© 2006 American Society for Blood and Marrow Transplantation
1083-8791/06/1212-0001$32.00/0
doi:10.1016/j.bbmt.2006.07.007

Rituximab Induces Effective Clearance of Minimal Residual Disease in Molecular Relapses of Mantle Cell Lymphoma

Marco Ladetto,[1] Michele Magni,[2] Gloria Pagliano,[1] Federica De Marco,[1] Daniela Drandi,[1] Irene Ricca,[1] Monica Astolfi,[1] Paola Matteucci,[1] Anna Guidetti,[1] Barbara Mantoan,[1] Chiara Lobetti Bodoni,[1] Manuela Zanni,[1] Mario Boccadoro,[1] Alessandro M. Gianni,[2,3] Corrado Tarella[1]

[1]Divisione di Ematologia, Dipartimento di Medicina ed Oncologia Sperimentale, Università di Torino, Torino, Italy; [2]Medical Oncology Unit, Istituto Nazionale Tumori, Milan, Italy; [3]Department of Medical Oncology, University of Milan, Milan, Italy

Correspondence and reprint requests: Marco Ladetto, MD, Cattedra di Ematologia, Via Genova 3, 10126 Torino, Italy (e-mail: marco.ladetto@unito.it).

Received April 5, 2006; accepted July 17, 2006

ABSTRACT

Molecular remission (MR) is associated with improved outcome in mantle cell lymphoma (MCL). If MR is not achieved, patients are at high risk of relapse. We retrospectively describe the molecular and clinical follow-ups of 4 patients with molecular relapses (M-rels) who were treated with rituximab. The 4 patients received rituximab-supplemented, high-dose sequential chemotherapy and autologous stem cell transplantation as induction treatment and achieved clinical remission and MR. M-rel was defined as polymerase chain reaction (PCR) positivity in 2 consecutive samples in the absence of clinical relapse. M-rels occurred at 3, 6, 39, and 52 months and were always confirmed by direct sequencing of the clonal rearrangement. Minimal residual disease was monitored by qualitative and real-time quantitative PCR. All patients received 4 courses of rituximab, with 2 additional infusions if PCR positivity remained. After 4-6 courses of rituximab, all patients re-entered MR. No clinical relapses were recorded at 3, 6, 18, and 62 months from treatment, although 1 patient had a second M-rel that was sensitive to rituximab. Our results indicate that rituximab is active against residual MCL cells and suggest that molecularly tailored maintenance therapy needs to be investigated in clinical trials.

KEY WORDS

Mantle cell lymphoma • Minimal residual disease • Molecular remission • Monoclonal antibodies • Maintenance therapy

INTRODUCTION

Mantle cell lymphoma (MCL) is 1 of the worst subtypes of B cell lymphoma, with a median overall survival that does not exceed 4 years with conventional chemotherapy [1]. Aggressive treatment with autologous stem cell transplantation (ASCT) in the era before rituximab was attempted to improve outcome [2-4]. Although some good results were reported, ASCT was not curative as documented by the nearly constant persistence of molecularly detectable disease [1-5]. Rituximab has been employed in MCL as in most CD20-positive neoplasms [6,7]. However, the value of this drug is not exhaustively established. It is an effective single-agent drug that induces short-term responses in approximately 25% of rituximab-naive relapsed/refractory patients at diagnosis or at relapse [8,9]. When combined with conventional chemotherapy, rituximab is also beneficial. The German Low-Grade Lymphoma Study Group has observed a better response rate and time to treatment failure in rituximab-treated patients. However, this was not associated with an improved progression-free survival and overall survival [10]. The combination of rituximab and ASCT has been attempted in phase II trials, with excellent results [11,12], although randomized studies are lacking. One important observation was the frequent achievement of molecular remission (MR). This was rarely observed in patients with MCL treated with rituximab-free autografting regimens [2,4].

