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First line treatment with rituximab- Hyper-CVAD alternating with rituximab- Methotrexate- Cytarabine and followed by consolidation with 90Y-Ibritumomab-Tiuxetan in patients with mantle cell lymphoma. Results of a phase 2 pilot multicenter trial from the GELTAMO group

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First line treatment with rituximab- Hyper-CVAD alternating with rituximab-Methotrexate- Cytarabine and followed by consolidation with ^{90}Y-Ibritumomab-Tiuxetan in patients with mantle cell lymphoma. Results of a phase 2 pilot multicenter trial from the GELTAMO group

Running heads: RHyperCVAD/MtxAraC and ^{90}YIbritumomab in MCL

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ABSTRACT

The prognosis for fit patients with mantle cell lymphoma has improved with intensive strategies. Currently, the role of maintenance/consolidation approaches is being tested as relapses continue appearing. In this trial we evaluated the feasibility, safety and efficacy of R-Hyper-CVAD alternating with R-MtxAraC followed by consolidation with ^{90}Y-Ibritumomab-Tiuxetan. Patients received 6 cycles followed by a single dose of ^{90}Y-Ibritumomab-Tiuxetan. Thirty patients were enrolled. Median age was 59 years. Twenty four patients finished the induction treatment, 23 achieved complete remission (77%, 95% confidence interval 60-93) and one patient had progressive disease (3%). Eighteen patients (60%), all in complete remission, received consolidation. In the intent- to- treat population, failure free, progression free and overall survival at 4 years were 40 % (95% confidence interval 20.4-59.6), 52% (95% confidence interval 32.4–71.6) and 81% (95% confidence interval 67.28– 94.72), respectively. For patients who received consolidation, failure free and overall survival were 55% (95% confidence interval 31.48–78.52) and 87% (95% confidence interval 70–100), respectively. Hematological toxicity was significant during induction and responsible for one death (3.3%). After consolidation, grade 3-4 neutropenia and thrombocytopenia were observed in 72% and 83% of patients, with median duration of 5 and 12 weeks, respectively. Six (20%) patients died, 3 due to secondary malignancies (myelodisplastic syndrome and bladder and rectum carcinomas). In conclusion, our experience with R-Hyper-CVAD/R-MtxAraC followed by consolidation with ^{90}Y-Ibritumomab-Tiuxetan is efficacious although less feasible than expected. The unacceptable toxicity observed, specially secondary malignancies, advise against the indication of this strategy. Trial registration: clinical.gov identifier: NCT2005-004400-37

INTRODUCTION

Mantle cell lymphoma (MCL) treatment is a clinical challenge. Patient's overall survival has improved over recent years, but median survival still remains poor, around 5 years in the majority of the patients (1). With the standard R (rituximab)-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), complete remission (CR) rates are lower than 50% and median failure free survival (FFS) is short, from 16 months to 2 years (2, 3).

The outcome has particularly improved for young and fit patients with the use of intensive strategies in first line, particularly with those containing high dose Ara-C (HDAC) followed or not, by autologous stem cell transplantation (ASCT) (4-7). With intensive treatments, CR rates raise to 80- 100% and median FFS of up to 5 years are reported (5-10). In spite of this improvement, the updated long term results obtained with these approaches have shown a continuous pattern of relapse (11, 12). After relapse, the outcome turns dismal for the majority of patients, frequently requiring sequential treatments until death.

Maintenance and consolidation strategies are a matter of active study in MCL in order to achieve more prolonged response duration. In fact, the European Mantle Cell Lymphoma Network recently demonstrated a significant improvement in progression free survival (PFS) and overall survival when maintenance R is used after R-CHOP whereas no benefit could be documented for patients treated with R- Fludarabine-Cyclophosphamide (R-FC) (13).

Radioimmunotherapy (RIT) with an anti-CD20 antibody conjugated to a beta-emitting radioisotope is a treatment with demonstrated efficacy in follicular lymphoma (FL) (14-17) Efficacy data for RIT in MCL is more limited although it has demonstrated its efficacy as monotherapy in relapsed or refractory disease (18). More recently, its efficacy appears to be heterogeneous as good results have been reported when used as consolidation post standard therapy (19, 20) whereas no benefit has been communicated by the Nordic Group introducing RIT in the conditioning regimen in their MCL3 trial compared to their previous MCL2 trial (21). To our knowledge, only another preliminary communication has been reported so far with RIT as consolidation after intensive treatment (22).

Herein we report the results of a prospective and multicentre pilot phase II study, conducted by the Grupo Español de Linfomas y Transplante Autólogo de Médula Ósea (GELTAMO) in patients with untreated MCL, who received induction therapy with Rituximab (R)-HyperCVAD / R-MethotrexateAraC (R-MA) followed by consolidation with ^{90}Y-Ibritumomab-Tiuxetan.

METHODS

The study was performed in 12 Spanish institutions. It was approved by the local and central committees and registered at the Clinical Trials Gov web-site (NCT00505232). For more detailed information see on- line supplement.

Patients and assessments

Patients between 18 and 70 years-old and diagnosed with MCL (23) were eligible. Cyclin D1 or t(11;14))(q13;q32) translocation positivity was required for diagnosis.

Inclusion and exclusion criteria, pre-treatment evaluations and work-up studies are described in detail in the on-line supplement.

Complete disease evaluation was carried out before treatment, after the 4th cycle and at the end of the induction treatment. After consolidation, disease was evaluated every 4 months until the 2nd year and every 6 months thereafter.

Treatment

Induction phase

Patients received R-HyperCVAD therapy alternating with R-MA every 21 days (5). Dose adjustments were considered for patients older than 60 years, creatinine value >1.5 mg/dl or after development of febrile neutropenia, hematological toxicity (platelet count <100.000/mm3 or granulocyte count <1000/mm3 on day 21 of each cycle) or non-hematological grade 3 toxicity at any moment. Treatment was discontinued if patients did not reach hematological recovery 5 weeks after chemotherapy, acquired less than partial response (PR) after 4 cycles or developed grade 4 non-hematological toxicity. The number of induction cycles was fixed at 6.

Support therapies with Peg-Filgastrim and pneumocistis jirovecci prophylaxis were required by protocol. Peripheral blood stem cell collection as back-up was mandatory in the first six patients and recommended for the remaining patients.

