Project No.:
503351

Project acronym:
European MCL Network

Project title:
**"*European Mantle cell Lymphoma network*:
Translational determination of
molecular prognostic factors and pharmacodynamics
in a European interdisciplinary collaboration"**

Instrument:	**Specific targeted Research Project (STREP)**
Thematic Priority:	**Translational research on promising predictive & prognostic markers (LSH-2002-2.2.0-8)**

Final report

Period covered: 1.7.2004 to 31.12.2007 Date of revision: 13. 02 2009

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Publishable executive summary

Mantle cell lymphoma (MCL) is a distinct, clinically very aggressive subentity of malignant lymphoma with a median survival of 3 years. However, a small subset of patients represents long-term survivors. So far, the discriminative power of different prognostic parameters has been limited and did not allow the reliable identification of the individual patient's risk profile. Thus, a better understanding of the underlying molecular mechanisms is eagerly warranted.
In the previous few years, European-wide MCL networks of clinicians (European MCL clinical intergroup), pathologists (European MCL Pathology Panel) and basic researchers (European MCL Research Network) have been established to investigate the clinical as well as molecular aspects of malignant transformation and progression in MCL.
Based on these extensive prerequisites and the recent development of innovative molecular techniques (matrix CGH, RNA array chips, RQ-PCR, proteomics), we performed a global approach to investigate innovative treatment options of MCL and evaluate new predictive (pharmacogenomics, minimal residual disease) and prognostic molecular markers (genomic alterations, RNA/proteome profiles).
In 2004 a set of prospective randomized trials have been initiated which meanwhile have recruited more than 600 patients:

patients <65 years: R-CHOP vs. R-CHOP/R-DHAP followed by myeloablative consolidation

patients >60 years: R-CHOP vs. R-FC followed by Rituximab vs. IFN maintenance

In all of these patients, diagnosis of MCL was confirmed and subclassified by the European MCL Pathology panel. Markers of proliferation have been confirmed as the most powerful prognostic marker.

In addition, patient samples for the systematic evaluation of MRD has been successfully collected and analysed for the vast majority of these study cases. Finally, molecular results of extensive RNA array, PCR and proteome analyses do not only indicate more individualized therapeutic strategies based on the molecular risk profile but will also finally elucidate the way to future molecular targeted treatment options in a subtype of malignant lymphoma with an otherwise dismal clinical outcome.

For detailed description of the *European MCL Network*, please check www.european-mcl.net

Section 1

Project objectives and major achievements during reporting period

WP I: In 2004 a set of prospective randomized trials have been initiated which meanwhile have recruited more than 600 patients:

- *R-CHOP vs. R-CHOP/R-DHAP followed by myeloablative consolidation*
- *R-CHOP vs. R-FC followed by Rituximab vs. IFN maintenance*

WP II: In all of these patients, diagnosis of MCL was confirmed and subclassified by the European MCL Pathology panel. In addition, an extensive reanalysis of several proliferation-associated molecular markers (Ki67, repp86, topoisomerase-II-alpha and MCM6) has been performed. Based on these results, a combined biological and clinical risk score (MIPI) has been developed..

WP III/1: More than 2700 samples of 446 study patients have been successfully collected for the systematic evaluation of MRD. In addition, consensus primers, FISH probes and FACS analysis have been standardized and applied to more than 250 clinical samples. Our analyses suggest that PCR and 4 color FACS are comparable concerning predictivity in initial staging before start of treatment. In addition, bone marrow biopsy may be substituted by peripheral blood samples without major loss of accuracy.

WP III/2: Different experimental approaches towards pharmacogenomics have been applied:

- a consensus set of SNP probes have been established and applied in a control cohort as well as first patient samples.
- comparative gene array analysis of paired MCL samples (a first diagnosis and relapse) have been performed applying the Agilent technology to identify the gene expression profile of chemoresistance.
- A nude mouse model has been generated and various molecular targeted compounds have been analysed in this *in vivo* model.

