Rituximab-augmented myeloablation for first-line autologous stem cell transplantation for mantle cell lymphoma: effects on molecular response and clinical outcome

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ABSTRACT

Background and Objectives

Autologous stem cell transplantation (ASCT) is effective in mantle cell lymphoma (MCL). We investigated whether incorporation of rituximab into the high-dose regimen might further improve the results of ASCT in patients with MCL.

Design and Methods

In a prospective phase II study, patients with newly diagnosed MCL were treated with a sequential dose-escalating therapy comprising standard chemotherapy for remission induction, intensive ara-C-containing chemotherapy for mobilization of stem cells, and myeloablative therapy followed by ASCT. The myeloablative regimen consisted of total body irradiation and high-dose cyclophosphamide supplemented with two doses (375 mg/m^3) of rituximab. Outcome parameters (toxicity, clinical and molecular response as assessed by allele-specific *IGH* real-time quantitative polymerase chain reaction (RQ-PCR), event-free survival, and overall survival) were compared with those of 34 historical controls treated identically but without rituximab.

Results

Thirty-four patients were accrued. Whereas engraftment, toxicity and clinical response were not different from those in controls, event-free survival was significantly increased with rituximab (not reached vs. 43 months; hazard ratio 0.38; p=0.036). This was associated with a trend for a superior molecular response rate in 11 study vs. 10 control patients with a marker available (73% vs. 30%, p=0.086) despite similar levels of lymphoma contamination of the stem cell inocula infused.

Interpretation and Conclusions

Incorporation of two standard doses of rituximab into the myeloablative regimen might improve outcome of upfront ASCT for MCL, allowing long-term disease control to an extent previously not reached in this disease.

Key words: MCL, autologous stem cell transplantation, rituximab.

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Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma which is characterized by early dissemination and an unfavorable clinical course. In order to improve the dismal prognosis of patients with MCL, myeloablative therapy with autologous stem cell transplantation (ASCT) has been extensively studied.[1-8] Despite some evidence that ASCT might improve the outcome of MCL, in particular if used as part of first-line treatment, relapses continue to occur with the transplant strategies currently employed although follow-up of published studies is limited. Considering the results of minimal residual disease (MRD) analyses post-transplant, it is not clear whether complete MCL eradication can be achieved at least in a subset of patients,[9-11] thereby emphasizing the need to analyze the long-term outcome after ASCT.

The chimeric monoclonal anti-CD20 antibody rituximab is active in MCL and has yielded high response rates in particular if used in combination with polychemotherapy.[12-14] However, sustained disease control cannot be reached with median times to treatment failure rarely exceeding 2 years. Given the superiority of both ASCT and rituximab-supplemented therapy over conventional treatment,[7,15] we wondered whether a combination of these two modalities might exert synergistic activities, resulting in sustained disease control and possibly cure. For this reason, we performed a prospective study using peri-transplant rituximab as an adjunct to a standard myeloablative regimen with total body irradiation (TBI) and high-dose cyclophosphamide for consolidation of MCL in first remission and compared the results with those of our original series treated identically but without rituximab.[5] This study, the first using relevant controls and both clinical and molecular endpoints, shows that the addition of only two doses of rituximab to TBI and high-dose cyclophosphamide might increase the antineoplastic effect of upfront ASCT in MCL significantly. Even without peri-transplant rituximab, however, a substantial proportion of patients enjoys long-term progression-free survival after ASCT, as reflected by the outcome of the original cohort with the longest observation time ever reported.

Design and Methods

Patients' eligibility and diagnosis

Patients with a diagnosis of stage II-IV MCL were eligible if they were between 18 and 69 years old, and had an adequate performance status (Karnofsky score ≥80%) and organ function. The diagnosis was established in all cases according to the criteria for MCL of the REAL and WHO classifications.[16,17] Diagnoses were originally made or confirmed by at least one member of the hematopathology reference panel of the German Low-Grade Lymphoma Study Group.[14] Conventionally and immunohistochemically stained paraffin sections of lymph node biopsies from all cases were available. Minimal requirements for a immunohistochemical diagnosis included positivity for CD20, CD5, and cyclin D1 with negativity for CD23 and CD10. The diagnosis of MCL was verified by assessment of the t(11;14)(q13;q32) translocation by polymerase chain reaction (PCR) and/or interphase/conventional cytogenetics if appropriate diagnostic material was available. All histopathological subtypes including the blastoid variant were eligible. A single case lacked cyclin D1 expression but was included as a cyclin D1-negative MCL because of the presence of t(11;14) along with a typical immunophenotype.[18]