Consolidation phase

Consolidation with ^{90}Y-Ibritumomab-Tiuxetan was scheduled 12 weeks after the 3rd cycle. The drug was kindly provided by Bayer-Shering and administered following brochure instructions. Although a dose escalation to 0.4 mCi/kg was planned, the dose for ^{90}Y-Ibritumomab-Tiuxetan was fixed at 0.3 mCi/kg.

Study endpoints and definition of study variables

The main objective was to evaluate the feasibility, safety and efficacy of the whole treatment. Clinical efficacy was evaluated in terms of response and survival. The toxicities were evaluated according to the National Cancer Institute's Common Toxicity criteria (CTCAE v3.0).

Response criteria were assessed according to The International Workshop to Standardize Response Criteria for Non-Hodgkin's Lymphoma (24). Patients without response assessment were considered non responders.

Failure free survival (FFS) was defined as the time from the date of study entry until date of recurrence, progression, death from any cause or any toxic event that prohibited treatment. Responding patients who did not complete the whole treatment were censored in the moment this deviation occurred.

Progression free survival (PFS) was defined as the time from the inclusion in the trial until progression, recurrence or death as a result of lymphoma**.**

Overall survival (OS) was defined as the interval between the date of study entry until death from any cause.

Variables were calculated in the intent- to treat- population, defined as patients who had received at least one cycle. For survival and serious adverse events (SAE) analysis all patients were followed until the closure of the study regardless of treatment discontinuation.

Statistical analysis

Statistical methods are described in the supplement (25) (26).

RESULTS

Patient characteristics

Between February 2006 and July 2008, 38 patients with untreated MCL from 12 institutions were registered. There were 8 screening failures due to: positive serology for hepatitis B virus (HBV) (2 patients), central nervous system (CNS) involvement (1 patient), older age (1 patient), localized disease (1 patient), patient's refusal (1 patient), protocol deviation (1 patient) and urgency for treatment (1 patient). Therefore, 30 patients were evaluable for results.

Patient characteristics' are described in Table 1. Median age was 59 years (range, 41-70 years). Blastic MCL variant was diagnosed in 21% of the cases. Most patients had advanced disease (97%), with documented bone marrow involvement (93%) and 40% were in the intermediate-high risk group of mantle international prognostic index (MIPI) (27). Cytogenetic studies on bone marrow/peripheral blood samples to detect t(11;14)(q13;q32) and other cytogenetic abnormalities (del 13q14, +12, del 17p, del 11q22.3) are shown in Table 2.

Treatment outcome

Response to induction therapy: Twenty eight (93.3%) out of 30 patients were evaluable for response after the 4th cycle and 24 (80%) after the 6th. After the 4th cycle, 19 patients achieved CR or unconfirmed CR (uCR) (68%, 95% IC 67%-69%) and 9 partial response (PR) (32%, 95% IC 31%-33%). Response at 4th cycle could not be assessed in two patients due to grade 4 infection and an unexpected suicide in a patient without previously known psychiatric disorder. Four additional patients discontinued treatment between the 4th and 6th cycle due to: grade 4 and grade 5 bacterial infection (1 patient each), pulmonary aspergillosis (1 patient) and grade 4 neurological toxicity (1 patient).

At the end of induction treatment twenty-three patients (77%, 95% IC 60%-93%) achieved CR/uCR and one patient had progressive disease (PD) (3%).

Consolidation therapy

The dose of ^{90}Y-Ibritumomab-Tiuxetan was maintained at 0.3 mCi/kg. Five out of the first 6 patients treated had lower peripheral blood counts than those required for full dose at 12 weeks after the completion of induction therapy.

Eighteen patients (60%), all in CR/uCR, received consolidation treatment. Six patients failed due to grade 4 infection (1 patient), patient's decision (1 patient), delayed peripheral blood counts recovery (2 patients), protocol deviation (1 patient) and progressive disease (1 patient). The study throughput is shown in Figure 1.

FFS, PFS and overall survival

Median follow-up for survivors was 3.9 years (range 0.45 – 5.4 years). Two and 4 year FFS were 57 % (95% CI 37.4%-76.6 %) and 40 % (95% CI 20.4%-59.6%) respectively. PFS at 2 and at 4 years were 72 % (95% CI 54.36% – 89.64%) and 52 % (95% CI 32.4% – 71.6%) respectively. Median PFS was 4.9 years (range 2.7 to 7.1 years). Overall survival (OS) at 2 and at 4 years were 89% (95% CI 79.2% – 98.8%) and 81% (95% CI 67.28 %– 94.72%) (Figures 2A-2C).

In the group of 18 patients who received the whole treatment, FFS at 2 and at 4 years were 78 % (95% CI 58.4% – 97.6%) and 55 % (95% CI 31.48 – 78.52) respectively. Overall survival at 2 and at 4 years were 93.8 % (95% CI 82% – 100%) and 87% (95% CI 70% – 100%).

HDAC has been proven to be outstanding for outcome; therefore we studied the survival variables according to a cut-off age of 60 years, where significant Ara-C dose adjustments are made. Four- year FFS for older patients was 30% (95% CI 4.5%– 55.5%) versus 50% (95% CI 22.5% – 77.4%) for the younger ones (p= 0.57). Four year- OS was not affected by age. In fact, older patients' OS was 92% (95% CI 78% – 100%) versus 73% (95% CI 51.4% – 94.5%) for those younger than 60 years. Other characteristics were similarly distributed between the groups.

FFS for low and intermediate-high risk groups of MIPI were significantly different (p=0.003). Median FFS for low risk was 4.8 years (95% CI 2.7- 6.9) versus 1.4 years (95% CI 0.15- 3.2) for the intermediate-high risk group. OS was also significantly different (p= 0.003), and was 93% (95% CI 81.24% – 100%) for the low-risk group, versus 31% (95% CI 0% – 76%) for the intermediate-high risk group (Figures 3A, 3B). No other patient's characteristics had impact on survival.

Six (20%) out of 30 patients have died during the study. Causes of death were unexpected suicide (1 patient), infection (1 patient due to septic shock), relapsed disease (1 patient) and secondary malignancies in 3 patients (myelodisplastic syndrome, bladder carcinoma and rectum carcinoma). Two of these patients died without evidence of lymphoma.

Toxicity

Six out of 30 patients (20%) did not complete their intended number of cycles during induction treatment because of toxicity (5 patients) and suicide (1 patient).

