and infectious complications and is therefore generally not recommended for the majority of patients or as front-line treatment.[13] There is, therefore, an urgent need for innovative therapeutic strategies with increased lymphoma specificity and reduced treatment-related toxicity. Several epitopes virtually restricted to lymphoproliferative malignancies and normal lymphoid tissues represent valid targets for immunotherapeutic approaches.

Immunotherapy with monoclonal antibodies promises increased lymphoma specificity, reduced toxicity and synergistic efficacy with conventional chemotherapy, based on their different modes of action. Monoclonal antibodies may be used as direct anti-lymphoma agents or can serve as carriers for either cytotoxins (immunotoxins) in the setting of targeted cytotoxic therapy[14] or radioisotopes in the setting of a targeted radiation therapy (radioimmunotherapy).[15] The introduction of the monoclonal chimeric anti-CD20 antibody rituximab and the emerging concepts of radioimmunotherapy have already substantially added to the therapeutic repertoire and clearly changed the standard clinical approach for patients with indolent lymphoma. Additional antibodies with promising activity and different target molecules as well as multimodal approaches are now being widely tested in preclinical and clinical trials. This review will focus on the current status in FL emphasizing the importance of

incorporating rituximab into up-to-date standard care. A perspective of antibody therapy in FL will also be provided, incorporating the most recent developments in this rapidly evolving field.

Mechanisms of action of monoclonal antibodies

The use of monoclonal antibodies and the approval of rituximab clearly represent major advances in the treatment of FL. However nearly one in three patients at front line[16] and about half of the patients at relapse[17] do not respond to single agent rituximab treatment and virtually all patients suffer from recurrent disease or progress at some time after rituximab treatment, revealing the critical need to improve and enhance the efficacy of monoclonal antibody activity. Understanding the underlying mechanisms of rituximab activity is, therefore, crucial. Rituximab is a chimeric IgG_1 anti-CD20 monoclonal antibody created by fusing the light- and heavy- chain variable domain of the murine IgG_1 anti-CD20 antibody 2B8 and the human κ light chain and γ1 heavy- chain constant region. The targeted antigen CD20 is expressed on virtually all B-cell lymphomas, does not internalize or shed from the surface in response to antibody binding and is absent on plasma cells and hematopoietic stem cells, thus representing the prototypic target antigen for antibody – based therapy of malignant B-cell lymphomas. The exact *in vivo* function of CD20 remains largely unknown. No physiologic ligand has been described and the structure of CD20 does not display typical features of a usual receptor.[18] In addition CD20-deficient knock-out mice do not show any obvious B-cell defect.[19]

Suggested mechanisms of antilymphoma activity of monoclonal antibodies include direct induction of growth arrest and apoptosis as well as complement-dependent cytotoxicity (CDC), and antibody-dependent cellular cytotoxicity (ADCC) based on experimental and clinical data.[18] However, the contribution of these different mechanisms to the cytotoxicity of the antibody remains controversial. Despite the demonstration that rituximab induces apoptosis in certain B-cell lines[20,21] the *in vivo* relevance of this pro-apoptotic activity for its anti-lymphoma effect remains unclear.[18] In contrast, there is clinical evidence for the relevance of CDC, coming from the observation that complement is activated and consumed during rituximab treatment and that remaining cells seem to express increased levels of the complement defensive molecule CD59.[22,23] In addition, rituximab failed to eliminate human CD20-positive lymphoma cells in C1q-deficient knock-out mice.[24] Furthermore, it has been shown that binding of certain CD20 monoclonal antibodies triggers translocation of CD20 into lipid rafts and that this is strongly associated with the ability of the antibody to induce CDC.[25] Based on their ability to eradicate lymphoma cells by complement-mediated mechanisms, two distinct types of anti-CD20 monoclonal antibodies were suggested:[26] the activity of type I reagents, including rituximab and IF5, is related directly to their binding and activation of the first component of complement, C1q, whereas type II reagents such as B1 are unable to translocate CD20 into lipid rafts and apparently do not use complement as their effector mechanism. In contrast B1 can directly induce potent apoptosis, probably accounting for its efficacy *in vivo*. Notably, B1 was approved for the treatment of non-Hodgkin's lymphoma as an iodine-131 conjugate, tositumomab (Bexxar®). ADCC is largely mediated by effector cells expressing immunoglobulin G fragment C receptor (FcγR), as demonstrated by significant reduced antilymphoma activity of rituximab in FcγR-deficient knock-out mice.[27]