WP III/3: The objective of Workpackage III/3 was a global approach to evaluate the predictive value of large scale analysis of genomic alterations and gene expression profiles at mRNA and protein levels using innovative molecular techniques (matrix CGH, RNA array chips, RQ-PCR, proteomics) in MCL. In this project we have developed different strategies to analyze the expression profile and genomic alterations in these tumors. We have examined the mRNA expression profile of a large series of MCL using a panel of genes previously recognized to be related to prognosis in this lymphoma. A new macroarray technology based on quantitative PCR has allowed us to determine a set of 5 genes that form a predictive model applicable in routinely processed tissue samples. At genomic level, we have established array-CGH and expression array hybridizations of peripheral blood samples. In our studies comparison of the genomic profile and global expression profile of MCL examined by CGH and microarrays identified a set of genes deregulated by gene dosage as well as a number of chromosomal alterations that have prognostic significance, two of them (3q gains and 9q losses) have a prognostic significance that is independent of the proliferation signature and therefore add prognostic information to the model based on the expression profile. However, based on the gene expression profile proliferation signature remains the strongest parameter for survival rate in typical MCL cases. We have also achieved preliminary proteome results using high throughput technologies. In addition, proteomics identified a p53-centered network of interactions after proteasome inhibition.

Section 2

Workpackage I: Clinical intergroup

Background

The clinical Intergroup working party was founded in 1996 and is currently involving national study groups from all over Europe (representatives in brackets):

BNLI	(S. Rule, Plymouth)
Catalanian lymphoma group	(A. Lopez-Guillermo, Barcelona)
CLSG	(M. Trneny, Prag/Czech Republic)
EORTC Lymphoma Group	(H. Kluin-Nelemans, Groningen/Netherlands)
GELA	(O. Hermine, Paris/France)
GLSG	(W. Hiddemann, Munich/Germany)
GOELAMS	(R. Gressin, Grenoble/Suisse)
HOVON	(M. van t'Veer, Rotterdam/Netherlands
Israelian lymphoma group	(Ofer Shpilberg, Haifa/Israel)
Nordic Lymphoma Group	(C. Geissler, Copenhagen/Denmark)
OSHO	(M. Herold, Erfurt/Germany)
PLRG	(J. Walewski, Warzawa/Poland)
SAKK	(N. Ketterer, Lausanne/Suisse)

Project objectives and progress

Following first line therapy optimization studies haven been activated in 2004:

R-CHOP vs. R-CHOP/R-DHAP followed by myeloablative consolidation

This prospective randomised, international multicentre phase III trial compares the efficacy of two different immuno-chemotherapy schedules followed by myeloablative consolidation with subsequent autologous stem cell transplantation in MCL patients <65 years.

Study aim:
To determine the efficacy of 3 cycles of CHOP/DHAP plus rituximab and high dose ARA-C myeloablative radiochemotherapy regimen followed by blood stem cell transplantation versus 6 cycles of CHOP plus rituximab and myeloablative radiochemotherapy followed by blood stem cell transplantation.

Objectives: (primary) Time to treatment failure
(secondary) Complete response rate and overall survival

Statistical hypothesis:
48% risk reduction of relapse which corresponds to an improvement of 20% in failure-free survival after 3 years.

Duration of study:

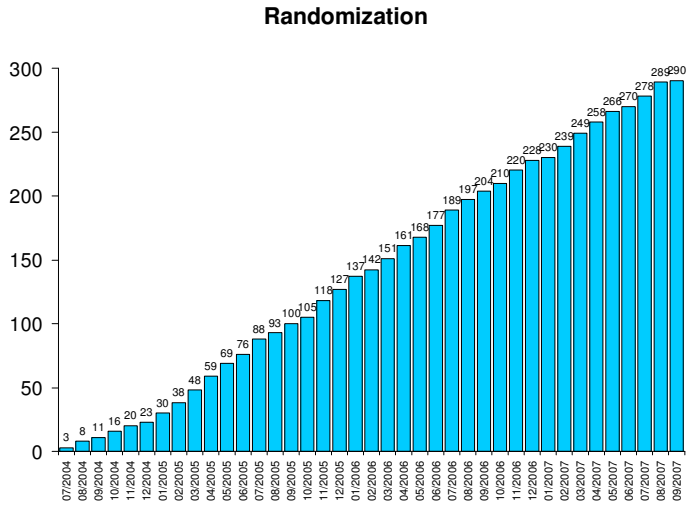
Based on the statistical hypothesis and our previous studies, a recruitment period of approximately 4 years and an annual patient recruitment of 90 patients and has been estimated.

Progress
Since the study was also activated internationally in 2004 the number of recruited patients continuously rose and is higher than the expected rate. Until the end of 2007, 314 patients from Germany, France, Belgium and Poland have been recruited. Following, data of an interim analysis in September 2007 are presented.