One hundred and seventy cycles were administered during this phase. Adverse (AE) and serious adverse events (SAE) are reported in Table 3 and 4. The principal toxicity was hematological, and significantly higher during R–MA cycles, where more than 90% of the cycles were associated with grade 3-4 neutropenia and thrombocytopenia, and 50% with grade 3- 4 anemia ($p < 0.0001$). Neutropenic fever and infection accounted for 56 out of 69 SAEs (81%) reported during this phase and were again significantly higher in R-MA cycles (49% versus 18%; $p< 0.05$). There was one toxic death due to septic shock during this period.

Hematological toxicity was the main adverse event during consolidation. Grade 3-4 neutropenia was seen in 72% of the patients with a median duration of 5 weeks (range 2- 24 weeks). Grade 3-4 thrombocytopenia was observed in 83% of the patients with a median duration of 12.5 weeks (range 4- 56 weeks). No patient received the back-up peripheral blood stem cells collected.

Secondary malignancies (7 events) and infection with normal neutrophils (5 events) accounted for the total of 16 SAEs communicated after consolidation treatment. Six of these neoplasms were reported after the first line treatment and one occurred after subsequent patient's exposure to other treatments for relapsed disease. A more detailed description of these malignancies is given in Table 5. The crude incidences of myelodisplastic syndrome/acute myeloid leukemia (MDS/AML) and solid tumors were 10% each. The 2 and 4- year cumulative incidences were 4% and 17%, respectively. Cytogenetic analysis in 2 cases with MDS revealed complex karyotypes, with abnormalities of chromosomes 5 and 7. For solid tumors, the 2 year

cumulative incidence was 13%. No further solid tumor was diagnosed later on, with a median follow up for the survivors of 3.9 years.

DISCUSSION

The use of intensive treatment approaches in young and fit patients with MCL has significantly improved their outcome. However, the best strategy remains uncertain so far, although the more extended practice includes the performance of ASCT as consolidation of first response. Our trial considered the up front intensive regimen HyperCVAD alternating with MA in association with rituximab, developed by the MD Anderson Cancer Center (MDACC), due to the good response rates and their prolonged duration reported. Of notice, updated MDACC results showed a PFS of 43% and an OS of 56%, quite similar to those reported by the Nordic Group using consolidation with ASCT, with PFS of 43% and OS of 58%, respectively.

In this study, treatment with R-HyperCVAD alternating with R-MA (3 cycles of each) followed by consolidation with ^{90}Y-Ibritumomab-Tiuxetan achieved poorer results than expected. Toxicity was unacceptably high and the CR rate, median FFS and PFS were 77%, 2.6 years and 4.9 years, respectively, without plateau.

In our hands, the feasibility of the R-HyperCVAD alternating with R-MA was lower than previously reported (5, 7). At the MDACC, 70% of the patients received 8 cycles. In a multicenter setting, the Gruppo Italiano Studio Linfomi administered 6 cycles to 75% of their patients without mandatory growth factor support. In our study, safety withdrawal events account for 83% of the reasons for discontinuations throughout the induction phase. Hematological toxicity was high, particularly in the R-MA cycles where grade 3- 4 neutropenia and thrombocytopenia were practically universal. In accordance, febrile neutropenia and infection episodes were observed in 17% and 32% respectively, which are significantly higher than the toxic events detected in the R-HyperCVAD cycles (7% and 11%, respectively). The high toxicity detected in the R-MA cycles was higher than previously reported with the same schedule treatment (5, 7) and significantly higher than those reported with other intensive strategies (6, 28). Probably, the concomitant administration of methothrexate with the HDAC in the R-MA cycles increases the hematological toxicity and impairs renal clearance. In spite of the hematological toxicity, mortality due to infection was 3.3%, similar or even inferior to that communicated with this treatment.

HDAC seems crucial for improving remission rates and FFS in MCL, as has been recently reported by the Mantle Cell Lymphoma Network. In this trial (28), the introduction of HDAC within the R-CHOP treatment improved significantly patients' outcome when compared with that of patients treated only with R-CHOP and followed by consolidation with ASCT. With this information, it would be desirable to clarify the best dose and schedule of this agent. In this study, the total dose of HDAC planned to be administered was 36 g/ m^2, similar to that used by the Nordic Group in the MCL2 trial (6) and higher than the doses of 14 g/ m^2 to 16 g/ m^2 used in other treatment strategies (4, 8, 28) that report treatment compliances up to 89% with similar median FFS, around 4.5 years.

Even using intensive approaches to treat MCL, a continuous pattern of relapse has been documented (11, 12). Currently, consolidation and/or maintenance approaches are a matter of active clinical research in order to improve the response duration. In this study, consolidation with a single dose of ^{90}Y-Ibritumomab-Tiuxetan was planned and administered to 60% of the patients. This data is in agreement with the results recently communicated by Beaven et al using consolidation with ^{131}I-Tositumomab after an intensive induction schema (22) in patients with MCL and diffuse large B cell lymphoma, and significantly lower than the described when RIT is administered after a non intensive regimen as R- CHOP (19). To our knowledge, there is no other published report with RIT used as consolidation after first line treatment in MCL patients.

In this pilot study, consolidation with RIT after this intensive treatment did not increase the CR rate neither FFS nor PFS when compared with other studies. However, in our study the achieved 4 year FFS of 55%, which is inferior to that observed by other authors (4, 5, 7, 8), might be hampered by the inferior feasibility of the treatment and the higher TRM. The high incidence of secondary malignancies observed in patients who received consolidation with RIT is of major concern, since three patients (10%) developed MDS/AML and 3 (10%) solid tumors at a median time of 1.22 years (range 0.4-3.12 years) after RIT.

Regarding solid tumors, the observed crude and cumulative incidences were 10% and 12.7%, respectively. An unusual increased incidence of second malignancies was suggested for 156 patients with MCL treated with R-HyperCVAD/R-MA, 37% also transplanted and 32% treated with total body irradiation (29). With a median follow up of 2 years, a crude incidence of 4.5% of invasive neoplasms was detected soon after the treatment. However, this observation has not been confirmed by the same authors in more recent reports, neither has it been mentioned by others using the same induction treatment (5, 7). Geisler et al report a crude

incidence of 3% with a median follow up of more than 6 years although they did not consider them to be treatment-related. With ^{131}I-Tositumomab, Bennet et al reported a crude incidence of less than 5% of solid tumors in MCL patients with relapsed and refractory disease previously treated with a median of 3 chemotherapy lines.