Interestingly, patients with non-Hodgkin's lymphoma expressing the high-affinity variant 158V of the FcγRIIIa gene had better responses to rituximab treatment than those carrying low-affinity allotypes.[28] In addition, two independent polymorphisms of this receptor predicted response rate and freedom from progression in a clinical trial enrolling patients with advanced FL treated with rituximab as a single agent.[29] Recently, novel roles of rituximab as a signal-inducing antibody and as a chemosensitizing molecule have been described and extensively reviewed.[30] Various molecular signaling pathways have been shown to be modified by rituximab using *in vitro* B-cell non-Hodgkin's lymphoma (B-NHL) cell lines, including the p38 mitogen-activated protein kinase, the Raf-1/mitogen activated protein kinase/extracellular signal-regulated 1/a and the nuclear factor κB^{s} pathways. These modifications induced by rituximab were associated with down-regulation of the anti-apoptotic gene products Bcl-2/Bcl-xL and chemosensitization of drug-resistant B-NHL cell lines to various cytotoxins.[31]

There is a major concern about inducing CD20-negative escape mutants by rituximab treatment. Despite conflicting data about rituximab-induced downregulation of CD20 by a combination of internalization and RNA regulation,[32] there is an abundance of evidence suggesting relatively stable expression of the CD20 on the cell surface in response to rituximab binding.[26] However emergence of CD20-negative tumor variants in rituximab treated B-cell lymphoma patients have been reported occasionally.[33-36] Therefore CD20 expression should be reconfirmed at relapse or at progressive disease in lymphoma patients after rituximab treatment.[37] This progress in our understanding of the exact mechanisms of rituximab-induced killing of lymphoma cells will help to optimize the use of this antibody in the clinical setting and will also provide valuable information for the development of novel anti-CD20 antibodies.

Current status of monoclonal antibody therapy in indolent lymphoma

Rituximab as single agent therapy

In the first clinical trials the efficacy of rituximab was tested as single agent therapy in patients with relapsed or refractory indolent lymphoma (mainly follicular-type). The high anti-lymphoma activity of this antibody combined with its low toxicity profile and immunogenicity were confirmed in the pivotal study of 166 patients with refractory or relapsed low - grade B - NHL with an overall response rate of 48% (6% complete responses [CR], 42% partial responses [PR]) and a median time to progression of 13 months (median observation time of 13 months). Side effects were moderate and consisted mainly of infusion–associated flu-like symptoms.[17] These promising results were confirmed in several subsequent clinical trials with response rates between 21 to 63% (CR rate 6% to 24%).[35,38-41] On the basis of these data rituximab 375 mg/m^2 given four times at weekly intervals was approved for the treatment of refractory or relapsed FL. Rituximab monotherapy has also proven to be highly effective in first-line treatment of indolent lymphomas with response rates of 47% to 73% and complete remission rates of 7% to 20%.[16,40,42] Table 1 summarizes the results of important clinical trials in patients with indolent, mostly follicular lymphoma, treated first-line or at relapse with rituximab. Despite these encouraging results the duration of response in patients treated with rituximab monotherapy at relapse is quite short. One possible way of improving treatment outcome in these patients is to give rituximab as maintenance therapy after the initial four weekly rituximab infusions. The feasibility and efficacy of this approach was investigated in a phase III clinical trial by the *Swiss Group for Clinical Cancer Research* (SAKK), which randomized patients with FL, who achieved at least stable disease after four weekly rituximab infusions, into a rituximab maintenance arm (single rituximab infusions 375 mg/m^2 at 3, 5, 7 and 9 months after starting therapy) versus an observation arm.

After a median observation period of 35 months the event-free interval was 23 months in the maintenance arm and 12 months in the observation group (Figure 1). This difference in favor of the maintenance arm was even more pronounced for patients treated first line (36 months versus 19 months, respectively).[40] A similar approach was evaluated by Hainsworth *et al.*, who combined rituximab induction therapy (four-weekly infusions of 375 mg/m^2) with a rituximab maintenance regime, consisting of four-weekly rituximab infusions (375 mg/m^2) every 6 months until progression or a maximum of 2 years, in patients with advanced stage FL. The median progression-free survival of 34 months was significantly longer than the reported 12 months from a historical comparison for patients previously treated with the standard rituximab regimen.[42] A randomized phase II trial compared the benefit of rituximab either as maintenance treatment or re-treatment at progression in patients with relapsed indolent NHL. All 114 enrolled patients received a standard 4-week course of rituximab and those with either objective responses (28%) or stable disease (51%) underwent subsequent randomization. Progression-free survival was significantly longer in the maintenance group (31.3 vs. 7.4 months, respectively) and this was associated with higher final overall and complete response rates. However, the duration of the benefit of rituximab (i.e. the time from first rituximab treatment until the next required treatment) was statistically similar in the maintenance and the re-treatment groups, being 31.3 months and 27.4 months, respectively.[43] In conclusion, rituximab is clinically active a single agent in first-line as well as salvage therapy of advanced stage FL. However, the duration of response is short. Thus, rituximab maintenance strategies seem promising, but confirmatory data from large prospective trials are needed before this approach can be generally recommended. Due to the favorable toxicity profile of rituximab, its combination with conventional chemotherapy represents an attractive approach to enhance anti-lymphoma activity.