Inclusion criteria for our original series of patients, who served as controls, were identical except for restriction to patients with stage III or IV disease and to those without blastic or pleomorphic morphology.[5]

Protocol design

The primary objective of this study was to assess the feasibility of adding two doses of rituximab (375 mg/m^2 on days –8 and –2) to standard high-dose therapy with TBI and cyclophosphamide for ASCT in patients with MCL. Secondary objectives were to study clinical response rate, molecular response rate, event-free (EFS) and overall survival in comparison to those of a historical control group of patients treated similarly except that they were not given rituximab.[5]

Pre-transplant sequential intensification of treatment of the study patients and the Kiel/Hamburg patients from the control group uniformly comprised induction with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) until maximum response followed by stem cell mobilization with Dexa-BEAM (dexamethasone 3×8 mg on days 1 through 10, carmustine 60 mg/m^2 on day 2, etoposide 75 mg/m^2 on days 4 through 7, ara-C 100 mg/m^2 every 12h on days 4 through 7, and melphalan 20 mg/m^2 on day 3) or DHAP (dexamethasone 40 mg days 1 through 4, high-dose ara-C 2 g/m^3 every 12h on day 2, and cisplatin (100 mg/m^3/d continuous infusion on day 1). For the Heidelberg patients from the control group, mobilization was performed with HAM (high-dose ara-C 2 g/m^2 every 12h on days 1-2, mitoxantrone 10 mg/m^2/d on days 2 and 3).[5] *Ex vivo* purging was not allowed in the study group, whereas 12 of 34 patients of the control group received CD34$^+$ selected grafts.[5]

The protocol including the informed consent form was approved by the responsible institutional review board (Ethics Committee of the University of Kiel, approval # A121/99). Patients gave written informed consent using study-specific forms.

High-dose therapy

Myeloablative therapy consisted of fractionated TBI (12 Gy) and high-dose cyclophosphamide (2×60 mg/kg) as previously described.[5] In addition, two standard doses of rituximab (375 mg/m^2) were administered on days –8 and

–2 (immediately prior to and after myeloablative treatment, respectively). All study patients received granulocyte colony-stimulating factor (G-CSF) daily from day +5 after stem cell reinfusion until neutrophil recovery. Neutrophil recovery was defined as the first of three consecutive days with a neutrophil count >0.5×10^9/L; platelet recovery was defined as the first day with an unsupported platelet count >20×10^9/L. Patients were discharged from hospital after the white blood count had recovered (3 days above 1×10^9/L) in the absence of fever, parenteral nutrition or intravenous antibiotics. Supportive care was given as described elsewhere.[5]

Evaluation of clinical response

The clinical response was evaluated according to NCI criteria.[19] In addition, very good partial remission was defined as a partial remission with residual masses smaller than 2cm and bone marrow infiltration of 20% or less.

Identification of minimal residual disease (MRD) markers and RQ-PCR

Molecular analyses were performed centralized by one of the authors (*CP*). Extraction of genomic DNA from diagnostic samples and clonality analysis by *IGH* multiplex PCR was performed as published elsewhere.[20,21] Sequencing analyses were done on an ABI PRISM 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were obtained by direct sequencing using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

For *IGH* RQ-PCR, two consensus probes complementary to germline JH1, JH4 and JH5 (JH1/4/5-probe) and JH6 (JH6-probe) were used in combination with four germline reverse primers (JH1, JH4, JH5 and JH6, respectively). PCR reactions and albumin normalization of MRD values were performed following a published protocol.[11]

Evaluation of molecular response

In patients with a clonal *IGH* rearrangement accessible for RQ-PCR, peripheral blood, and/or bone marrow follow-up samples were collected at 3-monthly intervals and analyzed for MRD. Complete molecular response required the absence of *IGH* RQ-PCR-detectable lymphoma cells in peripheral blood or bone marrow at any time point during the first year after ASCT. At least two samples taken at different time points had to be investigated with a minimum sensitivity of 1×10^{-4}.[11]

Statistical analysis

Employing GraphPad Prism software (version 3.02 for Windows, GraphPad Software, San Diego, USA), non-parametric Mann-Whitney tests were used to compare quantitative parameters between subgroups of patients. Qualitative parameters were analyzed with Fisher's exact test. If appropriate, quantitative variables were transformed into binary variables using the median as the cut-off value. Survival was calculated using the Kaplan-Meier method. Survival curves were compared by log rank analysis. Significance levels were set at 0.05. Data were analyzed as of April 30, 2006.