MR, particularly when associated with a clinical remission (CR), is a sign of major cytoreduction, although it does not indicate disease eradication. Nevertheless, a recent report by Pott et al [13] has demonstrated that the achievement of MR is a major favorable prognostic indicator in MCL. Although many patients with MCL achieve MR with rituximab-supplemented ASCT programs, molecular relapse (M-rel) is frequently observed and represents an early warning for subsequent clinical relapse. We retrospectively describe the clinical and molecular results of 4 patients who developed M-rel after a documented molecular response achieved with a rituximab-supplemented, high-dose sequential chemotherapy (R-HDS) program. Patients were analyzed with qualitative and real-time quantitative polymerase chain reaction (PCR).

METHODS

Patients' Clinical Features and Treatment Modality

Four patients were treated with R-HDS outside the context of a clinical trial (Table 1): 3 patients were never enrolled in a clinical trial and the fourth participated in a small preliminary study whose endpoints were exclusively feasibility, toxicity, and early molecular outcome. At the time of M-rel, their treating physicians decided that rituximab retreatment was an appropriate choice from a clinical viewpoint based on several published and personal unpublished observations suggesting that M-rel is rapidly followed by clinical relapse in MCL [4,13] (Ladetto, unpublished observation; Magni, unpublished observation). The R-HDS regimen has been reported elsewhere [11,12]. Briefly, patients were initially treated with 2 to 3 courses of doxorubicin (75 mg/m^2 of body surface area given intravenously on day 1), prednisone (40 mg/m^2 orally given from days 1 to 21), and vincristine (1.4 mg/m^2 given intravenously on day 1). After the initial standard dose phase, all patients received the 4-step high-dose sequence, including intravenous administration of high-dose cyclophosphamide (7 g/m^2), high-dose cytarabine (1.5 or 2 g/m^2 every 12 hours for 6 consecutive days), high-dose melphalan (180 mg/m^2), and high-dose mitoxantrone plus melphalan (60 and 180 mg/m^2, respectively). Stem cell collections were taken after high-dose cyclophosphamide and high-dose cytarabine. Minimum delay between courses was 3 weeks but delays were considered depending on hematologic and nonhematologic toxicities. It should be pointed out that this program includes 6 rituximab infusions at the dosage of 375 mg/m^2. Two doses were given after high-dose cyclophosphamide the day after chemotherapy and the day before stem cell collection. Two doses were given after high-dose cytarabine with a similar schedule. The last 2 doses were given at the end of the entire program. To hasten hematopoietic recovery, each patient received 3 progenitor/stem cell reinfusions after the nonmyeloablative course of high-dose cytarabine and after the 2 subsequent myeloablative courses of melphalan and mitoxantrone plus melphalan. The minimum target doses of CD34$^+$ cells/kg of body weight to be reinfused after each of the 3 autografts were 2, 3, and 5 $\times$ 10^6, respectively. PCR-negative stem cell collections were always preferred for autografting procedures. Complete CR was defined according to the criteria of Cheson et al [14]. Follow-up evaluations including physical examination, computed tomographic scan, bone marrow biopsy, cell blood counts, and blood chemistry were performed at 3, 6, and 12 months during the first year, at least twice in the second and third years after transplantation, and then at least yearly. M-rel was defined as reappearance of PCR positivity in $\geq$2 subsequent bone marrow samples taken with an interval of $\geq$30 days. Three patients were treated at the University of Torino and 1 at the Istituto Tumori of Milan. Risk and advantages of the planned treatment were carefully dis-