Rituximab in combination with chemotherapy

In vitro data early suggested the synergistic activity of cytotoxic chemotherapy and rituximab, based on their different modes of action. The anti-lymphoma activity of an immunochemotherapy approach consisting of rituximab combined with a conventional cytotoxic regimen in first-line treatment of advanced FL was investigated in several clinical trials. In one of the first phase II trials the combination of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) with rituximab induced responses in all evaluable patients with a complete remission rate of 63%.[44] A recent update of this study reported an impressive median time to progression and duration of response of 82.3 months and 83.5 months, respectively with 16 of 38 patients still in ongoing remission 6 to 9 years after treatment. Of note, seven of eight patients tested for the bcl-2 translocation achieved molecular remissions, of which three were sustained for several years after treatment.[45] The anti-lymphoma activity of rituximab plus chemotherapy was compared with that of chemotherapy alone in several large randomized phase III clinical trials. In the largest trial the *German Low-grade Lymphoma Study Group* (GLSG) randomized patients with previously untreated advanced stage FL to either 6 to 8 cycles of rituximab plus CHOP (R-CHOP) or to CHOP alone.[46] Responding patients were offered a second randomization to either myeloablative treatment with autologous stem cell transplantation (ASCT) or interferon-α maintenance. Although it cannot be excluded that post-remission therapy influenced treatment outcome of the initial cytoreduction, there was a balanced distribution of treatment in remission in both the R-CHOP and the CHOP arms. R-CHOP was superior with regards to overall response rate (96% versus 90%, respectively; p=0.011), time to treatment failure(p<0.0001) and duration of response (p<0.0006) (Table 2, Figure 2). Considering only patients receiving interferon-α maintenance (n=243), duration of response was significantly prolonged by the addition of rituximab, such that the median had not been reached compared to 26 months after CHOP (p=0.0004). There was even a trend towards improved overall survival within the first three years. The advantage of immunochemotherapy was evident both in younger (<60 years) (p=0.0024) and older patients (> 60 years) (p=0.003), as well as in low-risk (according to an IPI score of 0-2) (p<0.001) and high-risk patients (IPI score 3-5) (p=0.006). This improvement of treatment outcome was accompanied by a slight increase in the occurrence of grade 3/4 granulocytopenia but not in the rate of clinically relevant infections or other therapy-associated toxicity in the R-CHOP arm.

In a second large phase III trial, Marcus *et al.* also demonstrated the advantage of a rituximab/chemotherapy combination compared to chemotherapy alone in previously untreated patients with advanced stage FL. The addition of rituximab to the moderately intensive CVP regimen

Table 1. Rituximab as single agent therapy.

Disease	Number of patients (evaluable)	Response rate (CR)	Time to progression (months)	Reference (year)
Salvage therapy				
Indolent lymphoma (refractory or relapsed)	166 (151)	48% (CR 6%)	13	[17](1998)
Indolent lymphoma (relapsed with tumor bulk > 10 cm)	31 (28)	43% (CR 4%)	8.1	[35](1999)
FL (refractory or relapsed)	38 (30)	47% (CR 17%)	6.7	[38](2000)
FL (first line or relapsed)	78 (76)	52% (CR 6%)	na	[39](2000)
Indolent or transformed lymphoma	70 (70)	56% (CR 16%)	10.1	[41](2002)
FL (refractory/ relapsed)	128 (for response rate) 46 (for EFS)	46 (CR 8%)	10 (EFS)	[40](2004)
First-line therapy				
FL	50 (49)	73% (CR 20%/CRu 6%)	na	[16](2001)
Indolent lymphoma (rituximab-induction + rituximab maintenance)	62 (60)	47% (CR 7%) 73 %(CR 37%) after maintenance	34 (after maintenance)	[42](2002)
FL	57 (for response rate) 26 (for EFS)	67 (CR 9%)	19	[40](2004)

na: not available; FL: follicular lymphoma; EFS: event-free survival.

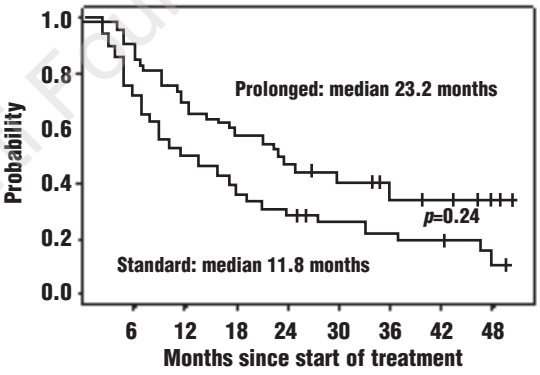

Figure 1. Event-free survival after rituximab maintenance or observation.[40]