Results

Patients

Between June 1999 and October 2004, 34 patients were enrolled. The characteristics of the study patients were largely comparable to those of the control group except for age which was higher in the study group (Table 1). Moreover, three study patients but none of the control cohort had the blastoid variant of MCL histology. Two study patients had stage II disease, whereas all the others had more advanced disease.

Clinical response

All 34 patients received standard first-line therapy with CHOP (median number of cycles, 4; range, 2-8), with addition of rituximab in the last 14 patients. The maximum response was complete remission in 8 cases and partial remission in 21 cases, giving an overall response rate of 88% which was not significantly different from that of the historic controls (84%)(Table 2).

All patients proceeded to intensification with Dexa-BEAM (n=28), R-DHAP (n=5), or R-HAM (n=1). Whereas in most instances only one intensification cycle was administered, ten patients received a second Dexa-BEAM course for improvement of response quality, and a single patient was treated with three cycles of R-DHAP. Although a true complete remission was achieved in only 33%, the criteria of very good partial remission were fulfilled by the vast majority of patients (88%). Since the remaining four patients reached partial remission, the overall response rate prior to transplantation was 100%. Restaging 3 months after myeloablative treatment documented residual masses in only two patients, corresponding to a complete remission rate of 94%.

Engraftment and toxicity

Following rituximab-supplemented myeloablative treatment and peripheral blood stem cell reinfusion, engraftment was generally prompt and durable with no significant differences between the study group and the control group in terms of platelet recovery. However, neutrophil reconstitution was slightly but significantly slower in the study group (Table 3). A single patient showed delayed neutrophil and platelet engraftment despite having received a high number of CD34$^+$ cells (18.1×10^6/kg).

All patients developed mucositis (median WHO grade 2) which necessitated parenteral nutrition in 79% of them (Table 3). Neutropenic fever occurred in 85% of study patients but was easily controlled by empiric antibiotic therapy in all instances except for the case with delayed

Table 1. Patients' characteristics at diagnosis.

	TBI/CY	*R-TBI/CY*	*p*
Number	34	34	
Sex (M/F)	23/11	25/9	n.s.
Age (years)	53 (30-64)	58 (39-67)	0.004
Ann Arbor Stage IV	34 (100%)	30 (88%)	n.s.
BM involvement	30 (88%)	24 (71%)	n.s.
Involvement of extranodal sites	21/30 (70%)	20/34 (59%)	n.s.
Elevated LDH	2/13 (15%)	6/27 (22%)	n.s.
Blastoid subtype	n.i.	3/34 (9%)	

TBI: total body irradiation; CY: cyclophosphamide; R: rituximab; BM: bone marrow; LDH: lactate dehydrogenase; n.i.: not included as per protocol; ns: not significant.

Table 2. Pre-transplant treatment and response.

	TBI/CY	*R-TBI/CY*	*p*
n.	34	34	
Induction (CHOP-like)			
median n. of cycles (range)	4 (3-6)	4 (2-8)	n.s.
n. of patients receiving rituximab	0/34	14/34 (41%)	<0.0001
Status after induction			n.s.
Complete remission	6	8	
Partial remission	20	21	
Less than PR	5	4	
Untreated primary disease	2	0	
Unknown	1	1	
Mobilization			
Dexa-BEAM	20	28	
HAM or DHAP	14	6	n.s.
N. of patients receiving rituximab	0/34	6/34 (18%)	0.025
Status after mobilization			n.s.
Complete remission	15	11	
Partial remission	17	23	
Less than PR	0	0	
Unknown	2	0	

TBI: total body irradiation; CY: cyclophosphamide; R: rituximab; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisolone; Dexa-BEAM: dexamethasone, carmustine, etoposide, ara-C, melphalan; DHAP: dexamethasone, high-dose ara-C, cisplatin; HA: high-dose ara-C; HAM: high-dose ara-C, mitoxantrone; PR: partial remission.