Table 1. *Main Clinical Features*

Patient Identification	Age	Sex	Stage	aaIPI	BM Involvement	Molecular Marker	Follow-up: PCR Status at End of R-HDS	Follow-up: End of R-HDS/M-rel	Reinduction of MR/Final Follow-up Visit	Clinical Status at Final Follow-up Visit
MCL-77	42	M	IVA	1	10%	t(11;14)	Negative	3	16	CR
MCL-81	63	M	IVB	2	10%	t(11;14)	Negative	42	12	CR
MCL-57	56	M	IVB	2	40%	IgH	Negative	6*	58‡	CR
								36†	24§	
MCL-51	57	M	IVA	1	1%	IgH	Negative	55	11	CR

aaIPI indicates age-adjusted international prognostic index; BM, bone marrow; PCR, polymerase chain reaction; R-HDS, rituximab-supplemented, high-dose sequential chemotherapy; M-rel, molecular relapse; MR, molecular remission; M, male; IgH, immunoglobulin H; CR, complete remission.

*Follow-up from the end of R-HDS to first M-rel.

†Follow-up from the end of R-HDS to second M-rel.

‡Follow-up from first post-rituximab PCR-negative result after the first M-rel to final clinical observation.

§Follow-up from first post-rituximab PCR-negative result after the second M-rel to final clinical observation.

cussed with the patients and informed consent was signed by all. Four weekly doses of rituximab were delivered at the dosage of 375 mg/m^2. In case of persistent PCR positivity, 2 additional infusions were delivered.

PCR Amplification of t(11;14) Translocation by Qualitative and Quantitative PCR

Qualitative PCR amplification of the t(11;14) translocation was performed by using a seminested PCR approach as described by Andersen et al [2]. Reaction conditions for the first and second rounds of amplification were a first cycle at 94°C for 3 minutes, followed by 30 cycles with denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension time of 10 minutes. PCR products were then run in 2% agarose gels containing ethidium bromide and visualized under UV light. Each reaction was performed using appropriate negative and positive controls. The nature of the rearrangements was confirmed by direct sequencing. Real-time quantitative PCR was performed with an adapted version of the method described by Olsson et al [15] and the same primers and TaqMan probes. Real-time PCR conditions have been described; briefly, 400 ng of target DNA was amplified in 50 μL using the TaqMan PCR MasterMix (PE Applied Biosystems, Foster City, Calif). Reactions were performed in an AbiPrism 7900 sequence detector system (PE Applied Biosystems) under the following conditions: after an incubation of 2 minutes at 50°C and an incubation of 10 minutes at 95°C, the following reaction was run: 42 cycles of denaturation at 95°C for 15 seconds and annealing at 60-62°C for 1 minute. Absolute quantification of the number of rearrangements was done using serial 10-fold dilutions of plasmid containing the cloned rearrangement, starting from 10^6 plasmid copies, as described elsewhere [16]. Quantitation standards were obtained by cloning the tumor-specific t(11;14) rearrangement using the TA Cloning Kit as published elsewhere [16]. Normalization of samples for DNA quality and quantity was done using the glyceraldehyde 3-phosphate gene, as previously described [17].

When a sample was scored to be PCR positive by nested PCR and PCR-negative by real-time PCR, the score was defined as "below the sensitivity threshold" of real-time PCR (BST). Triplicate analyses of real-time PCR can detect a single clonal rearrangement in each tube. Thus, based on the amount of DNA tested, the sensitivity of real-time PCR is 5 clonal rearrangements (cr)/10^6. This leads to a postulate that the clonal burden of a BST sample might range from 5 cr/10^6 cells (sensitivity threshold of real-time PCR) to 1.0 cr/10^6 cells (sensitivity threshold of nested PCR). Thus, for the purpose of this analysis, the clonal cell burden of BST samples was placed equal to the mean of the 2 values, ie, 3 Bcl-1/immunoglobulin heavy chain gene (IgH) cr /10^6 dg.