Table 1.1. patient recruitment (MCL younger until 9/2007)

	R-CHOP	R-CHOP/R-DHAP
randomized	144	146
Belgium	6	11
France	57	50
Germany	73	77
Poland	8	8

Figure 1.1 patient recruitment (MCL younger until 9/2007)



Clinical characteristics were as expected. Especially, no unexpected toxicity has been observed so far. Toxicity (grade III/IV during R-CHOP/alternating R-DHAP) was mainly hematological: leukocytopenia 58/77%, thrombocytopenia 14%/74%, but only rare febrile neutropenia (11%/22%) or infections (5%/7%) were observed (table 1.2).

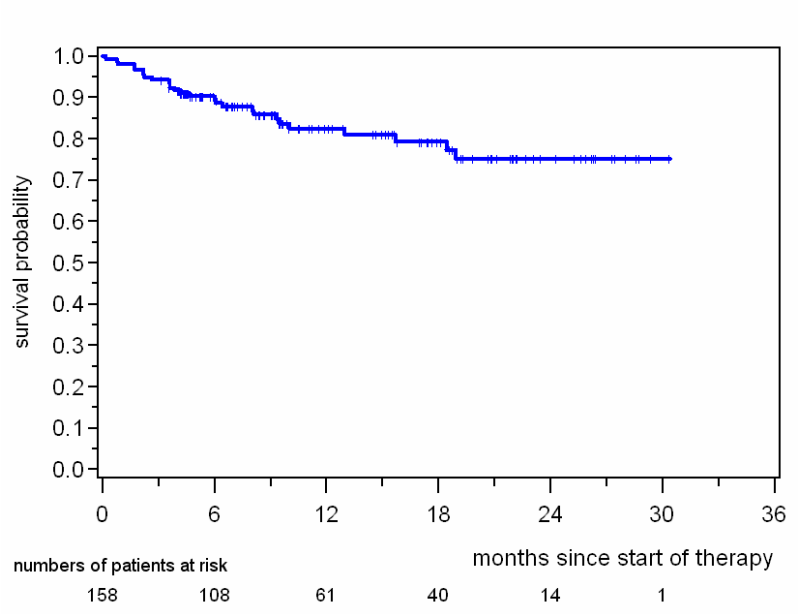
Table 1.2. Toxicity during induction (MCL younger)

		R-CHOP		R-DHAP				R-CHOP		R-DHAP	
Toxicity	Grade	freq	%	freq	%	Toxicity	Grade	freq	%	freq	%
Hemoglobin	1 or 2	40	51	47	56	Arrhythmia	1 or 2	1	1	2	3
	3 or 4	8	10	33	39		3 or 4	2	3	1	1
Leukocytes	1 or 2	23	29	14	16	Cardiac Function	1 or 2	0	0	2	3
	3 or 4	47	59	63	74		3 or 4	0	0	0	0
Granulocytes	1 or 2	9	13	7	9	Pulmonary Function	1 or 2	3	4	4	5
	3 or 4	52	72	60	78		3 or 4	3	4	1	1
Platelets	1 or 2	18	23	13	15	Hematuria	1 or 2	0	0	3	4
	3 or 4	11	14	63	75		3 or 4	0	0	0	0
Lymphocytes	1 or 2	9	13	11	14	Neuropathy	1 or 2	28	36	22	27
	3 or 4	54	75	58	73		3 or 4	5	6	1	1
Creatinine	1 or 2	7	9	34	41	Depression	1 or 2	6	8	9	11
	3 or 4	0	0	3	4		3 or 4	0	0	1	1
Bilirubin	1 or 2	6	8	6	7	Allergy	1 or 2	4	5	4	5
	3 or 4	1	1	1	1		3 or 4	0	0	0	0
Transaminases	1 or 2	21	28	26	33	Weight loss	1 or 2	6	8	10	12
	3 or 4	1	1	2	3		3 or 4	2	3	0	0
Nausea	1 or 2	28	36	48	57	Bleeding	1 or 2	0	0	2	3
	3 or 4	1	1	5	6		3 or 4	1	1	1	1
Vomiting	1 or 2	8	10	28	34	Alopecia	1 or 2	19	26	23	32
	3 or 4	1	1	4	5		3 or 4	24	32	22	30
Diarrhea	1 or 2	19	25	17	21	Fatigue	1 or 2	29	38	47	57
	3 or 4	0	0	1	1		3 or 4	2	3	1	1
Constipation	1 or 2	14	18	16	20	Infections	1 or 2	24	31	17	21
	3 or 4	0	0	0	0		3 or 4	5	6	7	9
Mucositis	1 or 2	18	23	19	23	Myalgia/Arthralgia	1 or 2	3	4	11	14
	3 or 4	0	0	1	1		3 or 4	0	0	1	1
						Febrile neutropenia	1 or 2	0	0	0	0
							3 or 4	9	12	16	20

