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Key points: Addition of lenalidomide to R-B is highly active in untreated MCL patients, but associated with unexpected high rates of infections and SPMs

Abstract

For elderly patients with mantle cell lymphoma (MCL), there is no defined standard therapy. In this multi-centre open-label phase I/II trial we evaluated the addition of lenalidomide (LEN) to rituximab-bendamustine (R-B) as first-line treatment to elderly MCL patients. Patients >65 years with untreated MCL, stage II-IV were eligible for inclusion. Primary endpoints were maximally tolerable dose (MTD) of LEN, and progression-free survival (PFS). Patients received six cycles q4w of L-B-R (L D1-14, B 90 mg/m^2 iv D1-2 and R 375 mg/m^2 iv D1) followed by single LEN (D1-21, q4w, cycles 9-13).

51 patients (median age 71 years) were enrolled 2009-2013. In phase I, the MTD of LEN was defined as 10 mg in cycles 2-6, and omitted in cycle 1. After six cycles, the complete remission rate (CRR) was 64% and 36% were MRD negative. At a median follow-up time of 31 months, median PFS was 42 and overall survival 53 months. Infection was the most common non-haematological grade 3-5 event and occurred in 21 (42%) patients. Opportunistic infections occurred in three patients; 2 PCP and 1 CMV retinitis. Second primary malignancies (SPM) were observed in eight patients (16%). LEN could safely be combined with R-B, when added from the second cycle in patients with MCL, and was associated with a high rate of CR and molecular remission. However, we observed a high degree of severe infections and an unexpected high number of SPMs which may limit its use. http://clinicaltrials.gov: NCT00963534.

Introduction

Mantle cell lymphoma is associated with poor prognosis with a reported median overall survival of 5 years.[1] The MCL International Prognostic Index, MIPI, which divides patients into three prognostic risk groups based on the parameters age, performance status (PS), lactate dehydrogenase level and white blood cell count, was proposed in 2008 and has been validated retrospectively as well as in a prospective randomized study. [2-5]

Survival rates of MCL have improved during the last decade, mainly due to the addition of rituximab (R) and, for the young patient population frontline intensive treatment including cytarabine [1,6-9] However, for the older patients, who constitute the majority of the MCL population, there is no defined standard therapy. For this group, R-CHOP followed by rituximab maintenance was associated with prolonged survival in comparison to R-FC.[10] The German STiL group compared R-bendamustine (R-B) and R-CHOP in a randomized trial with the conclusion that R-B was associated with higher PFS and less toxicity, making this regimen preferable.[11,12] Lenalidomide (LEN), an immuno-modulating agent, has shown activity in relapsed/refractory MCL as well as in first-line. [13-15]

Consequently, the Nordic Lymphoma Group designed a trial to investigate efficacy and safety of LEN in combination with R-B as first-line treatment for patients >65 years with mantle cell lymphoma.

Methods

This multi-centre, open-label, non-randomized phase I/II study was carried out in nineteen centres in Sweden, Norway, Denmark and Finland. The study was performed in agreement with the declaration of Helsinki and subsequent updates until 2008 and conducted according to the guidelines for Good Clinical Practice, issued by The International Conference on Harmonisation (ICH). The protocol was approved by all national Ethical Review Boards. All patients signed a written informed consent. The study was registered at http://clinicaltrials.gov as NCT00963534.

Study design/Objectives

Primary endpoints were in the phase I part to determine the maximally tolerable dose (MTD) for LEN in combination with R-B, and in the phase II expansion cohort, progression-free survival (PFS). Secondary endpoints included overall response rate (ORR), complete remission rate with and without PET, molecular remission rate measured by PCR, overall survival (OS), and safety.

Treatment

The regimen consisted of an induction phase with six cycles of LBR (LEN (po days 1-14), bendamustine (90 mg/m^2 iv, days 1-2), rituximab (375 mg/m^2 iv, day 1)), cycle duration 28 days, followed by a maintenance phase with single agent LEN (po day 1-21), cycle duration 28 days, up to a maximum of seven cycles (total duration 52 weeks).

In phase I, the treatment plan followed a sequential dose escalation according to a 3+3 design. The initial dose of LEN in cycles 1-6 was 5 mg, escalated by 5 mg in each step. In cycles 7-13, the dose of LEN was 25 mg.