(cyclophosphamide, vincristine, prednisone) resulted in a significant improvement of response rates, duration of response, time to treatment failure as well as progression-free survival compared to CVP alone (Table 2).[47] The superiority of a combination of rituximab with standard chemotherapy was further demonstrated by Herold *et al.*, who reported on the activity of rituximab plus the MCP-regimen (mitoxantrone 8 mg/m^3 days 3+4, chlorambucil 3×3 mg/m^3 days 3-7, prednisolone 25 mg/m^3 days 3–7, given every 28 days) versus the MCP regimen alone in patients with previously untreated advanced stage FL. In the subgroup of FL patients (n=201) immunochemotherapy induced an overall response rate of 92.4% and a CR rate of 49.5% versus 75% and 25%, respectively, for MCP alone (p<0.0001). Furthermore, the estimated 2-year event-free survival was 83% for R-MCP versus 43% for MCP (p<0.0001).[48] In another large randomized trial patients with advanced stage FL were treated first-line with either CHVP (cyclophosphamide 600 mg/m^2, doxorubicin 25 mg/m^2, etoposide 100 mg/m^2 on day 1 and prednisone 40 mg/m^2 days 1-5) plus interferon-α (175 patients) or with CHVP/interferon-α in combination with rituximab (184 patients). The first data analysis showed a significantly better response to the rituximab combination than to CHVP/interferon-α arm with a CR+CRundefined rate of 76% versus 49%, a PR rate of 18 versus 36% and rates of stable disease, progression or death of 6% versus 15%, respectively (p<0.0001)(Table 2).[49] Despite these encouraging results there are no definite data yet to answer the important question of whether immunochemotherapy also prolongs the overall survival of patients with advanced FL. For this a longer observation time in all the randomized studies mentioned above will be necessary. Another interesting approach is to incorporate rituximab into treatment protocols with the aim to clear minimal residual disease after successful chemotherapeutic cytoreduction. In an Italian multicenter trial, Zinzani *et al.* reported on their experience of sequential rituximab in patients with PR and those with CR but detectable minimal residual disease by bcl2/IgH specific polymerase chain reaction analysis after a randomized first line treatment with CHOP or fludarabine/mitoxantrone (FM) for stage II to IV FL.[50] The results, from 149 patients suggested a superiority of FM over CHOP in terms of CR rates (68% vs. 42%, respectively, p=0.003) and bcl-2/IgH negativity (38% vs. 19%, p=0.001). Sequential rituximab was capable of improving response status, both in clinical as well as molecular terms. However, the clinical significance of these findings remains unclear, as there were no significant differences in progression-free survival or overall survival between the various treatment arms. The question of the optimal time to administer rituximab was addressed in another randomized trial enrolling patients with stage IV indolent (follicular and small lymphocytic) lymphoma. Patients were treated with eight cycles of fludarabine/mitoxantrone/dexamethasone followed by interferon for 1 year and six doses of rituximab given either concurrently with chemotherapy or during the maintenance period. While there was no significant difference for the total cohort, subgroup analysis for patients with FL suggested better failure-free survival (76% vs. 60% at 3 years, respectively, p=0.12) and improved molecular responses for rituximab given concurrently with chemotherapy.[51] These data underline that the concept of concurrent rituximab – chemotherapy application should be followed to achieve the best treatment outcome. In an effort to minimize chemotherapy-related hematologic toxicity as well as cumulative non-hematologic toxicities a phase II clinical trial evaluated the feasibility, toxicity and efficacy of four weekly doses of rituximab (375 mg/m^2) followed by rituximab plus a short-duration chemotherapy regimen consisting of only three cycles of either CHOP or CVP in 86 previously untreated patients with stage II to IV FL.[52] Response rates were within the range of those reported for immunochemotherapy regimens of longer duration with overall and CR/CRundefined rates of 93% and 55%, respectively and 67% of patients remaining progression-free after a median follow-up period of 42 months. Of note all patients received the full dose of rituximab and scheduled chemotherapy could be used in the vast majority of patients without treatment delay. As expected therapy was well tolerated and treatment-related toxicity, including cardiotoxicity and peripheral neuropathy were clearly reduced in comparison to those produced by longer-duration chemotherapy, making this approach highly attractive for elderly patients and those with significant comorbidity. However randomized trails comparing this approach to standard immunochemotherapy are pending. In patients with relapsed FL the combination of rituximab with chemotherapy has also been proven to be superior to chemotherapy alone. In a randomized phase III clinical trial of the GLSG, patients with relapsed FL were treated either with four cycles of rituximab plus FCM (fludarabine 25 mg/m^2 day 1-3, cyclophosphamide 200 mg/m^2 day 1-3, mitoxantrone 8 mg/m^2 day 1) or with FCM alone.[53] The simultaneous use of the antibody in combination with FCM resulted in a significantly improved response rate (94% vs. 75%; p=0.047) and CR rate of 44% vs. 25% compared to FCM alone. Furthermore, the median progression-free survival was significantly prolonged by R-FCM with a median not reached after more than 3 years observation time compared to a median of 21 months in the FCM arm (p=0.013).[53] In addition, patients in this trial achieving com-

Table 2. Rituximab plus polychemotherapy in the first line therapy of advanced stage follicular lymphoma.