Table 3. Dose of peripheral blood stem cells, hematopoietic recovery, and acute toxicity.

	TBI/CY	*R-TBI/CY*	*p*
Ex vivo $CD34^+$-selected grafts (%)	12 of 34 (35%)	0 of 34 (0%)	0.0002
$CD34^+$ cells infused ($\times 10^6$/kg)	8.5 (0.8-25.7)	9.2 (2.5-34.5)	n.s.
ANC >0.5×10^9/L (days)*	9 (8-11)	10 (8-24)	0.0012
Platelets >20×10^9/L (days)	10 (6-98)	11 (6-28)	n.s.
Mucositis (WHO grade)	n.a.	2 (1-4)	–
Parenteral nutrition (days)	n.a.	6 (0-10)	–
Neutropenic fever (days)	n.a.	3 (0-10)	–
Hospitalization (days)	15 (11-27)	15 (11-28)	n.s.
Toxic deaths (n)	1	0	–

**only patients receiving post-transplant G-CSF are considered; TBI: total body irradiation; CY: cyclophosphamide; R: rituximab; ANC: absolute neutrophil count; na: not available.*

neutrophil recovery. This patient developed grade 3 pneumonia which finally resolved after hematopoietic reconstitution. Grade 4 toxicities and treatment-related deaths were not observed. Less severe acute non-hematologic toxicities (grade 2) included exanthema in 24%, diarrhea in 18%, cardiac arrhythmia in 9%, and engraftment syndrome in 6% of the patients. The duration of hospital stay was not different between patients in the study and control groups (Table 3). Three patients had to be re-admitted to hospital after completion of the engraftment phase due to pneumonia (grade 3), hip osteonecrosis (grade 3), and emesis (grade 2). Two patients developed grade 2 toxic pneumonitis 4 and 7 months post-transplant, which in both cases responded well to steroid treatment. There were no cases of fatal toxicity.

Two cases of secondary neoplasms were observed: One 65-year old patient was diagnosed with prostate cancer (T3 N0 M0 G2) 36 months after the stem cell transplant, and another patient showed relapsing basalioma starting 29 months post-transplant. These two tumors were controlled by hormonal and surgical measures, respectively, until the last follow-up. No cases of treatment-related myelodysplasia or acute myeloid leukemia were observed.

Survival

With a median follow-up of 33 (6-82) months post-transplant, five patients of the study group have relapsed (including one of the three patients with blastoid MCL), and two of these have succumbed to progressive lymphoma. Overall, the median event-free and overall survival at 4 years post-transplant was 83% (95%CI 68%, 99%) and 87% (95%CI 69%, 100%), respectively. These figures compare favorably with those of the control group (4-year event-free survival 47% (95%CI 30%, 64%); 4-year overall survival 77% (95%CI 63%, 92%); although only the difference in event-free survival (EFS) was statistically significant (HR 0.38 (95%CI 0.18, 0.94); p=0.036) (Figure 1). The median follow-up of the controls was 90 (4-135) months. Of note, among 13 control patients at risk, only two relapses occurred beyond 5 years after ASCT (at 89 and 90 months).

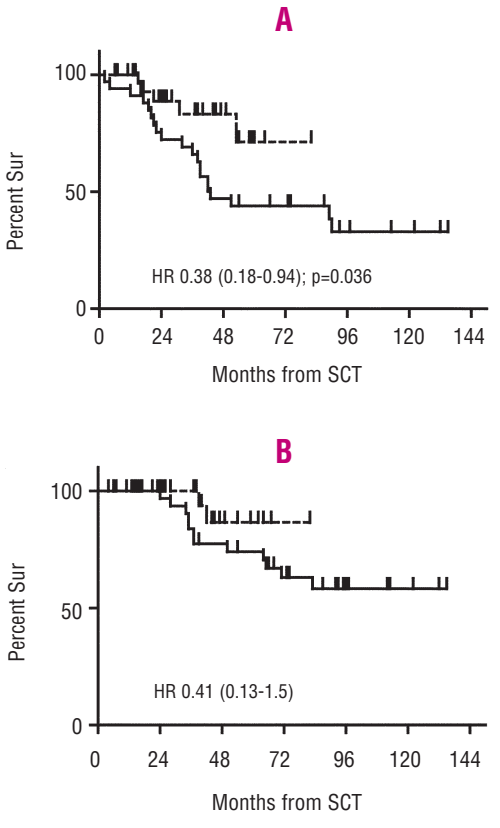

Figure 1. Event-free (A) and overall survival (B) of study patients (broken line, n=34) and controls (solid line, n=34)