Amplification, Sequencing, Qualitative, and Real-Time Quantitative PCR for the IgH Rearrangement

Patient-specific IgH rearrangements were amplified and direct-sequenced from tumor-derived genomic DNA (ie, bone marrow or lymph node) obtained at diagnosis. For these reactions, consensus sense primers derived from the leader (L) and first framework region (FR1) and a consensus antisense primer derived from the FR4 were employed as previously described [16]. Based on the IgH sequence, tumor-specific primers derived from the second and third complementarity-determining region (CDR) were designed and synthesized to perform qualitative minimal residual disease (MRD) evaluation by nested amplification, as described elsewhere [18]. Real-time quantification of the number of IgH rearrangements was performed on genomic DNA samples using tumor-specific primers and consensus probes, as previously reported [16]. The sense primer was derived from the CDR2 or FR3, and the antisense primer was always generated from the highly hypervariable CDR3, as previously reported [16]. Consensus probes for real-time PCR analysis were derived from the FR3 as previously reported. Sequences of these probes have been already detailed elsewhere [16].

Absolute quantification was performed by cloning the patient-specific IgH rearrangement and by preparing six 10-fold dilutions of cloned rearrangements as previously described [16]. DNA normalization and quantification of samples scoring PCR positive by nested PCR and PCR negative by real-time PCR were done as for the t(11;14).

RESULTS

Clinical Results

All patients were in CR and maintained their status throughout the entire observation period. All patients had normal complete blood cell counts and routine chemistry values. As planned in the R-HDS, these patients were kept on acyclovir and cotrimoxazole prophylaxis for the first year after transplantation. Rituximab infusion produced no side effects in any patient.

Molecular Follow-up by Nested PCR

The 4 patients were monitored for 76, 71, 58 and 18 months from transplantation. All 4 patients received PCR-negative grafts after the 2 submyeloablative procedures. Their molecular follow-up by nested PCR is shown in Figure 1. Overall, 42 MRD determinations were done by nested PCR on post-transplantation bone marrow samples. Two M-rels occurred early after transplantation at 3 and 6 months,