Dose-limiting toxicity (DLT) was defined as any grade 3-5 non-hematological adverse event (AE) within the first two cycles of LBR with the exception for thromboembolic events grade 3-4, non-persisting nausea, diarrhoea, elevated transaminases or events attributed to progressive disease. A recovery to ANC ≥ 1.0 x 10^9/L and platelet count ≥ 100 x 10^9/L was required before starting the next cycle.

Initially, the protocol included premedication with corticosteroids prior to rituximab infusion exclusively in cycle 1 but after protocol amendment (below), corticosteroids was administered prior to every rituximab infusion and in cycle 2, all patients received oral prednisone 20 mg d1-14, followed by one week tapering of the dose. The use of G-CSF was mandatory in cycles 1-6, as the addition of LEN was expected to augment haematological toxicity.

Antibiotic prophylaxis was not initially recommended. After the first case of pneumocystis pneumonia (PCP), co-trimoxazole was prescribed to all patients.

All patients received allopurinol 300 mg/day p o days 1-3 cycle 1 but not hereafter, due to the risk of cutaneous reactions in combination with bendamustine.

Thrombosis prophylaxis was recommended to all patients during the treatment phase, unless contraindicated (aspirin 75 mg/day, or low molecular weight heparin to patients with a history of a thromboembolic event and/or a known hypercoagulable state).

Eligibility criteria

Patients were eligible if they were >65 years or ≤65 years but unable to tolerate high-dose chemotherapy, with a confirmed diagnosis of MCL, stage II-IV and WHO Performance status 0-3, requiring treatment due to at least one of the following symptoms: bulky disease, nodal or extra nodal mass > 7 cm, B- symptoms, elevated serum LDH, involvement of ≥3 nodal sites (each with a diameter >3 cm), symptomatic splenic enlargement, compressive syndrome or pleural/peritoneal effusion. Further, patients should not have received any previous treatment (one cycle of chemotherapy and/or radiotherapy was accepted).

Assessment during study

At base-line, all patients underwent clinical examination, collection of blood samples, bone marrow (BM) biopsies and aspirates and computed tomography (CT) of neck, thorax, abdomen and pelvis. BM and peripheral blood (PB) samples were sent for MRD analyses and a formalin-fixed tissue sample was collected for central review. During treatment, patients were assessed with clinical examination prior to each cycle and blood samples were obtained at days 1, 7, 14 and 21 respectively.

Response evaluation was performed after three and six cycles of LBR as well as six weeks (1.5 months) after completion of therapy and included CT and BM examination including samples for MRD assessment. PET scan was recommended (not mandatory) at base-line, and after six and twelve months. Patients were subsequently assessed with clinical examination, labs and CT scan every six months until thirty-six months after end of treatment.

Response was evaluated according to the international response criteria of 2007.[16,17] Toxicity was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 3.0 (NCI CTCAE).

Detection of MRD was performed as previously described.[8] Briefly, DNA was extracted, sequenced and used as a template for patient specific primer design and standard nested PCR amplification of clonally rearranged immunoglobulin heavy chain (IGHV) genes and/or Bcl-1/IGHV rearrangement (translocation 11;14).

Statistical methods

A prolongation of PFS of 6 months in comparison to the reported median PFS of 30 months (at time of protocol design) in the R-B arm in the German STiL group trial was considered

significant. [11] Based on exponentially distributed PFS, a 95% confidence interval was calculated to 23.1 months by 40 observations, why the total sample size was determined as 60 patients with 20 patients in phase I and 40 patients in phase II.

Progression-free survival was defined as the interval between registration date and date of documented progression, lack of response, first relapse, or death of any cause. Overall survival was defined as time from registration to death from any cause. The Kaplan-Meier method was used to estimate survival curves for PFS and OS. Comparison of frequency of adverse events in different groups was based on chi-square-tests. Analysis on the incidence of infection in relation to lymphocyte subpopulations was conducted by using Mann Whitney U test. For statistical analyses, IBM SPSS 22 was used. All analyses were based on data collected through 27 February 2015.

Results

Fifty-one patients were enrolled between 12 October 2009 and 22 May 2013 from thirteen centres in four Nordic countries. The accrual was slower than expected and enrolment was stopped prematurely. One patient was excluded because of screen failure and was removed from all analyses. Baseline characteristics are shown in Table 1.