More information about the risk of MDS/AML is available. The incidence in our study is higher than that communicated by other authors using intensive approaches with/without ASCT but without RIT, ranging from 0% to 5%. Romaguera et al reported (5) a crude incidence of 4% with a median follow-up of 3.3 years, data that remained practically unchanged (5%) in the updated results, with a follow-up of 8 years (11). The Nordic Group reported an incidence of 0.6%, in the updated results of the MCL2 trial, with a median follow up of 6.5 years (6). Interestingly, the same group in their MCL3 trial, which adds ^{90}Y-Ibritumomab-Tiuxetan to the BEAM (BCNU, Etoposide, Ara-C, Melphalan) conditioning regimen, do not communicate an increase MDS/AML incidence with a median of follow-up of 3.2 years (21). Other authors, using intensive schemas and including ASCT as consolidation, do not mention any secondary malignancies in patients followed during a similar period of time (4, 8).

The crude incidence of MDS/ AML communicated in patients treated with RIT after R- CHOP or Fludarabine-Mitoxantrone-Rituximab (FMR) in first line treatment for MCL and follicular lymphoma is 0%- 0.5% (19, 30, 31). More accurate data of this complication comes from the registration or compassionate- use studies in patients exposed to a median of 2- 3 lines (32, 33). The 2 and 5 year cumulative incidence communicated ranged from 1.7% to 6.3%, respectively. Our figures were 4% and 15%, higher than those communicated by Guidetti et al in patients previously treated and autografted after conditioning with myeloablative doses of RIT (34).

The cytogenetic findings in the MDS diagnosed were those commonly associated with the prior exposure to cytotoxic drugs and radiation therapy. Complex karyotypes involving chromosomes 5 and 7 are genetic events associated with these therapies (35-37). None of the patients with MDS/AML had additional genetic abnormalities besides the Bcl-1 translocation, such as p53, ATM or 13q deletions, detected in 23% of our patients at diagnosis.

To our knowledge, neither an unacceptable increase in the incidence of secondary MDS/AML nor of solid tumors, have been described in spite of the wide use of HyperCVAD/MA, which requires the use of stem cell factors in order to decrease toxicity and maintain an adequate dose intensity. The toxicity found in our study could be due to the high cumulative dose of cyclophosphamide followed by a radiation of systemic nature as RIT, which may entail a second oncogenic event on previously sensitized cells.

In summary, R-HyperCVAD/R-MA followed by consolidation with ^{90}Y-Ibritumomab-Tiuxetan is effective although less feasible than expected. The important toxicity observed, advise against the use of this strategy. Currently, the number of new drugs available for MCL is increasing therefore it is crucial to focus on minimizing adverse events.

Authorship and Disclosures: RA coordinated the study, interpreted data analysis and wrote the manuscript. AGN and JCO helped to interpret the data and to the writing of the paper. RA, AGN, CG, MJT, JJS, JPC, MAC, JGM, CA, EC, AS, and DC were responsible for patient's clinical management. JCO was involved in the acquisition data and statistical analysis. JGM, AS, CG, AM and DC contributed to the interpretation of the data and to the revision of this manuscript. EA performed the cytogenetic analysis. All the authors approved the final version to be submitted. The authors report no potential conflict of interest.

Post-scritum: In memory of our dear colleague Javier Pérez-Calvo who passed away last year (r.i.p)

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TABLES

Table 1: Characteristics of the patients and cytogenetic findings at diagnosis

Age, years (range)	59 [41-71]
Male gender	23 (77%)
ECOG 0- 1	28 (93%)
Blastic histology	6 (21%)
Ann Arbor IV	29 (97%)
Bone marrow involvement	28 (93%)
Spleen involvement	17 (57%)
Gastrointestinal infiltration	19 (63%)
LDH (> UNL)	9 (30%)
ß2 macroglobulin (> UNL)	18 (62%)
MIPI - **Low risk** - **Intermediate risk** - **High risk**	 18 (60%) 10 (33%) 2 (7%)
Cytogenetic findings - Bcl 1 alone - Bcl 1 + p53 mutated - Bcl 1 + p53 mutated + del 13q - Bcl 1 + p53 mutated + del ATM - p53 mutated + del ATM - Negative	22 patients 68% (15) 9 %(2) 4.5 % (1) 4.5 % (1) 4.5 % (1) 9 % (2)

Table 3: Hematological toxicity during the induction treatment

Hematological toxicity	All grades		Grades 3-4	
	R- HyperCVAD (88)	R-MA (82)	R- HyperCVAD (88)	R-MA (82)
	n (%)	n (%)	n (%)	n (%)
Anemia	73 (83%)	81 (99%)	23 (26%)	41 (50%)
Leucopenia	57 (65%)	82 (100%)	43 (49%)	80 (98%)
Neutropenia	54 (61%)	81 (99%)	42 (48%)	74 (90%)
Thrombocytopenia	44 (50%)	81 (99%)	30 (34%)	79 (96%)

Table 4: Non hematological toxicity during the induction treatment

Non hematological toxicity	All grades	Grades 3-4		SAES	
	170 cycles	R- HyperCVAD (88)	R-MA (82)	R- HyperCVAD (88)	R-MA (82)
	n (%)	n (%)	n (%)	n (%)	n (%)
Neutropenic fever	77 (45%)	7 (8%)	15 (18%)	6 (7%)	14 (17%)

Infection	78 (46%)	26 (29%)	24 (29%)	10 (11%)	26 (32%)
Bleeding	26 (15%)		2 (2%)		2 (2%)
Nausea/vomiting	32 (19%)	2 (2%)	2 (2%)		
Diarrhea	18 (11%)	1 (1%)	2 (2%)	1 (1%)	
Mucositis	23 (14%)	1 (1%)	1 (1%)	1 (1%)	
Liver (transaminitis)	18 (11%)	1 (1%)			
Renal disorder	11 (6%)				
Cardiac	9 (5%)	1 (1%)	1 (1%)		1 (1%)
Pulmonary/pleural	8 (5%)	1 (1%)	2 (2%)		1 (1%)
Deep vein thrombosis	2 (1%)		2 (2%)		2 (2%)
CNS-cerebellum	1 (0.5%)		1 (1%)		1 (1%)
Stroke	1 (0.5%)	1 (1%)		1 (1%)	
Personality disorder	1 (0.5%)	1 (1%)		1 (1%)	
Allergic reaction	2 (1%)	1 (1%)	1 (1%)	1 (1%)	1 (1%)
Pain	13 (8%)	3 (3%)	3 (3%)		