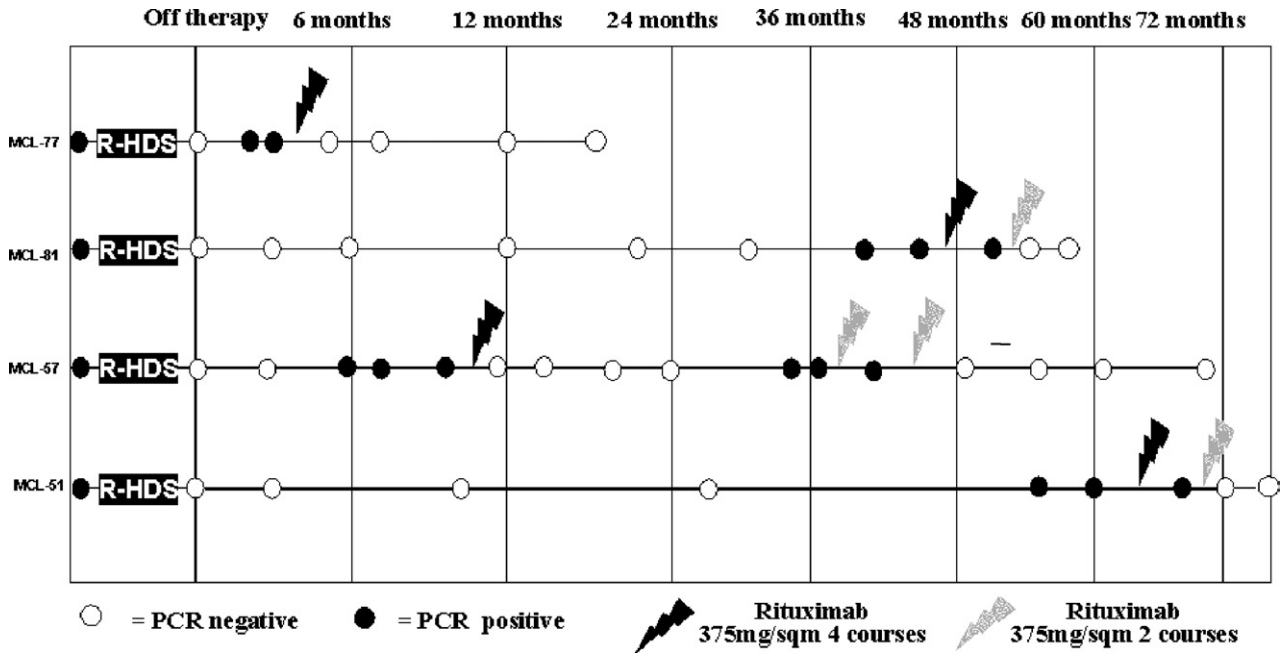

Figure 1. Molecular monitoring of patients with MCL and time points of rituximab delivery. The R-HDS program has been described elsewhere [11]. Rituximab was delivered at the dosage of 375 mg/m^2.

respectively. Two subsequent M-rels occurred at 39 and 52 months from the end of treatment. All M-rels were confirmed to be related to the original tumor clone by direct sequencing of the t(11;14) translocation or IgH rearrangement.

All 4 M-rels responded to rituximab treatment as demonstrated by a reversion to MR in the 4 patients after 4 or 6 rituximab courses (Figure 1). This status has been maintained in 3 patients at 5, 6, and 18 months, whereas patients MCL-57 developed a second M-rel 24 months later. Of note, the second relapse was responsive to rituximab and PCR negativity was achieved and has been maintained at 24 months from the final rituximab infusion. From a clinical viewpoint at the final follow-up visit, patients showed no sign of active lymphoma at 11, 12, 16, and 24 months from reinduction of M-rel (when considering a second reinduction for patient MCL-57).

Molecular Follow-up by Quantitative Real-Time PCR

Figure 2 shows the results of quantitative monitoring of clonal cells at the time of M-rel and after

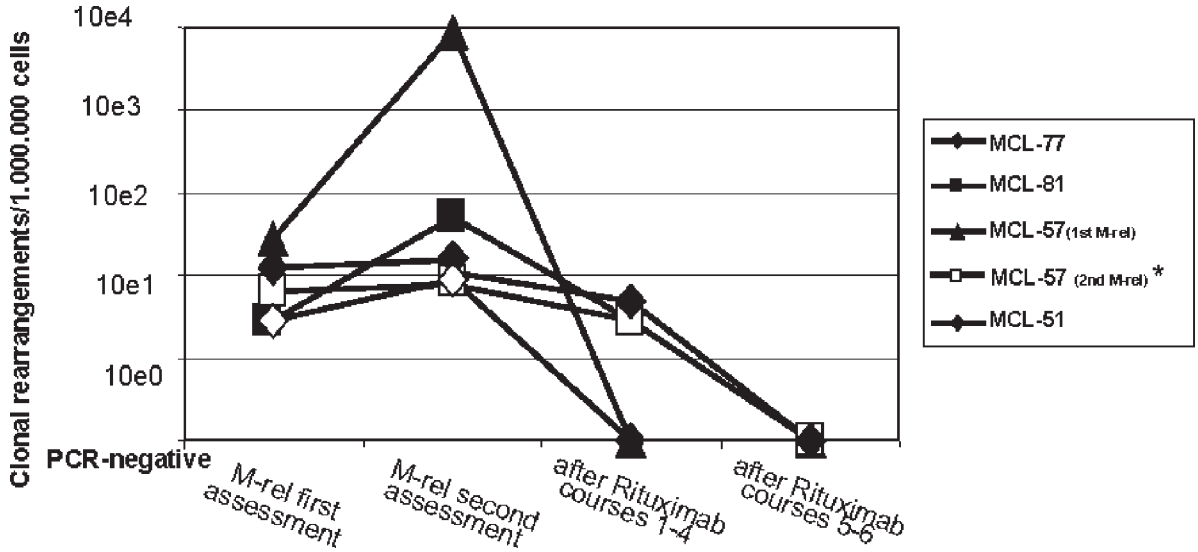

Figure 2. Quantitative monitoring of MRD at M-rel and after rituximab treatment. Both M-rels of patient MCL-57 were studied. *During his second M-rel, this patient received only 4 rituximab courses, 2 before the first post-treatment time point and 2 before the second time point.

rituximab treatment. For patient MCL-57, both M-rels were studied.