Hiddemann *et al.*[46]	CHOP	R - CHOP	p value
Patients evaluable	205	223	
Response rate	90%	96%	p=0.011
Median time to treatment failure	31 months	Not reached	p< 0.0001
Marcus *et al.*[47]	CVP	R-CVP	p value
Patients evaluable	159	162	
Response rate	57%	81%	p< 0.0001
Median time to treatment failure	7 months	27 months	p< 0.0001
Herold *et al.*[48]	MCP	R-MCP	*p* value
Patients evaluable	96	105	
Response rate	75%	92%	p<0.001
Median event-free survival	19 months	Not reached	p< 0.0001
Salle *et al.*[49]	CHVP/IFN-α	R-CHVP/IFN-α	
Patients evaluable	175	184	
Response rate (CR/CRu)	85% (49%)	94% (76%)	P< 0.0001
Median event-free survival	Not reached	Not reached	

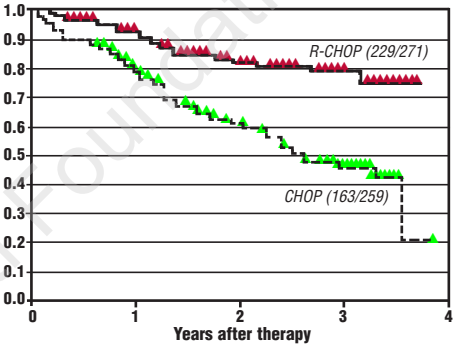

Figure 2. Time-to-treatment failure in follicular lymphoma: rituximab plus CHOP(R-CHOP) versus CHOP in first-line therapy.[46]

plete or partial remission (82%) underwent a second randomization for observation only or rituximab maintenance. While the median duration of response was 19 months for observation only at 3 years, it had not been reached in the rituximab maintenance arm (p=0.0171). Importantly, this beneficial effect was also observed in patients receiving R-FCM initially (19 months vs. not reached at 3 years) (p=0.0208).[54] These results were confirmed by another large phase III intergroup trial that enrolled patients with stage III or IV FL at initial diagnosis who relapsed after or were resistant to a maximum of two non-anthracycline containing systemic chemotherapy regimes. The addition of rituximab to the CHOP regimen yielded similar partial response rates (52.5% and 53.7%), but complete response rates were significantly improved by immunochemotherapy (30.4% for R-CHOP vs. 18.1% for CHOP) (p=0.0004). Again, patients achieving objective responses were randomly assigned to observation only or rituximab maintenance. Progression-free survival was significantly prolonged in the rituximab maintenance group with 80.2% and 67.7% free of progression at, respectively 1 and 3 years vs. 54.9% and 31.2% in the observation only group.[55] Combinations of rituximab with other cytostatic agents, including bendamustine[56] and fludarabine,[57] were also well tolerated and achieved excellent response rates in relapsed and refractory disease but longer follow-up and comparative trials are required. In summary, clinical trials have convincingly demonstrated that combined immuno-chemotherapy is superior to chemotherapy alone in first line as well as salvage therapy of patients with advanced stage FL in terms of improving response rates and prolonging response duration. Rituximab plus chemotherapy should be considered the new treatment standard in this patient group, achieving long-term responses without adding additional treatment-associated toxicity in comparison to chemotherapy alone. In this context R-CHOP may be the preferred treatment option in patients with advanced stage symptomatic disease in whom a high remission rate and long-lasting remissions are the primary goals of therapy. For patients with contraindications to anthracyclines or for medically unfit patients less intensive regimens such as CVP or short-term chemotherapy or prolonged use of rituximab as a single agent might be more appropriate. The key question still is whether immunochemotherapy also prolongs the overall survival in patients with advanced stage FL. Clinical trials are under way to answer this question, but a longer follow-up is needed to draw definite conclusions. However, there is no doubt that rituximab in combination with standard chemotherapy has a long-term beneficial effect for patients with this disease. Encouragingly, a 9-year follow-up of a phase II trial of patients treated with R-CHOP first-line for indolent lymphoma showed sustained disease-free intervals,[45] and there is hope that by designing multimodal concepts, such as combined immuno-chemotherapy, myeloablative treatment and rituximab maintenance, a cure can be potentially approached in a subset of patients with advanced FL.