Impact of pre-mobilization rituximab

The last 14 patients received a standard dose of rituximab along with each CHOP cycle, five of them also with the mobilization regimen. Altogether, six (range, 3-7) rituximab doses were administered during the remission induction and mobilization phase in these 14 individuals. Although the pre-transplant complete remission rate tended to be higher after pre-mobilization rituximab (50% vs. 21% in the 20 study patients without pre-mobilization rituximab; p=0.063), this did not translate into superior EFS after transplantation (HR 1.2; 95%CI 0.11-15.4; p=0.84). However, the median follow-up of the patients with pre-mobilization rituximab was only 16 months.

On the other hand, EFS among the patients treated with peritransplant rituximab remained significantly better than that of the control group when only the 20 patients without pre-mobilization rituximab were considered (HR 0.36; 95%CI 0.17-0.98; p=0.044).

Molecular response

Twenty-one patients (10 from the control group, 11 from the study group, all without pre-mobilization rituximab exposure) for whom a molecular marker could be established from diagnostic material had at least two independent follow-up samples available for MRD assessment within the first year post-transplant. A complete molecular response, defined as MRD negativity in all samples investigated, was achieved in 8 of 11 study patients (73%) and 3 of 10 controls (30%) (p=0.086, Fisher's exact test). With only two relapses among the patients who had a complete molecular response, achievement of MRD negativity was strongly predictive for superior EFS (HR, 0.09; 95%CI 0.01-0.23; p<0.0001) and overall survival (HR undefined, p=0.0012). The EFS benefit in the study group was exactly maintained in the 21 patients with a molecular marker although no longer significant with this small sample size (HR, 0.33; 95%CI 0.08-1.1; p=0.066). Molecular data for patients receiving pre-mobilization rituximab were not available.

Molecular analyses of graft MRD levels

Quantitative MRD assessment of the stem cell inoculum was possible in all 21 patients with a molecular marker. MRD levels in unmanipulated peripheral blood stem cell products were not significantly different between patients in the study and control groups (0.0054 (0–0.207) vs. 0.0029 (0–0.064); p=0.97). $CD34^+$ selection was performed in six of ten patients with a marker in the control group, reducing the graft MRD content to 0.00057 (0–0.021) in these six individuals (p=0.39 vs. study patients).

Discussion

Therapeutic options in MCL were very limited until the mid 1990s when ASCT became available for this especially poor-risk subtype of lymphoma. The Omaha group was the first to show the potential efficacy of this intensive modality in MCL.[1] Since then, numerous studies have been published documenting the feasibility and potent anti-lymphoma activity of ASCT in this entity, in particular if used as part of first-line treatment. However, almost all trials were uncontrolled and suffered from small patients numbers and limited observation times. Only recently Dreyling and co-workers were able to demonstrate the superiority of ASCT over standard CHOP chemotherapy with interferon maintenance in terms of progression-free survival in a prospective randomized phase-III study.[7] Nevertheless, with a median follow-up of 34 months, a plateau in the survival curve was not seen even in this trial, and a significant survival benefit could not be shown. Thus, one of the aims of the present study was to investigate the long-term outcome after ASCT by re-evaluating our first series of patients who had been treated with upfront ASCT consolidation between 1992 and 1998.[5] With a median observation time of surviving patients of now 90 (4-135) months (two patients were lost to follow-up at 4 and 16 months after ASCT), EFS remains similar to that originally predicted.[5] Although the decrease of the survival curve seems to slow

Table 4. Intensified strategies in the primary management of mantle cell lymphoma (results of prospective studies).