Real-time PCR indicated that, from the first PCR-positive result to the confirmatory assessment, tumor burden was stable or increasing. Interestingly, at the time of his first M-rel, patient MCL-57 was not treated immediately but only after 5 months. This was associated with a more pronounced increase in tumor cell burden compared with the other patients. This increase clearly indicated the presence of active disease. Rituximab treatment always induced a reduction of residual tumor cell burden that resulted in PCR negativity after 4 courses in 2 cases and after 6 courses in 3.

DISCUSSION

This retrospective report indicates, for the first time, that rituximab can reinduce MR in patients with MCL who develop M-rel after intensified rituximab-containing autografting regimens. None of the patients re-entering MR developed clinical relapse. MR is reinduced for periods that are non-negligible, although it is probably not permanent. One patient with a long follow-up had a second M-rel that was again sensitive to rituximab.

Persistence of molecular disease is a major negative prognostic feature in several B cell lymphomas. Although this observation was initially established in follicular lymphoma [19], several studies have shown that the same is true for MCL. In the age before rituximab, Andersen et al [2] and Corradini et al [4] showed that patients with MCL remain PCR positive in the vast majority of cases and develop early clinical relapse [2,4]. In contrast, allogeneic transplant recipients are often characterized by persistent MR [2,20]. After the introduction of regimens such as R-HDS, which combine rituximab and high-dose sequential chemotherapy with ASCT, a significant increase in the rate of MR was observed [11,12]. The clinical value of R-HDS is difficult to define because it has not been assessed in randomized trials. However, these PCR-negative patients had an outcome that compared favorably to the typical outcome of MCL. Recent work from Pott et al [13] has clearly shown that achievement of MR ensures a significantly better long-term outcome in patients with MCL.

The clinical decision of delivering rituximab in the presence of a documented M-rel without additional signs of disease activity was made after taking into account the good toxicity profile of rituximab and the increased risk of relapse typical in patients with this condition. These considerations were further supported by the sharp increase in tumor burden observed between the first and second molecular assessments by real-time quantitative PCR.

Our study shows that, in case of M-rel, rituximab allows reinduction of a PCR-negative status. This occurs even if patients have already received several courses of rituximab, suggesting that residual tumor cells are still sensitive to this agent. Because our series of patients is small, we cannot demonstrate that PCR negativity restores the low-risk status associated with MR. However, it is worth mentioning that none of our patients has so far relapsed at 11, 12, 16, and 24 months from reinduction of M-rel (considering second reinduction for patient MCL-57). Undoubtedly a larger experience and a longer follow-up will help clarifying this issue.

Rituximab has been commonly employed as maintenance treatment in lymphoid malignancies [21-23]. The benefit of this treatment has been clearly demonstrated in several phase III trials for patients with follicular lymphoma [22,23]. In MCL, the use of rituximab in the context of a "prolonged treatment" schedule has been investigated by Ghielmini et al [24]. This study has clearly shown that prolonged delivery of rituximab as a single agent is not able to improve the outcome of patients with MCL [24]. However, patients included in this study were not in a phase of extensive cytoreduction. This indicates that rituximab alone is probably not able to control disease in the presence of large amounts of tumor cells. This might provide an additional rationale for the use of maintenance rituximab in a MRD setting in MCL. Our data suggest that a customized delivery, based on molecular results, might be an alternative strategy worthwhile of investigation in clinical trials to improve clinical efficacy and ensure rational and cost-effective use of expensive drugs.