Radioimmunotherapy

Radioimmunotherapy (RIT) represents a novel therapeutic approach that combines the tumor-targeting attributes of lymphocyte-specific monoclonal antibodies with therapeutic radioisotopes to be delivered to sites of disseminated disease. The most encouraging results have been reported for the ^{90}Y- labeled IgG1 κ anti-CD20 antibody ibritumomab (Zevalin®) and the ^{131}I– labeled IgG2 κ anti-CD20 antibody tositumomab (Bexxar®). In a non-myeloablative approach both conjugates demonstrated comparable activity with response rates of 60% to 80% and CR/CRu-rates between 15% to 44% in series of patients including some who relapsed after or were refractory to previous chemotherapy and rituximab.[15] In a randomized trial a single infusion of ^{90}Y-ibritumomab tiuxetan to patients with relapsed or refractory follicular lymphoma was superior to the standard 4 weekly infusions of rituximab with regard to overall response rate (80% vs. 56%, respectively) and CR-rate (30% vs. 16%, respectively); although the median time to progression was not greathy different (11.2 months and 10.1 months for ^{90}Y-ibritumomab tiuxetan and rituximab, respectively) (p=0.173) between the two treatment arms in the total cohort, the patients who achieved a CR after ^{90}Y-ibritumomab tiuxetan had a particularly long-term benefit.[41,58] Interestingly, durable long-term responses (i.e. time to progression ≥12 months) were achieved in 37 % of patients with relapsed or refractory B-cell lymphoma treated with ^{90}Y-ibritumomab tiuxetan in four clinical trials.[59] Kaminski *et al.* reported on the experience with ^{131}I-tositumomab in 76 previously untreated patients with FL.[60] Despite the favorable prognostic profile of this patient population, the high response rates (overall response rate 95%, CR rate 75%) and the nature of the durable remissions, with 59% of the patients remaining in ongoing complete remission at a minimum follow-up of more than 4 years after treatment, are encouraging. The efficacy and safety of ^{131}I-tositumomab therapy were also demonstrated in 21 patients with non-bulky FL, who developed progressive disease after rituximab, with an overall response rate of 86% and a median progression-free survival of 18.6 months.[61] Table 3 summarizes the data about the clinical activity of conventional doses of both radioconjugates.

Table 3. Radioimmunotherapy.

Study (Reference)	*Radio immunoconjugates*	*Number of patient*	*CR/CRu (%)*	*Response rate (%)*	*PFS/TTP* (months)*
Salvage Therapy					
Phase II[78]	^{131}I-tositumomab	59	34	71	12
Phase II[79]	^{131}I- tositumomab	47	32	57	11.6 (PFS)
Phase II[80]	^{131}I-tositumomab	60	20	65	8.4 (PFS)
Phase II[81]	^{131}I-tositumomab	273	27	58	NR (PFS)
Phase I/II[82]	^{90}Y-ibritumomab tiuxetan	34	26	82	12.9 (TTP)
Phase II[83]	^{0}Y-ibritumomab tiuxetan	54	15	74	8.7 (response duration)
Phase III[41]	^{0}Y-ibritumomab tiuxetan	73	34	80	11.2 (TTP)
Phase II[84]	^{90}Y-ibritumomab tiuxetan	30	44	84	12.6 (TTP)
First-ine therapy					
Phase II[85]	^{131}I-tositumomab	76	74 (CR)	95	Median not reached
Phase II[86]	CHOP followed by ^{131}I-tositumomab	90	67	90	Median not reached

**PFS: progression-free survival; TTP: time to progression.*

Perspectives of monoclonal antibody therapy in FL

Impact on overall survival

In the recent years there has been enormous progress in antibody–based therapy in patients with indolent lymphomas. In particular the introduction of the chimeric anti-CD20 antibody rituximab has dramatically changed our approach to patients with advanced stage FL and has become standard care in combination with simultaneous cytotoxic chemotherapy in the clinical management of

patients with FL and other lymphoma subtypes. In addition, targeted radiation therapy, applying radio-immunoconjugates such as ^{90}Y-ibritumomab tiuxetan or ^{131}I-tositumomab, is another promising approach because its mechanism of action is complementary to that of conventional chemotherapy and exploits the high radiosensitivity of malignant lymphomas. However, despite the encouraging results a still open question is whether immunochemotherapy or radioimmunoconjugates also prolong the overall survival of patients with advanced stage FL. Because of the indolent course of FL longer observation times are necessary to provide definite data from randomized trials in this respect. Importantly, Hiddemann *et al*. demonstrated for the first time that overall survival can be prolonged by adding rituximab to the FCM salvage regimen compared to FCM alone in patients with recurrent FL.[62] Data demonstrating that patients live longer when they receive rituximab combination therapy will play an important part in the discussion about the high costs of treatment approaches such as antibody–based therapies and for the acceptance of this expensive treatment approach outside of large clinical centers.

Rituximab as maintenance therapy

Another potential approach to improve treatment outcome in advanced stage FL is a maintenance strategy with rituximab after the best possible initial cytoreduction by rituximab in combination with a standard induction regimen. Data have shown that rituximab maintenance can improve duration of response after initial cytoreduction with rituximab alone.[40,42] Furthermore, in a randomized phase III study Hochster *et al*. demonstrated that rituximab maintenance significantly prolonged progression-free survival, such that the estimated 4-year progression-free survival was 58% in the maintenance arm compared to 34% in the observation arm after initial cytoreduction with conventional chemotherapy.[63] Recently the GLSG reported that rituximab maintenance improved progression-free survival compared to observation only (not reached at 3 years, vs 19 months, respectively) in patients with objective responses to induction therapy, which importantly also included prior rituximab use in combination with the FCM regimen.[54] The important question of whether rituximab maintenance remains beneficial after initial immunochemotherapy consisting of rituximab plus standard chemotherapy is currently being addressed in various other prospective randomized studies. Because of the cost implications of the maintenance approach, other important questions are how long and at what time intervals rituximab should be given for optimal efficacy in a maintenance regimen. Therefore, clinical trials aiming at defining a highly active, but also cost-effective maintenance regimen are needed.