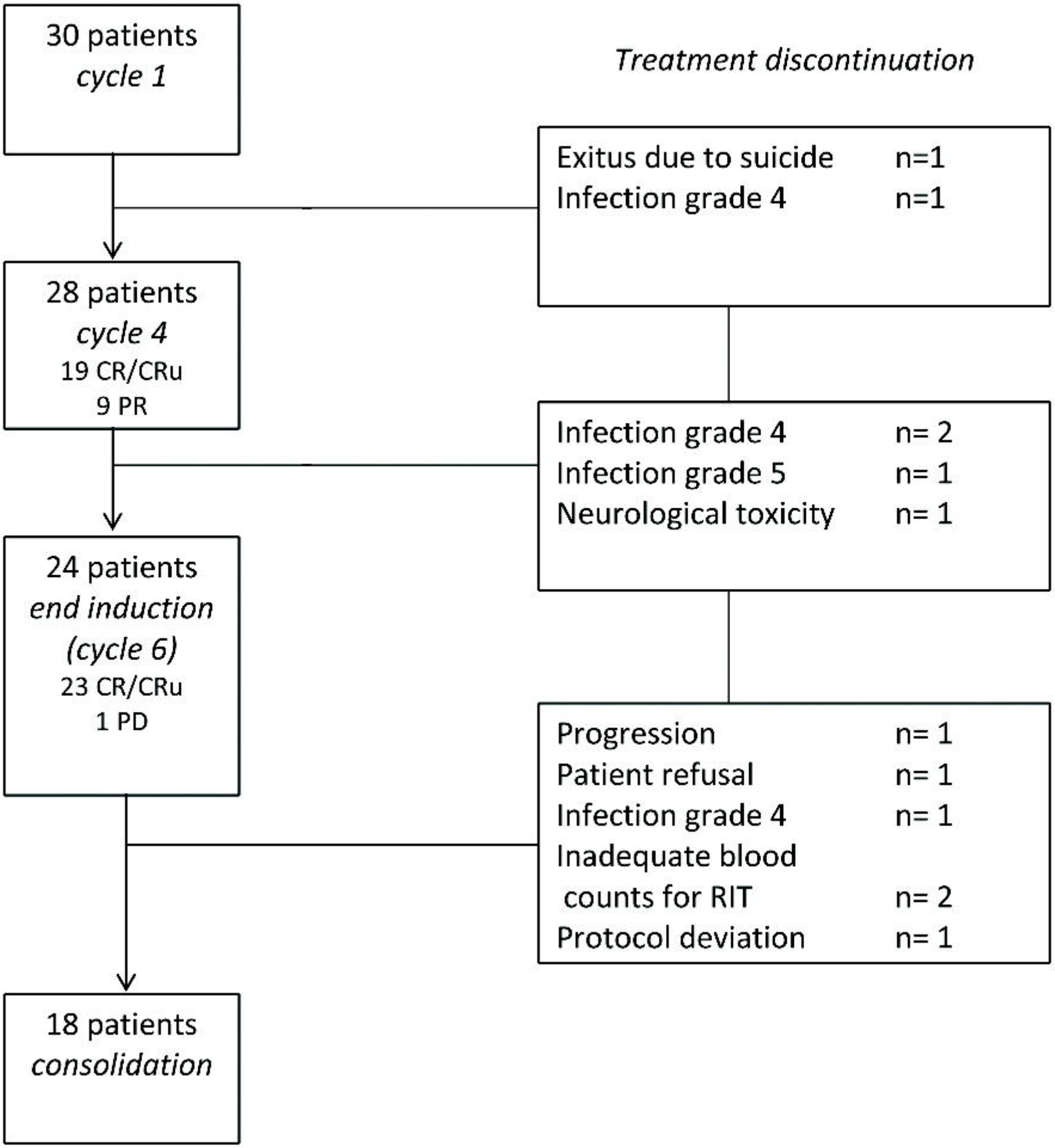
Table 5: Description of the second malignancies diagnosed during the study

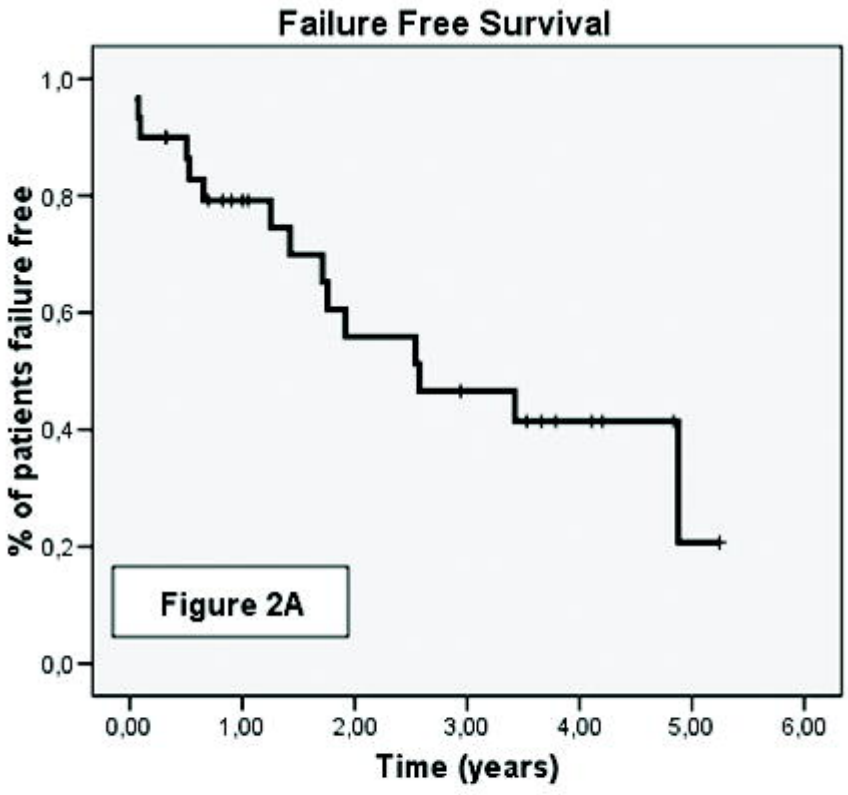
UPN	Age	Molecular findings at diagnosis	Years from diagnosis	Years from ^{90}Y-Ibritumomab	Previous lines	Secondary malignancy	Genetic 2nd malignancy
102	67	BCL1	1.6	0.81	1	Bladder carcinoma	
301	53	BCL1	1.99	1.25	1	Rectum adenocarcinoma	
402	47	BCL1	1.08	0.40	1	MDS/AML-6	t(16;16)
905	65	BCL1	2.06	1.19	1	Endometrial cancer	
1101	63	NA	3.67	3.12	1	MDS	Complex Karyotype*
1302	69	IgH	2.97	2.17	1	MDS	Complex Karyotype**
1303	59	BCL1	3.56	2.79	3 (including ASCT)	MDS	No metaphases due to marrow fibrosis