Molecular monitoring of MRD, particularly by real-time PCR, has gained considerable success in several hematologic cancers, in particular chronic myelogenous leukemia [25] and acute promyelocytic leukemia [26]. Its use in mature lymphoproliferative disorders is less established. We have shown that real-time PCR using the t(11;14) or IgH rearrangement allows accurate disease monitoring. In 2 cases, the clonal cell burden appeared to be increasing before rituximab delivery, thus providing good kinetic evidence of the presence of active disease. In addition, rituximab effectively induced a progressive disease reduction that was effectively monitored by real-time PCR. Interestingly, some patients reverted to PCR negativity after 4 courses, whereas others required 6 courses. Of note, the patient with the greatest tumor load had a prompt response after only 4 cycles of rituximab. This suggests the existence of different levels of sensitivity to rituximab, although the numbers are too small to draw definite conclusions.

This study is only 1 of several reports that indicate that molecular monitoring of MRD is becoming robust enough for widespread use in mature lymphopro-

liferative disorders. Obviously, many well-designed prospective clinical trials need to be performed in future years to build a strong basis for the use of MRD as a routine clinical tool in non-Hodgkin lymphoma. Several of these studies employing PCR results as major endpoints of clinical activity and for treatment tailoring are ongoing at our institution and worldwide in lymphoma and multiple myeloma. As far as MCL is concerned, a prospective trial assessing the value of molecularly tailored treatment is under discussion in the context of our cooperative group.

ACKNOWLEDGMENTS

The study was supported in part by the Compagnia di San Paolo, Turin; Italy; Regione Piemonte, Turin, Italy; and Ministero dell' Università e della Ricerca, Rome, Italy. GP received a fellowship from the Fondazione Angela Bossolasco, and MA received a fellowship from the Associazione Italiana Leucemie.