Treatment

Among all patients in phase I+II, 37 patients (74%) completed the induction (c1-6) and 12 patients (24%) completed the maintenance phase (c1-13). Thirty-six patients (68%) received the established MTD dose of LEN 10 mg in combination with R-B. In summary, all 50 patients received 266 cycles of L-B-R and 28 patients received 131 cycles of single LEN. The causes for treatment discontinuation were in descending order: toxicity (n=28 (74%), 15 during the induction phase), progressive disease (n=6 (16%), 5 during the induction phase), second primary malignancies (n=3 (8%)) and consent withdrawn (n=1). Among those who stopped treatment due to toxicity, two patients received treatment outside the study with rituximab maintenance and R-B respectively. For CONSORT diagram of phase I+II, see Figure 3 (suppl).

Safety

Phase I

Dose escalation and adverse events including DLT are showed in Table 4 (suppl). The starting dose of LEN in cohort 1 (n=3) was 5 mg. AE grade 3 or 4 occurred in two patients within the first two cycles. One patient had infection and one patient had cerebral infarction after cycle 1 and allergic reaction after cycle 2, reported as related to rituximab. These events were not considered related to study treatment by the data monitor committee and the next three patients (cohort 2) received the escalated dose of 10 mg. In cohort 2, AE grade 3 occurred in two patients; one patient developed allergic reaction and infection and one with rash and infection, none of them assessed as DLT. In cohort 3, one patient was reported with DLT, urticaria grade 3 as well as sensory neuropathy with oedema and hypotension, and the cohort was expanded to include another three patients. Among these, one patient developed hypotension grade 3, also regarded as DLT. Further, one patient had urticaria grade 3 and received a lower dose of LEN in the following cycle.

As described, a high number of adverse events was observed in the first three cohorts, including high rate of allergic and cutaneous reactions, predominantly in the first cycle. In combination with DLT in cohort 3 at 15 mg, the protocol was amended to exclude LEN from cycle 1. Further, to exclude a dose-dependent impact of bendamustine, the amended protocol included a de-escalation schedule of bendamustine (B) for the three following cohorts ("A-C") B 90 mg/m^2 + LEN 10 mg (cohort A, n=6), B 70 mg/m^2 + LEN 10 mg (Cohort B, n=6)) and B 70 mg/m$^{2\ +}$ 5 mg (Cohort C, n=4), respectively. Due to hematological toxicity, the protocol amendment also included a reduction of the dose of LEN in the maintenance part; 10 mg in the first two cycles following induction (cycles 7-8), and 15 mg in cycles 9-13. All patients received corticosteroids and PCP prophylaxis after protocol amendment.

In these three cohorts (A-C) of sixteen patients, grade 3 AEs occurred in three patients during cycle 1; rash (1), pneumonia (1) and tumour lysis syndrome (1) of which the pneumonia was recorded as DLT. After cycle 2, four patients were reported with DLT; three with rash (and mucositis grade 3 in one patient) and one with sepsis grade 4. Two patients had other adverse events grade 3; one acute coronary syndrome and one infection grade 3.

At this point, the assessment was made that by excluding LEN from cycle 1 and by adding corticosteroids during the L-B-R cycles, LEN could be combined with R-B and a dose reduction of bendamustine did not affect the incidence of DLT. MTD of LEN was determined to be 10 mg, given in cycles 2-6 in combination with bendamustine 90 mg/m^2 and rituximab 375 mg/m^2. The dose of LEN during maintenance was 10 mg in cycles 7-8 followed by 15 mg in cycles 9-13.

Adverse Events

The adverse events, including those previously described in the phase I part of the study, are summarized in Table 2. In total, 29 grade 3-5 infections were reported in 21 (42%) patients. The infections occurred during the induction phase in 19 patients and during the maintenance phase in 2 patients. Opportunistic infections were diagnosed in 3 patients; one fatal PCP due to ARDS during induction and one PCP after cycle 13 as well as one case of CMV retinitis.

When comparing the incidence of adverse events (grade 3-5) in the first cohorts (92 cycles) to the subsequent cohorts of 37 patients where LEN was omitted from cycle 1 (299 cycles), all allergic reactions occurred in the first three cohorts (n=5). Furthermore, 4 of 12 (33%) patients in the first cohorts receiving LEN in cycle 1 were reported with severe cutaneous reactions compared to 5 of 37 (14%) patients in the subsequent cohorts. Regarding other adverse events, no difference could be clearly distinguished.