Data on response rate of study arms are still blinded. However, overall response rate was promising (91%) with a 56% CR/CRu in the overall group. Based on the limited follow-up, both

progression-free and overall survival are remarkable with 82% and 88% at 12 months, respectively (figure 1.2).

Figure 1.2 Time to treatment failure (MCL younger until 9/2007)



R-CHOP vs. R-FC followed by Rituximab vs. IFN maintenance

This prospective randomised, international multi-centre phase III trial compares the efficacy of two different immuno-chemotherapy schedules followed by different maintenance regimens (interferon, anti CD20 antibody) in MCL patients >65 years.

Study aim:
To determine the efficacy of maintenance therapy with rituximab after induction chemotherapy (R-CHOP vs. R-FC) for elderly patients with mantle cell lymphoma not suitable for stem cell transplantation

Primary objectives:
(first randomisation) Reduction of lymphoma mass measured by the CR rate
(second randomisation) Progression-free survival after chemotherapy

Statistical hypothesis:
(first randomisation): Improvement of the CR rate by 15%
(second randomisation): 60% reduction of relapse risk

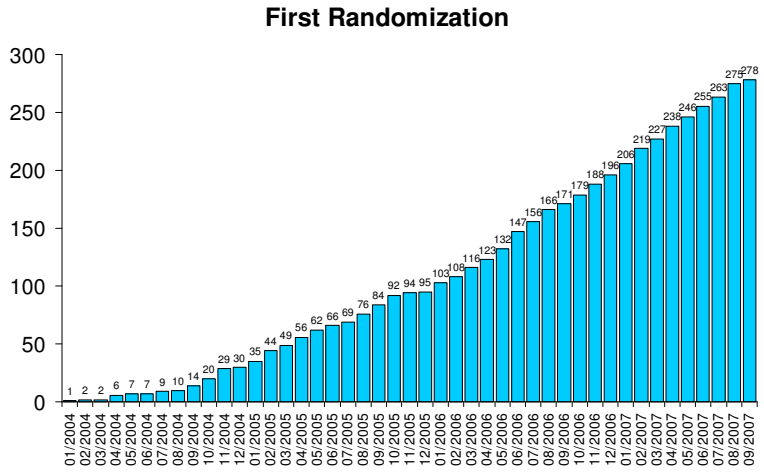
Duration of study:
Based on the statistical hypothesis and our previous studies, a recruitment period of approximately 4-6 years and an annual patient recruitment of 90 patients and has been estimated.

Progress
Since the study was also activated internationally in 9/2004 the number of patients recruited continuously rose and is currently almost matching the expected rate (90 patients/year). Until December 2007, 302 patients from Germany, France, Netherlands, Poland, Czech Republic, Denmark, Italy and Belgium have been recruited. Following, data of an interim analysis in September 2007 are presented.

Table 1.3. patient recruitment (MCL elderly until 9/2007)

	R-CHOP	R-FC
randomized	139	139
Belgium	5	5
Czech Republic	4	5
Denmark	4	3
France	34	36
Germany	67	67
Italy	3	3
Netherlands	14	13
Poland	8	7

Figure 1.3 patient recruitment (MCL elderly until 9/2007)