REFERENCES

1. Lenz G, Dreyling M, Hiddemann W. Mantle cell lymphoma: established therapeutic options and future directions. *Ann Hematol*. 2004;83:71-77.
2. Andersen NS, Donovan JW, Borus JS, et al. Failure of immunologic purging in mantle cell lymphoma assessed by polymerase chain reaction detection of minimal residual disease. *Blood*. 1997;90:4212-4221.
3. Freedman AS, Neuberg D, Gribben JG, et al. High-dose chemoradiotherapy and anti-B-cell monoclonal antibody-purged autologous bone marrow transplantation in mantle-cell lymphoma: no evidence for long-term remission. *J Clin Oncol*. 1998;16:13-18.
4. Corradini P, Astolfi M, Cherasco C, et al. Molecular monitoring of minimal residual disease in follicular and mantle cell non-Hodgkin's lymphomas treated with high-dose chemotherapy and peripheral blood progenitor cell autografting. *Blood*. 1997;89:724-731.
5. Ganti AK, Bierman PJ, Lynch JC, Bociek RG, Vose JM, Armitage JO. Hematopoietic stem cell transplantation in mantle cell lymphoma. *Ann Oncol*. 2005;16:618-624.
6. Witzig TE. Current treatment approaches for mantle-cell lymphoma. *J Clin Oncol*. 2005;23:6409-6414.
7. Brugger W. Clearing minimal residual disease with rituximab consolidation therapy. *Semin Oncol* 2004;31(suppl 2):33-37.
8. Foran JM, Rohatiner Ama ZS, Cunningham D, et al. European phase II study of rituximab (chimeric anti-CD20 monoclonal antibody) for patients with newly diagnosed mantle-cell lymphoma and previously treated mantle-cell lymphoma, immunocytoma, and small B-cell lymphocytic lymphoma. *J Clin Oncol*. 2000;18:317-324.
9. Ghielmini M, Schmitz SF, Burki K, et al. The effect of rituximab on patients with follicular and mantle-cell lymphoma. Swiss Group for Clinical Cancer Research (SAKK). *Ann Oncol* 2000;11(suppl 1):123-126.
10. Lenz G, Dreyling M, Hoster E, et al. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). *J Clin Oncol*. 2005;23:1984-1992.
11. Magni M, Di Nicola M, Devizzi L, et al. Successful in vivo purging of CD34-containing peripheral blood harvests in mantle cell and indolent lymphoma: evidence for a role of both chemotherapy and rituximab infusion. *Blood*. 2000;96:864-869.
12. Gianni AM, Magni M, Martelli M, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo rituximab-purged stem cell autografting (R-HDS regimen). *Blood*. 2003;102:749-755.
13. Pott C, Schrader C, Gesk S, et al. Quantitative assessment of molecular remission after high-dose therapy with autologous stem cell transplantation predicts long-term remission in mantle cell lymphoma. *Blood*. 2006;107:2271-2278.
14. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. *J Clin Oncol*. 1999;17:1244-1253.
15. Olsson K, Gerard CJ, Zehnder J, et al. Real-time t(11;14) and t(14;18) PCR assays provide sensitive and quantitative assessments of minimal residual disease (MRD). *Leukemia*. 1999;13:1833-1842.
16. Ladetto M, Donovan JW, Harig S, et al. Real-Time polymerase chain reaction of immunoglobulin rearrangements for quantitative evaluation of minimal residual disease in multiple myeloma. *Biol Blood Marrow Transplant*. 2000;6:241-253.
17. Donovan JW, Ladetto M, Zou G, et al. Immunoglobulin heavy-chain consensus probes for real-time PCR quantification of residual disease in acute lymphoblastic leukemia. *Blood*. 2000; 95:2651-2658.
18. Voena C, Ladetto M, Astolfi M, et al. A novel nested-PCR strategy for the detection of rearranged immunoglobulin heavy-chain genes in B cell tumors. *Leukemia*. 1997;11:1793-1798.
19. Gribben JG, Neuberg D, Freedman AS, et al. Detection by polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. *Blood*. 1993;81:3449-3457.
20. Corradini P, Ladetto M, Astolfi M, et al. Clinical and molecular remission after allogeneic blood cell transplantation in a patient with mantle-cell lymphoma. *Br J Haematol*. 1996;94:376-378.
21. Hainsworth JD, Litchy S, Shaffer DW, Lackey VL, Grimaldi M, Greco FA. Maximizing therapeutic benefit of rituximab: maintenance therapy versus re-treatment at progression in patients with indolent non-Hodgkin's lymphoma—a randomized phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol*. 2005;23:1088-1095.
22. Hochster HS, Weller E, Gascoyne RD, et al. Maintenance rituximab after CVP results in superior clinical outcome in advanced follicular lymphoma (FL): results of the E1496 phase III trial from the Eastern Cooperative Oncology Group and the Cancer and Leukemia Group B. *Blood*. 2005;106:349.
23. Van Oers MHJ, Van Glabbeke M, Teodorovic I, et al. Chimeric Anti-CD20 monoclonal antibody (rituximab; mabthera) in remission induction and maintenance treatment of relapsed /resistant Follicular Non-Hodgkin's lymphoma: final analysis of a phase III randomized intergroup clinical trial. *Blood*. 2005;106:353.
24. Ghielmini M, Schmitz SFH, Cogliatti S, et al. Effect of single-agent rituximab given at the standard schedule or as prolonged treatment in patients with mantle cell lymphoma: a study of the Swiss Group for Clinical Cancer Research (SAKK). *J Clin Oncol*. 2005;23:705-711.

25. Paschka P, Merx K, Hochhaus A. Molecular surveillance of chronic myeloid leukemia patients in the imatinib era—evaluation of response and resistance. *Acta Haematol*. 2004; 112:85-92.

26. Lo Coco F, Breccia M, Noguera N, Miller WH Jr. Diagnostic value of detecting fusion proteins derived from chromosome translocations in acute leukaemia. *Best Pract Res Clin Haematol*. 2003;16:653-670.