Nine second primary malignancies SPM) were found in eight patients (16 %) during follow-up of which seven invasive malignancies; one chronic myelomonocytic leukemia (CMML), one Hodgkin lymphoma, one renal cancer, one squamous epithelial cancer of the skin, one squamous epithelial lung cancer in a heavy smoker, one hepatocellular carcinoma and one prostate cancer. Two patients had non-invasive malignancies, one with basal cell carcinoma (BCC) and one with squamous cell carcinoma in situ and BCC.

Deaths during study

Twelve deaths have been reported; 6 due to progressive disease, 3 due to infection during induction (of which one was reported as caused by myelosuppression) and 2 due to SPM (lung cancer and CMML). One patient with progressive disease died without a report of the cause of death.

Response

Response data is shown in Table 3. After six courses of LBR, overall response rate (ORR) was 80% based on intention to treat, Seven patients were not evaluated due to the following reasons: two deaths, two patients were withdrawn from study due to toxicity, one due to consent withdrawn, one patient who did not undergo CT/BM (but was in CR based on PET, not included as CR) and one patient who had stopped treatment after 4 cycles and were evaluated as CR, recorded at the point of 1.5 months after completed therapy. At evaluation 1.5 months after completing therapy, ORR was 64%. Complete remission/Complete remission undefined (CR/CRu) was achieved in 64% (n=32) of all patients after six months of LBR and in 62% (n=31) 1.5 months after completing therapy. PET was not mandatory in the study

protocol and was only performed in a minority of patients. After induction therapy, 16 of 20 evaluable patients were in complete remission (CRR 80%) and 1.5 months after completed therapy, 7 of 8 evaluated patients were in CR (CRR 88%).

MRD

A primer for assessment of MRD could be identified in 88% (43 of 49) of the patients prior to treatment, of which 42 of 43 (97%) patients were MRD positive in bone marrow (BM) and/or peripheral blood (PB). At three months, 18 of 36 (50%) analysed patients (36% of all patients) were MRD negative in BM and at six months, 18 of 32 (56%) analysed patients (36% of all patients) were MRD negative in BM. At 1.5 months after completing therapy, molecular remission was achieved in 64% (16 of 25 pts) in BM (32% of all patients). (Table 3)

Progression-free survival and Overall survival

At a median follow-up time of 31 months (13-59), median PFS was 42 months (95% CI 31-53) and median overall survival 53 months (Figures 1a+b). A separate analysis was performed on PFS and OS in relation to MIPI risk group, or age groups (≥75 years or ≥71 years respectively) but no significant correlation could be observed. In the MIPI low risk group, all 4 patients were alive (Figure 4a+b, suppl).

Lymphocyte populations

A significant decrease in median level of all lymphocyte subpopulations could be detected after three cycles compared to baseline levels except for CD8 (Table 5 suppl). Median values of CD4 count (10^9/L) was 0.6 at baseline and 0.12 after 3 months ($p<0.001$) and remained below the lower reference limit until 13 months after completed therapy (Figure 2). Patients with any infection during treatment had significantly lower median CD4 counts at baseline (0.52 [IQR 0.34] compared to patients with no infections (0.77 [IQR 0.45] ($p=0.037$).

Discussion

Although the survival for patients with mantle cell lymphoma has improved, the disease is still considered incurable. Bendamustine in combination with rituximab has become a commonly used regimen in first line for elderly patients, on the basis of a favourable safety profile and non-inferiority when compared to anthracycline-based regimens. [7,12,18,19] Our results show that LEN can be combined with R-B in untreated patients when omitted in the first cycle and with the addition of corticosteroids in subsequent cycles. We identified the MTD of LEN as 10 mg for 14 days in a 28-days cycle in combination with standard doses of rituximab and bendamustine. This combination was associated with a high response rate as evaluated by CT, PET and MRD in evaluated patients, although when based on intention to treat, the response rates are clearly lower, as a high proportion were not evaluable and/or not being able to complete therapy..