Intervention	*Study*	*Design*	*Rituximab*	*Ara-C Intensification*	*ASCT*	*n*	*Follow-up (months)*	*PFS (months)*
Rituximab/CHOP	Howard[13]	phase-II; prospective	6 doses	no	no	40	–	16.6
	Lenz[14]	phase-III; prospective	6 doses	no[a]	no[a]	62	18	21[b]
CHOP/ASCT	Geisler MCL1[29]	phase-II; prospective	no	no	BEAM	42	n.a.	24% (3y)
CHOP/ara-C/ASCT	Khouri[22]	phase-II; prospective	no	Hyper-CVAD/ Mtx-HA	TBI/CY	33	49 (14-77)	43% (5y)
	Dreyling[7]	phase-III; prospective	no[c]	Dexa-BEAM	TBI/CY	62	34	39
	Lefrere[24]	phase-II; prospective	no	DHAP	TBI/CY/E; TAM	23	66	51d
	This study[5]	phase-II; prospective	no	Dexa-BEAM;	TBI/CY	34	90 (4-135)	47% (4y)
Rituximab/Ara-C	Romaguera[25]	phase-II; prospective	6-8 doses	Hyper-CVAD/ Mtx-HA	no	97	40	64% (3y)
Rituximab/ASCT	Mangel[26]	phase-II; prospective	1 dose *in vivo* purge; 8 doses post-transplant	no	CBV	20	25 (6-41)	85% (2y)
	Brugger[27]	phase-II; prospective	4 doses post			10	42	70% (4y)
Rituximab/ara-C /ASCT	Gianni[28]	phase-II; prospective	4 doses pre 2 doses peri	HA	Mito/Mel	28	35 (21-54)	79% (4y)
	Geisler MCL2[29]	phase-II; prospective	2 doses *in vivo* purge	HA	BEAM	104	n.a.	68% (3y)
	Delarue[23]	phase-II; prospective	6 doses pre/ *in vivo* purge	DHAP	TAM	30	25	1 relapse
	de Guibert[30]	phase-II; prospective	4 doses pre/ *in vivo* purge	DHAP	TBI or BEAM	17	28 (12-48)	76% (3y)
	This study	phase-II; prospective	2 doses peri	Dexa-BEAM	TBI/CY	20	46 (17-82)	84% (4y)
			6 (3-7) ds. pre/ + 2 doses peri	Dexa-BEAM; DHAP	TBI/CY	14	16 (6-38)	n.a.

ASCT: autologous stem cell transplantation; BEAM: carmustin, etoposide, ara-C, high-dose melphalan; CBV: cyclophosphamide, carmustin, etoposide; Dexa-BEAM: dexamethasone, carmustin, etoposide, ara-C, melphalan; DHAP: dexamethasone, high-dose ara-C, cisplatin; HA: high-dose ara-C; HAM: high-dose ara-C, mitoxantrone; Mito/Mel: mitoxantrone, high-dose melphalan; Mtx: methotrexate; PFS: progression-free survival; TAM: total body irradiation, ara-C, high-dose melphalan; TBI/CY: total body irradiation, high-dose cyclophosphamide; TB/CY/E: total body irradiation, high-dose cyclophosphamide, etoposide. [a]23% underwent consolidation with Dexa-BEAM - ASCT; [b]Time to treatment failure; [c]28% received R-CHOP induction; [d]event-free survival from diagnosis.

down with time, relapses occurred more than 7 years post-transplant, indicating that with standard myeloablative treatment, permanent disease control or cure might be only rarely achieved, if ever. However, with the longest follow-up reported to date, our study demonstrates that substantial proportions of patients stayed in remission for more than 8 years, implying long-term therapeutic benefit at least for a subset of patients.

Apart from ASCT, other effective modalities for the treatment of MCL have been recognized during recent years. The addition of rituximab to standard CHOP chemotherapy yielded promising response rates[13] and resulted in significantly prolonged times to treatment failure in a prospective randomized study,[14] although neither progression-free survival nor overall survival was improved. Another modality with strong activity in MCL might be intensive ara-C-containing combination chemotherapy regimens, such as Hyper-CVAD/Mtx-HA, DHAP, HAM, and Dexa-BEAM. This conclusion was initially based on the observation that these regimens used alone or sequentially after CHOP consistently yielded response rates of 90% or more.[5,22-24] A second line of evidence derives from MRD studies documenting a median reduction of the tumor load of more than one log with Dexa-BEAM, whereas CHOP-like regimens had no significant impact on MRD levels.[11]