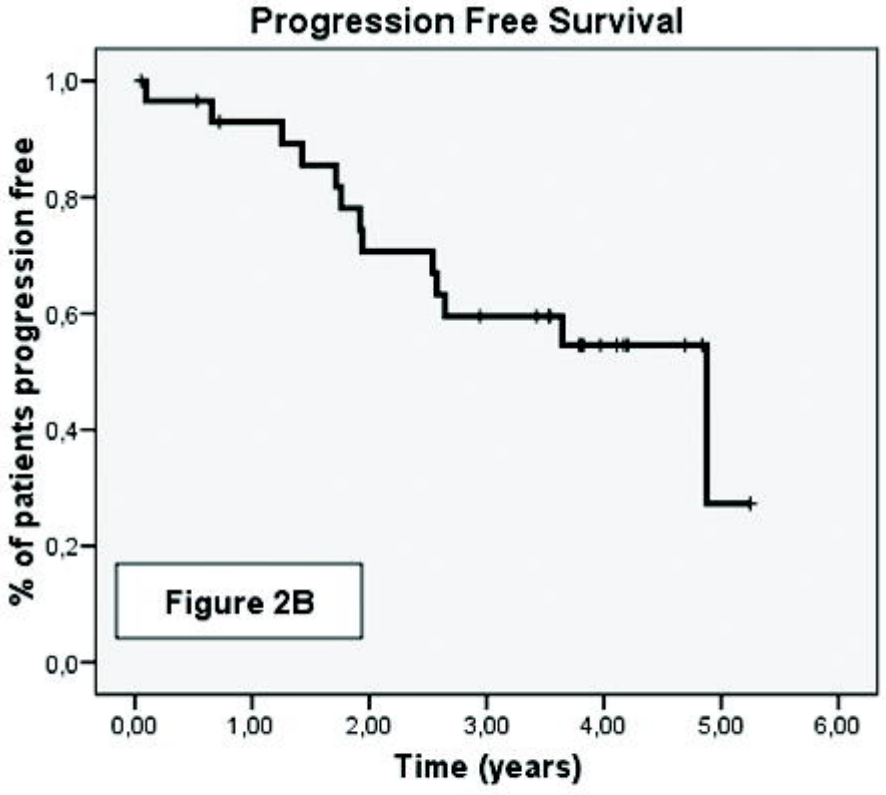
UPN: unique patient number; BCL- 1= FISH for Bcl-1 /IgH rearrangement; NA: non available; MDS: myelodisplastic syndrome; AML: acute myeloid leukemia; ASCT: autologous stem cell transplantation; (*): G-banding karyotype non available. FISH analysis: 46% del 5q31, 20% tris 8, 36% del 20q12, 40% del 5q33-34 CSF1R, 22% del 7 q31 ; (**): G-banding karyotype: 46, XY, r(7) [8]; 47, XY, r(7), +r(7)[8]; 48, XY, r(7), +r(7), +del(7q)[4]