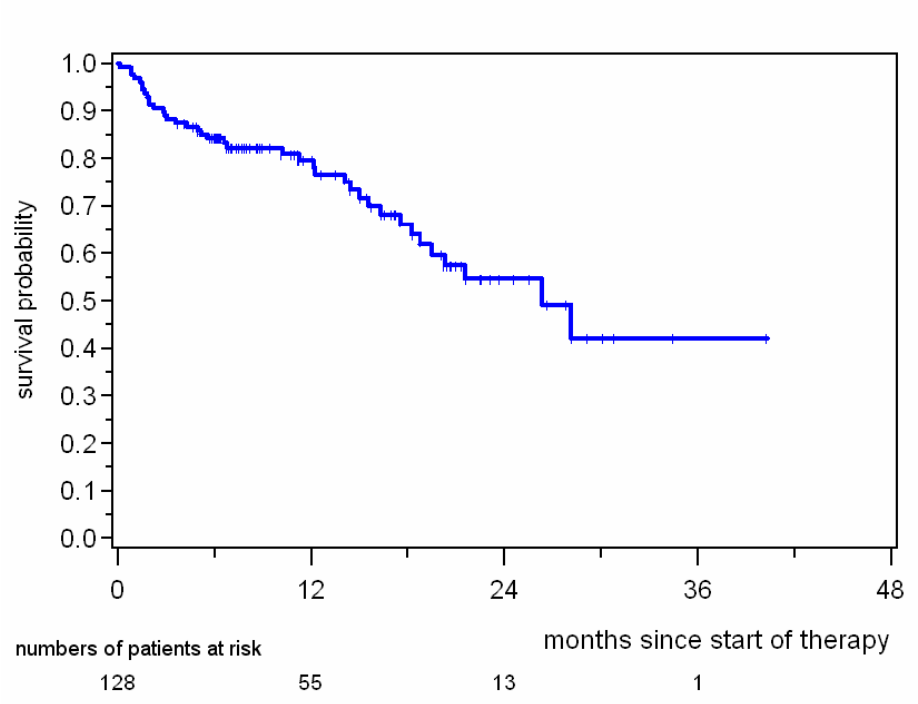
Clinical characteristics were as expected; median age was 70 years, with 93% of patients presenting in advanced stage III/IV and 42% with elevated LDH resulting in a very high risk patient population (65% intermediate high/high IPI).
Toxicity (grade III/IV during R-CHOP/R-FC) was mainly hematological: Leukocytopenia 62/72%, thrombocytopenia 13%/40%, but only rare febrile neutropenia (23%/7%) or infections (19%/23%) were observed (table 1.4).

Table 1.2. Toxicity during induction (MCL elderly)

		R-CHOP		R-FC				R-CHOP		R-FC	
Toxicity	Grade	freq	%	freq	%	Toxicity	Grade	freq	%	freq	%
Hemoglobin	1 or 2	49	73	37	53	Arrhythmia	1 or 2	4	6	2	3
	3 or 4	5	7	14	20		3 or 4	2	3	2	3
Leukocytes	1 or 2	16	24	13	18	Cardiac Function	1 or 2	4	6	1	2
	3 or 4	43	63	51	70		3 or 4	6	10	1	2
Granulocytes	1 or 2	9	16	15	25	Pulmonary Function	1 or 2	10	16	4	6
	3 or 4	33	57	40	66		3 or 4	4	6	5	7
Platelets	1 or 2	21	33	24	34	Hematuria	1 or 2	1	2	1	2
	3 or 4	9	14	29	41		3 or 4	0	0	0	0
Lymphocytes	1 or 2	13	21	7	11	Neuropathy	1 or 2	28	44	6	9
	3 or 4	38	62	45	71		3 or 4	1	2	1	2
Creatinine	1 or 2	13	21	15	23	Depression	1 or 2	9	14	7	11
	3 or 4	0	0	0	0		3 or 4	0	0	0	0
Bilirubin	1 or 2	6	10	9	15	Allergy	1 or 2	2	3	6	9
	3 or 4	1	2	1	2		3 or 4	1	2	1	1
Transaminases	1 or 2	13	22	12	19	Weight loss	1 or 2	13	20	11	17
	3 or 4	1	2	0	0		3 or 4	0	0	0	0
Nausea	1 or 2	17	27	28	42	Bleeding	1 or 2	2	3	2	3
	3 or 4	0	0	2	3		3 or 4	0	0	0	0
Vomiting	1 or 2	9	14	11	16	Alopecia	1 or 2	15	24	11	18
	3 or 4	0	0	1	1		3 or 4	26	41	3	5
Diarrhea	1 or 2	17	27	5	7	Fatigue	1 or 2	36	56	33	50
	3 or 4	3	5	2	3		3 or 4	8	13	3	5
Constipation	1 or 2	17	27	7	11	Infections	1 or 2	17	26	12	17
	3 or 4	0	0	0	0		3 or 4	14	22	14	20
Mucositis	1 or 2	15	23	14	21	Myalgia/Arthralgia	1 or 2	10	16	6	9
	3 or 4	1	2	0	0		3 or 4	3	5	0	0
						Febrile neutropenia	1 or 2	0	0	0	0
							3 or 4	15	23	5	7

Despite the poor risk profile, combined immuno-chemotherapy (total group) achieved an impressive 84% response rate (52% CR/CR_u). Although the impact of maintenance is not yet evaluable, both progression-free and overall survival rates were encouraging with 79% and 86% at 12 months, respectively (figure 1.4). Interestingly, especially the CR patients showed a favorable clinical course with only 2 relapses in 28 patients (7%) observed so far.