Monoclonal antibodies in the setting of myeloablative therapy

Another important strategy in the treatment of FL is myeloablative chemo- or radiochemotherapy followed by autologous stem cell transplantation (ASCT), which significantly prolonged progression-free survival in several phase III trials.[9,10,64] Driven by the concern not to reinfuse malignant cells with the autologous stem cell graft, the concept of *purging* has been developed, i.e. elimination of potentially contaminating lymphoma cells *in vitro* and more recently also *in vivo* with the use of monoclonal antibodies.[65] *In vitro* purging of the graft may be achieved by either complement-mediated or immunomagnetic techniques using a mixture of monoclonal antibodies targeting a variety of B-cell associated epitopes including CD19, CD20 and CD10. Most available data suggest that B-cell depletion does not adversely affect either engraftment or hematologic recovery.[66] However clinical data on the effectiveness of *in vitro* purging remain inconclusive. A prospective non-randomized trial in 153 patients with FL undergoing myeloablative conditioning with cyclophosphamide and TBI followed by an *in vitro* purged autologous bone marrow transplantation demonstrated that the time to relapse was significantly longer if the graft was PCR negative for the BCL2 rearrangement than if it remained PCR-positive after purging.[67] The importance of achieving a molecular response and a PCR negative autograft was confirmed by an Italian trial enrolling patients with follicular (n=40), mantle cell (n=16) and small lymphocytic (n=14) lymphoma. Using PCR to detect BCL2, BCL1 and IgH rearrangements 86% of the patients had an identifiable molecular marker. At the time of harvest 54% of patients with FL had PCR-negative grafts. After a median follow up of 75 months only 8% of patients who achieved a molecular response relapsed as compared to 88% of patients who did not,[68] with durable clinical and molecular responses seen in patients with FL. The only currently available randomized trial which compared purged with unpurged autografts, the European CUP (chemotherapy vs. unpurged vs. purged) trial, closed early due to poor patient accrual, resulting in an insufficient sample size to address the question of whether purging B cells from autografts improves disease-free and overall survival. A highly attractive approach is to purge *in vivo* with rituximab, because it is feasible in daily practice, has few side effects

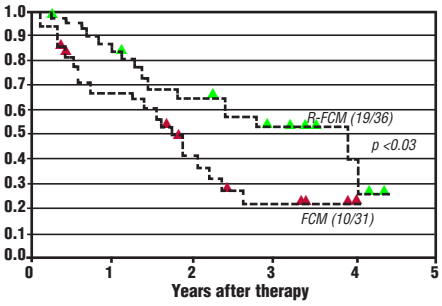

Figure 3. Duration of response in patients with relapsed follicular lymphoma: rituximab plus FCM (R-FCM) versus FCM.[53]

Table 4. Antibody constructs in pre-clinical and early clinical testing.

Antibody (Reference)	*Target*	*Structure*	*Pre-clinical testing*	*Clinical testing*
Epratuzumab[75]	CD22	Humanized, unconjugated + Radio-immunoconjugate	yes	Phase II, indolent lymphomas
CMC-544[14]	CD22	Humanized, conjugated to calicheamicin	yes	Phase I, B-NHL
HuMax-CD4[87]	CD4	Human	yes	Phase II, cutaneous T-cell lymphomas
Galiximab[88]	CD80	Chimeric	yes	Phase I/II, indolent lymphomas
Lym-1[89]	HLA-DR	Murine	yes	Phase I, NHL
HuMax-CD20[90]	CD20	humanized, unconjugated	yes	Phase I/II, relapsed or refractory FL
LL1[91]	CD74	humanized	yes	no
1D09C3[92]	HLA-DR	Human, unconjugated	yes	no
2F2 and 7D8[93]	CD20	Human, unconjugated	yes	no
IMMU 106[94]	CD20	Humanized, unconjugated	yes	no