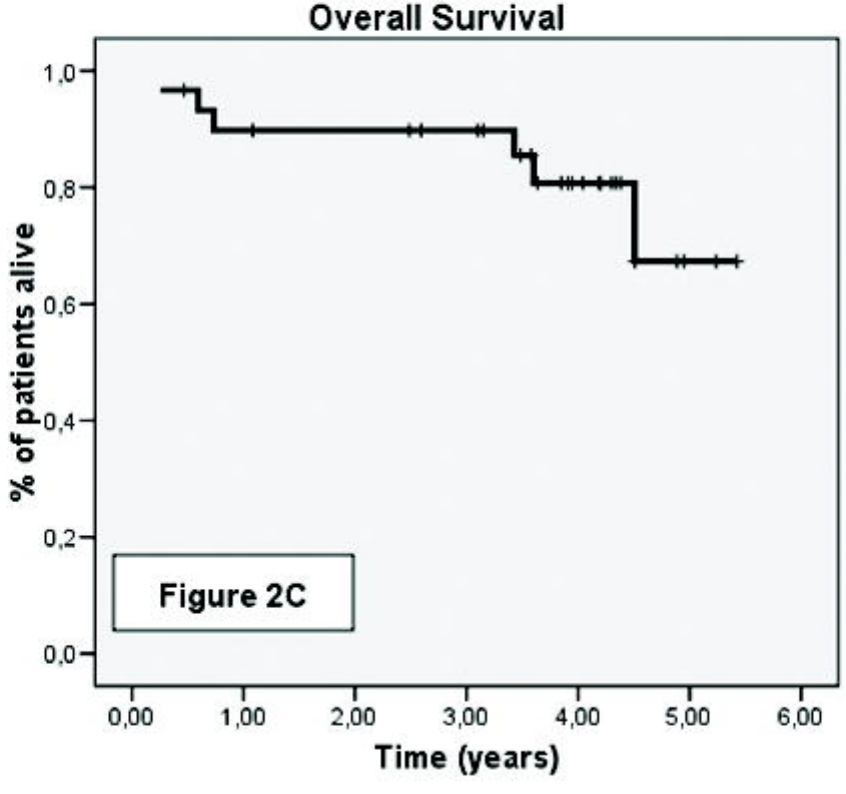
LEGEND TO FIGURES

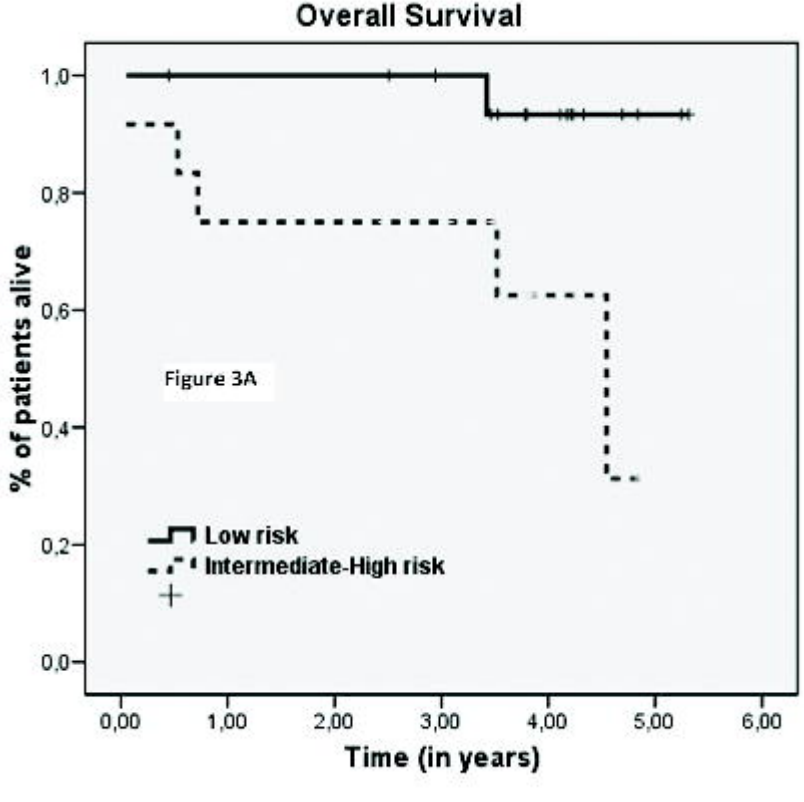
- **Figure 1:** Treatment flow chart
- **Figure 2:** FFS (2A), PFS (2B) and OS (2C) of the 30 patients by intent to treat analysis
- **Figure 3:** FFS (3A) and OS (3B) by MIPI: low risk versus intermediate- high risks

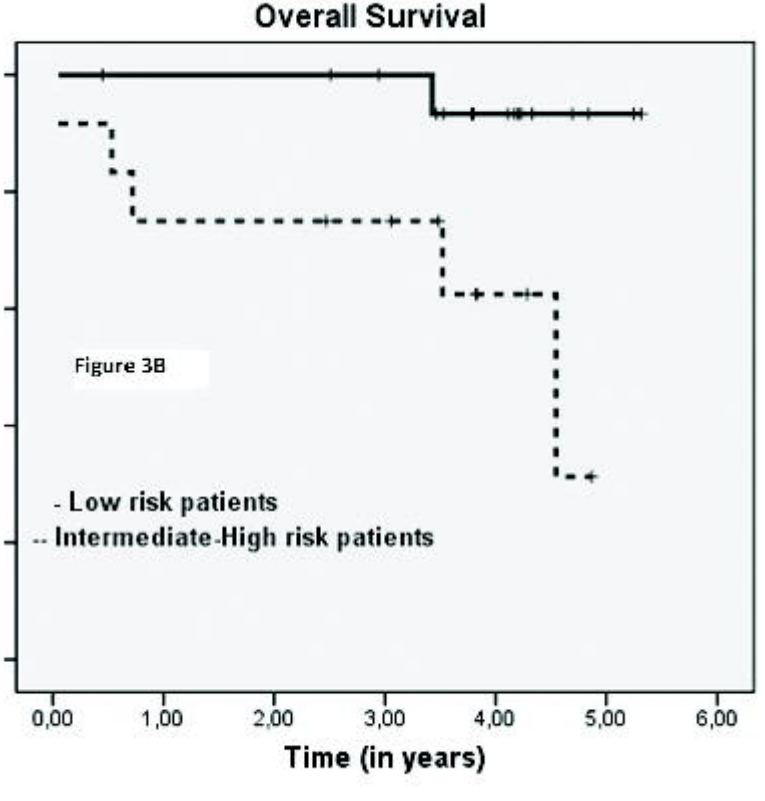

30 patients
cycle 1
Treatment discontinuation
Exitus due to suicide n=1
Infection grade 4 n=1
28 patients
cycle 4
19 CR/CRu
9 PR
Infection grade 4 n= 2
Infection grade 5 n= 1
Neurological toxicity n= 1
24 patients
end induction
(cycle 6)
23 CR/CRu
1 PD
Progression n= 1
Patient refusal n= 1
Infection grade 4 n= 1
Inadequate blood counts for RIT n= 2
Protocol deviation n= 1
18 patients
consolidation


Failure Free Survival
% of patients failure free
1,0
0,8
0,6
0,4
0,2
0,0
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Time (years)
Figure 2A


Progression Free Survival
% of patients progression free
1,0
0,8
0,6
0,4
0,2
0,0
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Time (years)
Figure 2B


Overall Survival
% of patients alive
1,0
0,8
0,6
0,4
0,2
0,0
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Time (years)
Figure 2C


Overall Survival
% of patients alive
1,0
0,8
0,6
0,4
0,2
0,0
Figure 3A
Low risk
Intermediate-High risk
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Time (in years)


Overall Survival
Figure 3B
- Low risk patients
-- Intermediate-High risk patients
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Time (in years)

SUPPLEMENTARY METHODS

The study was performed in 12 Spanish institutions. It was approved by the local and central committees and registered at the Clinical Trials Gov web-site (NCT00505232). All patients provided written informed consent before study entry. Patients were registered from February 2006 until July 2008 and were followed until September 2011.

Inclusion criteria and baseline assessment

Patients were considered eligible if they were between 18 and 70 years-old and diagnosed with MCL according the WHO classification (22). Cyclin D1 positivity of diagnostic lymph node or tissue was required for diagnosis. If only bone marrow was available, Fluorescence in situ hybridation (FISH) for the t(11;14))(q13;q32) translocation and immunophenotype with a CD19/CD20/CD5/CD23 panel, to rule out CLL, was needed.