Figure 1.4 Time to treatment failure (MCL elderly until 9/2007)



The conduction of these international phase III studies is based on the national study groups and the established data center at the Dept. of Medicine III/University of Munich. Thus, the workplan also included:

- central patient registration and randomisation
- coordination of national study centers
- development of CRFs as well as adequate *in silico* forms of the data bank
- data recrural and medical plausibility check
- regular (semi-automated) monitoring service of documentation
- registration and distribution of information on serious adverse events
- regular statistical evaluation (sequential test for phase III studies)
- close check for early stopping rules
- medical consultant service

Extension of project work programme

Relapsed MCL

In a prospective multicenter phase II study, another innovative option, radioimmunotherapy, has been evaluated in MCL.

Activated in May 2004, currently 41 patients have been recruited. In a joint analysis together with the Polish study results, our data indicate that upfront radioimmunotherapy is not very effective with an overall response of about 30% and a short remission duration of 3-5 months only. In contrast, radioimmunotherapy consolidation after initial lymphoma debulking with a fludarabine-containing regimen had significantly higher hematotoxicity, but also achieved a conversion to complete remission in 45% of PR patients; preliminary follow-up data suggest a prolonged remission duration after radioimmunotherapy consolidation (figure 1.5, 1.6).

Figure 1.5 Progression-free survival (A: RIT induction, B: RIT consolidation)

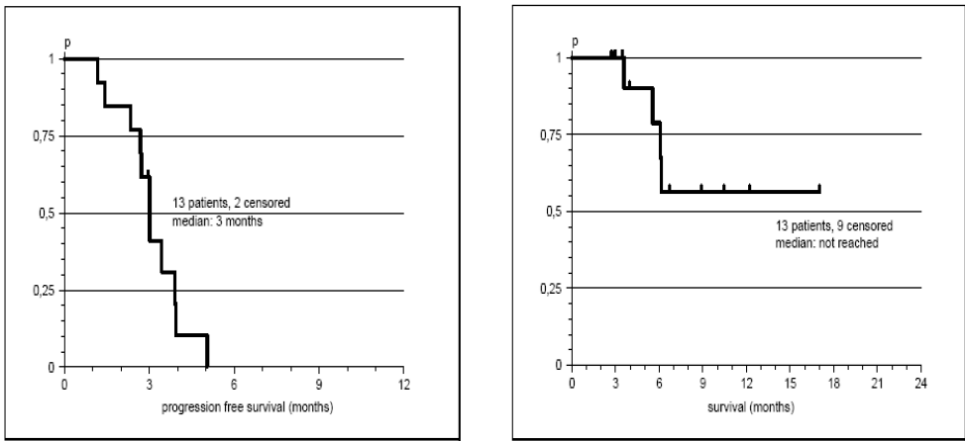
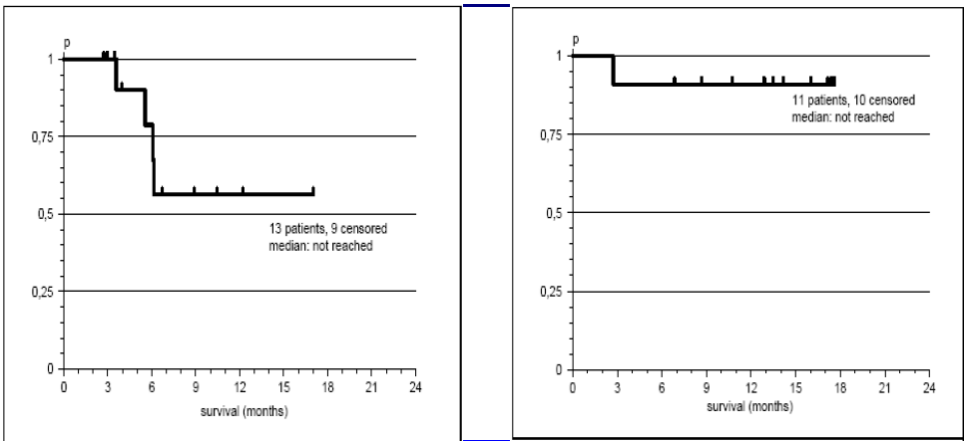