Further improvement of treatment results might be achieved by combination of these three modalities with proven efficacy in MCL (rituximab, high-dose ara-C (HA), and ASCT). The MD Anderson group examined the combination of rituximab with repetitive hyper-CVAD/Mtx-HA as first-line treatment of MCL in a large prospective phase II trial. With an 87% complete remission rate, failure-free survival after 3 years was 64% and thus comparable to results achieved with sequential HA-ASCT (Table 4). However, since the toxicity of this regimen was substantial, with 5% toxic deaths and a number of secondary therapy-related myelodysplastic syndrome, it does not

appear to have significant advantages over ASCT.[25] Two small studies investigated the combination of (post-transplant) rituximab with ASCT as part of first-line treatment not including HA.[26,27] Disease control was very promising although the results must be regarded as preliminary due to the low numbers of patients and short observation times. More experience has been reported with the triple combination rituximab-HA-ASCT using rituximab mainly pre-transplant for *in vivo* purging. Although follow-up was limited, the progression-free survival rate appeared to be consistently higher than 60% after 3 years (Table 4).[23,28-30]

The most compelling evidence for a beneficial effect of adding rituximab to the HA-ASCT sequence is, however, provided by the present study. Although the control group is historical only, it was treated identically to the study group except for rituximab, and differs merely by younger age and exclusion of blastoid type MCL. Neither of these two parameters should have biased the results in favor of the study group. In contrast, all other published trials were completely uncontrolled or used historical controls treated without ASCT[26,28] or HA.[29]

A secondary end-point of the study was molecular response as a more direct indicator of the anti-lymphoma activity of the individual high-dose regimens. We have previously shown that achievement of MRD negativity as assessed by *IGH* RQ-PCR, using clone-specific primers, is a very strong predictor for progression-free survival after ASCT.[11] Peri-transplant rituximab resulted in a marked increase of the molecular response rate (from 30% to 73%), although this improvement was not statistically significant given the small number of subjects investigated. This suggests that the better tumor control associated with the incorporation of rituximab into the high-dose regimen might be based on enhanced tumor cell clearance.

Because rituximab-CHOP became the accepted standard induction treatment for MCL during the course of the study,[14] the last 14 patients received six (range, 3-7) doses of rituximab prior to mobilization, which may have contributed to the superior outcome of the study patients. However, since the clinical benefit of peri-transplant rituximab was retained at the same order of magnitude when the analysis was restricted to patients without pre-mobilization rituximab, and only patients without pre-mobilization rituximab were included in the molecular analyses, the peri-transplant rituximab strategy seems to be effective on its own. It remains to be demonstrated whether disease control can be further improved by pre- and/or post-transplant rituximab intensification.

It is intriguing that the substantial clinical benefit observed was achieved with only two doses of rituximab. The rationale for the peri-transplant schedule employed here was the hypothesis that a single pretransplant dose given in a situation of minimal disease might render MCL cells more sensitive to the cytotoxic effects of TBI and high-dose chemotherapy, whereas the immediate post-conditioning dose might eradicate altered tumor cells surviving the high-dose regimen.[31] Moreover, rituximab administered immediately prior to graft reinfusion should also cause effective depletion of tumor cells delivered with the graft, thereby obviating the need for more complex means of purging. The observation that both molecular response and clinical outcome were superior after peri-transplant rituximab despite MRD contamination of virtually all grafts with no quantitative differences between study patients and controls is in line with this hypothesis. On the other hand, it still remains to be proven to what extent tumor cell contamination of the stem cell inoculum can contribute to relapse after stem cell transplantation for MCL.[11,32] To this end, the favorable results obtained with rituximab *in vivo* purging approaches[26,28] might rely on a systemic rather than a graft-related effect of rituximab.

In conclusion, two doses of peri-transplant rituximab seem to be an elegant and cost-effective way to significantly improve the results of sequential high-dose therapy including HA and ASCT in patients with MCL. With a 4-year progression-free survival rate of 83%, this strategy illustrates that the prognosis of MCL, at least in younger patients, is nowadays better than that in the era of CHOP monotherapy.

Author Contributions

PD, CP, and NS designed the project; PD, MR, BS, MH, ADH, and NS were involved in the care of patients and acquisition of samples and clinical data; CP and MK performed MRD analyses; PD and CP analyzed and interpreted the data; PD, CP, and NS wrote the paper; and all authors checked the final version of the manuscript.

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Conflict of Interest

The authors reported no potential conflicts of interest.

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