Inclusion criteria were: stage II-IV, ECOG < 3; adequate renal and liver function (creatinine, total bilirrubine, AST and ALT <2.5 upper normal limits), left ventricular ejection fraction $\geq$ 50% and appropriate bone marrow function (hemoglobin ≥10 g/dl, absolute neutrophil count ≥1500/mm^3 and platelets ≥100000 /mm^3), unless due to infiltration by lymphoma.

Patients previously treated with antineoplastic agents and those with active infection or non-controlled important concomitant diseases were excluded. Other exclusion criteria were positive serology for HBV (hepatitis B virus), HCV (hepatitis C virus) and HIV (human immunodeficiency virus) and central nervous system involvement by lymphoma

Pre-treatment and follow up evaluation

Pretreatment evaluation included physical examination; blood count with differential analysis; serum chemistry analysis; serum LDH, β2-microglobulin and immunoglobulins; peripheral blood phenotype; whole body computed tomography (CT) scan; bone marrow biopsy and aspiration; colonoscopy; cavum exploration and gastroscopy if Waldeyer ring involvement, echocardiography and/or radionuclide ventriculography. FISH to detect t(11;14) and other cytogenetic abnormalities (del 13q14, +12, del 17p, del 11q22.3) were performed on bone marrow/ peripheral blood samples at the Hospital Universitario de la Princesa.

Complete disease evaluation was carried out before treatment, after the 4^{th} cycle and at the end of the induction treatment. After consolidation, disease assessment was evaluated every 4 months until the 2^{nd} year and every 6 months thereafter.

Treatment

Induction phase

Patients were scheduled to receive R-HyperCVAD therapy alternating with R-MA every 21 days as described by Romaguera et al (5). Amendments of protocol were performed when the first 6 patients completed their treatment and the number of induction cycles was fixed at 6, due to the high response rate observed after the 4^{th} cycle.

Cytarabine dose was reduced to 1 g/m^2 in patients older than 60 years, and methotrexate was reduced to 75% in patients with creatinine value of 1.5-2 mg/dl and 50% in those with 2-3 mg/dl. Dose adjustments for subsequent cycles were required in the following situations: platelet count between 75000-100000/mm3 or absolute granulocyte count between (AGC) 750-1000/mm3 on day 21 of each cycle, development of febrile neutropenia or non-hematotological grade 3 toxicity at any moment. Dose -1 level entailed reductions of 75% of cyclophosphamide, 60% of methotrexate and 60% of cytarabine.

Treatment was discontinued if the patient did not reach the required hematological recovery after 5 weeks, (neutrophils $\geq$ 1500/ mm3 and/ or platelets > 100.000/ mm3) or developed grade 4 infection, any grade 4 non hematological toxicity or severe bleeding. Patients with less than partial response (PR) after 4 cycles discontinued the treatment.

Support therapy with Peg- Filgastrim after each cycle and pneumocistis jirovecci prophylaxis was mandatory. Prophylaxis with antibiotic, antiviral and antifungal therapy, the use of erythropoietin and transfusions were allowed according to each participating center's policy.

Peripheral blood stem cell (PBSC) collection as back-up was mandatory in the first six patients and recommended for the rest of patients.

Consolidation phase

Consolidation with ^{90}Y-Ibritumomab-Tiuxetan was scheduled 12 weeks after the 6^{th} cycle. Patients received a first dose of Rituximab 250 mg/m^2 and a week later, a second infusion immediately followed by a single dose of ^{90}Y-Ibritumomab-Tiuxetan was administered. The drug was kindly provided by Bayer-Shering.

Initial dose for ^{90}Y-Ibritumomab-Tiuxetan was 0.3 mCi/kg, and dose escalation to 0.4 mCi/kg was planned, according to a 3+3 design if no unacceptable toxicity was observed.

The dose of ^{90}Y-Ibritumomab-Tiuxetan was fixed at 0,3mCi/ Kg, as five out of 6 patients had sustained neutrophils and/or platelets below 1500/mm3 and/ or 150000/mm3 respectively, beyond 12 weeks after the completion of induction therapy.

The infusion of the back up stem cells was recommended if grade 4 hematological toxicity was not treatable with supportive care or at the discretion of the physician in charge of the patient.

Study endpoints and definition of study variables

The main objective was to evaluate the feasibility, safety and efficacy of the whole treatment. Clinical efficacy was evaluated in terms of overall response (OR) and CR rates, FFS, PFS and OS.

Response criteria were assessed according to The International Workshop to Standardize Response Criteria for Non-Hodgkin's Lymphoma (24). Patients without response assessment were considered non responders.

The toxicities of induction and consolidation treatment were evaluated according to the National Cancer Institute´s Common Toxicity criteria (CTCAE v3.0).

Failure free survival (FFS) was defined as the time from the date of study entry until date of recurrence, progression of disease, death from any cause or any toxic event that prohibited treatment according to protocol. Responding patients who did not complete the whole treatment due to personal reasons or whose treatment was delayed beyond recommended were censured in the moment this deviation occurred.

Off protocol responding patients who received any additional anti-lymphoma treatment were not considered failures, and were censored at the time of this new treatment.

Progression free survival (PFS) was defined as the time from the inclusion in the trial until progression, recurrence or death as a result of lymphoma**.**

Overall survival (OS) was defined as the interval between the date of study entry of inclusion in the trial until death from any cause.

All variables were calculated in the intent- to treat- population, defined as patients who had received at least one cycle of the chemotherapy regimen. For survival analysis all patients were followed until the closure of the study regardless of treatment discontinuation.

Statistical analysis

Sample size was calculated in order to estimate a feasibility rate of 70% (patients who complete the whole treatment schema), with a maximum error of 17.5% and 95% confidence level. The sample size was 27 patients and considering that 10% of patients would not be evaluable for efficacy for early drop-outs, the target for total enrollment was 30. Epidat v 3.1 program was used for calculations.

FFS, PFS, and OS were estimated using the Kaplan-Meier method (25) and were compared between groups by log-rank test. Tests were two-sided, and the level of statistical significance was a *P* value less than 0.05. Comparison between groups for categorical covariates was performed using the Fisher´s exact test. Statistical analysis was performed with the SPSS v 15.0 package.

The crude incidence of secondary malignancies was calculated as the proportion of patients diagnosed with secondary neoplasm in the whole population. The cumulative incidence was

estimated using a nonparametric method that considers death due to other causes as a competing risk (26). Stata v 12.1 program was used for calculations.