Figure 1.6 Overall survival (A: RIT induction, B: RIT consolidation)



Based on our own in vitro experiments (see also WP III/3), a European intergroup study for relapsed MCL (phase III) has been submitted to the authorities (table 1.3)

Table 1.3 Study design for patients with relapsed MCL after/not appropriate for autologous PBSCT (n=250)

Regimen:	Rituximab	375 mg/m2	day 1
	Ara-C	2x 2 g/m2	day 2
	vs.		
	Rituximab	375 mg/m2	day 1
	Ara-C	2x 2 g/m2	day 2
	Bortezomib	1,5 mg/m2	day 1,4
Study aim:	- time to treatment failure		
	- overall survival		
	- toxicity/feasibility		

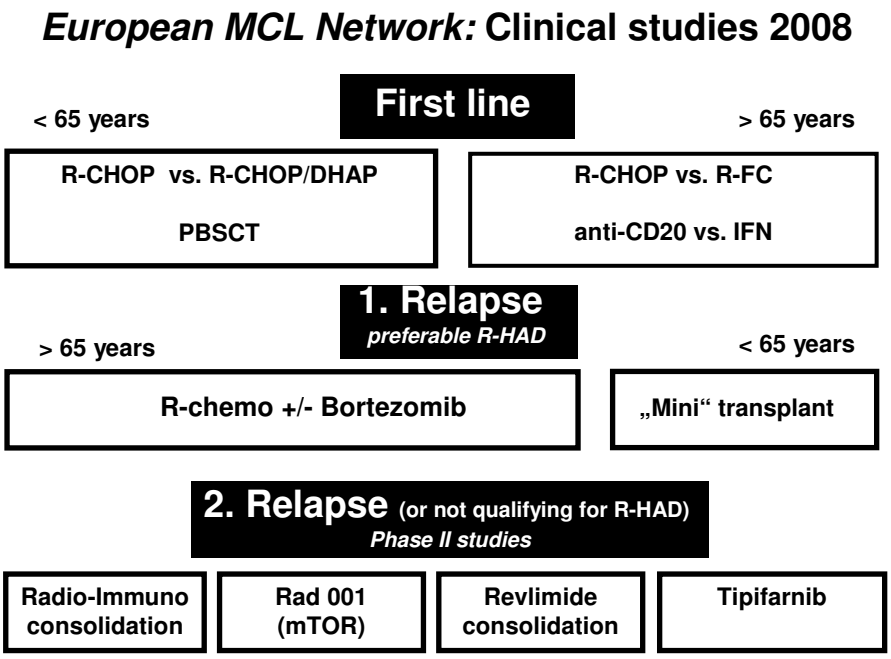
In the first 8 pilot patients, this regimen achieved disease control in all but two patients with relapsed MCL (table 1.4).

Table 1.4. Pilot study R-HAD (Bortezomib chemotherapy combination)

Recruited patients (obtained informed consent)	**8**
Evaluable patients (obtained documentation)	**8**
Median time since start of treatment	**265 days**
No. of applied cycles Patients with 2 cycles Patients with 4 cycles	**24** 4 4
Best response after 2 cycles (n=8) CR PR MR SD PD	 0 / 8 4 / 8 1 / 8 1 / 8 2 / 8
Best response after 4 cycles (n=4) CR PR	 1 / 4 3 / 4
Event (progression/relapse/death) Time to event	**6 / 8** 49, 51, 119, 130, 147, 271 days
Salvage therapy	**4 / 8**
Ongoing remission Time in remission	**2 / 8** 233+ (PR), 311+ (SD) days
Alive	**5 / 8**
Death	**3 / 8** **2 deaths** 75 and 314 days after start of treatment (both PD after 2 cycles) **1 death** 271 days after start of treatment (in PR after 4 cycles) after allogeneic hematopoetic stem cell transplantation from HLA identical sibling

In addition, based on recently published efficacy data, a phase II trial (IIT) evaluation the feasibility and efficacy of an immuno-modulatory compound (lenalidomide maintenance) will be initiated within the next months.
The current study generation of the *European MCL Network* is illustrated in figure 1.7.

Figure 1.7 Current study generation of the European MCL Network



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