and is clinically effective.[65] However, in some trials it is difficult to decide whether the observed clinical benefit is due to the *in vivo* purging or the anti-lymphoma effect of the antibody itself. In a small series enrolling patients with follicular and mantle cell lymphoma, the use of rituximab prior to stem cell harvest yielded 93% PCR negative autografts for the BCL2/IgH rearrangement as compared to only 40% in patients who did not receive rituximab.[69] These results were supported by an Italian trial enrolling patients with FL who were treated with the CHOP regimen followed by high-dose therapy with or without rituximab. With the use of rituximab 86% of patients had PCR negative autografts as compared to 14%, and 5-year progression-free survival was 100% as compared to 41%, respectively.[70] The concept of giving rituximab after ASCT proved to be feasible and safe in a phase II trial and suggested improved patient outcome.[71] These findings indicate that rituximab in the context of myeloablative therapy might be clinically important when used as an *in vivo* purging agent, or as consolidation after ASCT, However it is not known so far whether and if so to what extent the combination of rituximab with myeloablative therapy (e.g. with induction therapy, prior to stem cell harvest or as maintenance after ASCT) will further improve overall survival by eradicating or controlling minimal residual disease. An appealing approach for future studies will be to test whether a combination of initial immunochemotherapy, followed by myeloablation and ASCT in first remission and subsequent rituximab maintenance is able to improve overall survival and even cure patients with advanced stage FL.

Reducing treatment intensity in elderly and medically unfit patients

Taking into account that FL is a disease of the elderly and that in the future we will face a growing older population, one important issue will be to develop treatment strategies that are also applicable to those patients with their frequent comorbidities. Accordingly, a highly relevant aspect of antibody therapy with rituximab is its low toxicity profile, in particular in elderly patients who do not tolerate high-dose treatment or even conventional chemotherapy. Encouraging results have already been published for a combination regimen of full dose rituximab plus short duration chemotherapy yielding response rates comparable to those achieved with more aggressive chemotherapy while reducing treatment-related morbidity.[52] In this respect, a key task for the future will be todesign antibody-based strategies that allow effective lymphoma control without major treatment–related toxicity and impairment of quality of life.[72]

Enhancing the efficacy of monocloncal activity by biologicals

Despite its favorable safety profile, rituximab monotherapy has limited efficacy with a low CR rate and short duration of response in patients with FL.[17] Another attempt to enhance rituximab's antilymphoma activity is to stimulate the host's innate immunity by cytokines such as interferon, interleukin-2, granulocyte colony-stimulating factor[73] and granulocyte-macrophage colony stimulating factor (GM-CSF). The last approach was recently tested in a phase II clinical trial in 38 patients with FL grade receiving four cycles of standard rituximab monotherapy combined with GM-CSF for 8 weeks. The side effects of this treatment were not significantly greater than those of rituximab alone and response rates were better than expected from historical controls with an overall response rate of 70% and a CR rate of 45%.[74]

Investigational monoclonal antibody therapy

To further advance the field of lymphoma treatment additional novel monoclonal antibodies are currently being characterized and tested in the clinic. Preliminary results on fully humanized antibodies such as the anti-CD20 monoclonal antibody HuMax-CD20 demonstrated a favorable toxicity profile and significant depletion of peripheral blood B-lymphocytes in a phase I/II trial enrolling patients with relapsed or refractory FL.[73] Targeting other B-cell antigens also has therapeutic potential for malignant lymphomas. Epratuzumab is a humanized IgG1 antibody derived from the murine IgG2a antibody LL2. Though the function of CD22 is uncertain, it appears to play an important role in B-cell adhesion and activation. While virtually all developing B cells express CD22 in the cytoplasm, it is detectable on the cell surface only in mature stages of differentiation. FL is a subtype with strong expression of CD22. In a small phase I/II trial involving 55 patients with indolent lymphoma,[75] epratuzumab was well tolerated at doses up to 1,000 mg/m^2 weekly for 4 weeks. Fifty-one patients were assessable for response with three CR (6%) and six PR (12%) being observed only in patients with FL. Recently, combined treatment with epratuzumab 360 mg/m^3 and rituximab 375 mg/m^2 once weekly for 4 weeks was tested in 23 patients, including 15 who had FL.[76] The combination treatment was generally well tolerated and ten of the patients with FL achieved an objective (67%) response including nine CRs (60%). As CD22 is internalized in response to antibody binding, attempts have been made to conjugate cytotoxins to suitable antibodies. CMC-544 comprises a humanized IgG$_4$ anti-CD22 monoclonal antibody, G5/44, covalently linked to a potent DNA-binding cytotoxic antitumor antibiotic (N-acetyl-γ-calicheamicin dimethyl hydrazide).[14] After encouraging preclinical testing a dose escalation trial is currently ongoing in patients with various CD22 positive B-cell NHL. BL22, another recombinant immunotoxin containing an antibody-derived domain that recognizes CD22 and PE38, a truncated pseudomonas exotoxin A domain for inhibition of protein synthesis[77] is currently undergoing preclinical testing. Table 4 lists some of the continuously and rapidly growing group of antibodies screened for their anti-lymphoma activities *in vitro* and in first clinical trials. Taken together, the rapid progress of antibody-based therapy within the last few years has clearly changed treatment paradigms in FL. Thus hope is justified that in the near future curative treatment strategies will be available also for patients with advanced stages of this disease.

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