At a median follow-up time of 31 months, the median PFS was 42 months which is longer than the reported PFS of 35 months in the R-B arm of MCL patients in the German STiL study according to the update published in 2013.[11] In this paper, data on MIPI are not reported, but the median age of the MCL patients in the German trial was similar to our patient population. Although the difference in PFS of 7 months was the pre-determined improvement that would be considered clinically significant, the two confidence intervals are overlapping, and consequently we cannot conclude that there is a true difference. The lower number of included patients than the pre-calculated sample size makes the confidence interval wider, why a comparison is even more difficult to make.

In our study, CR/CRu was achieved in 64% after the induction phase and in 62% after maintenance with LEN, which is higher than the 50% CRR in the MCL subgroup of the R-B arm in the BRIGHT trial, although the latter included PET as part of the response evaluation [18] , but inferior to the CRR of 74% achieved after 6 cycles of R-B plus bortezomib (RiBVD) in untreated patients with similar patient characteristics as in our study population, as well as to the CRR of 93-95%, observed with R-B in combination with cytarabine (R-BAC) in the subgroup of untreated MCL patients after 4-6 cycles.[20-22]

Molecular remission (MR) after combined immunochemotherapy has been defined as an independent prognostic marker for long term remission in MCL and is associated with higher PFS in younger patients.[23,24] Our data shows that 36% of evaluated patients were MRD-negative in BM after induction with LBR, suggesting that molecular remission can be achieved with this regimen. However, the MR rate in BM is lower than what has been demonstrated in elderly untreated MCL patients after R-FC/R-CHOP (67%) and with RiBVD (74%).[22,24] R-B followed by R-high dose cytarabine in young patients showed an even higher MRD negativity already after 3 courses of R-B (77%) and almost complete negativity (97%)

after R-B+R-Ara-C, although, mainly due to a different age distribution, this study population was associated with a significantly more favourable prognostic profile with 70% low-risk MIPI patients[25]. Together, these results indicates that the addition of LEN to R-B does not increase the molecular remission rate more than has been showed with established immunochemotherapy combinations including alkylating agents, nucleoside analogues and anthracyclines.

In the phase I portion of this trial, we observed an unexpected high degree of severe adverse events, of which almost half were allergic or cutaneous reactions. By omitting LEN from cycle 1 and by adding corticosteroids in cycle 2, the allergic reactions observed in the first cohorts, could be prevented and the risk of severe cutaneous reactions was diminished, although not completely eradicated.

A major concern is the high incidence of grade 3-5 infections (42%), which caused treatment discontinuation in five (10%) patients. A similar rate of infection grade 3-4 was observed in the SAKK trial combining LBR.[26] The incidence of severe infections is higher in our study than what has been reported with R-B alone as well as with other combinations such as RiBVD and R-BAC which demonstrated grade 3-4 infections in 16% and 12% of patients, respectively. [1820,22,27]

Recently, results from a trial on L-R in first line to MCL patients were published by Ruan et al. This regimen was associated with a lower number of high grade adverse events, including 13% grade 3-4 infections in combination with high response rate with a reported CRR of 61% and superior median PFS and OS - not reached at 30 months. Notably, the median age of patients in our study was higher (71 vs 65) with more high-risk MIPI patients (52% vs 32%) and less patients with low-risk score (10% vs 34%)[15].

Rash is a common side effect of both bendamustine and LEN.[11,1628] R-B was associated with higher degree of cutaneous toxicity when compared to R-CHOP or R-CVP.[12,18,29,30] Concerning frontline LEN + rituximab in MCL, Ruan et al reported grade 3-4 rash in 29% of patients, in contrast to less than 10% in relapsed/refractory NHL.[15,29,31] In line with our results, this indicates that less treated patients may be more susceptible to the immunosensitizing effect of LEN, perhaps due to a more intact immune system, and that corticosteroids may be required to prevent severe reactions.

Low CD4 counts after primary treatment with R-B has previously been described.[32] Here, we demonstrate that the L-B-R regimen induces a longstanding reduction of CD4 counts which persists not only during the maintenance phase of single LEN but up to one year after completed treatment. Together with the incidence of opportunistic infections in three patients

of which one case of PCP occurred after 13 cycles, PCP prophylaxis is warranted when combining these agents. Possibly, the addition of prednisone during the induction may have contributed to the high incidence of opportunistic infections.

During the follow-up period, SPMs were recorded in eight (16%) patients. A higher risk of developing SPM has previously been observed after treatment with LEN.[33] Studies on LEN/D in untreated MCL patients have reported SPMs in 5% of the patients and studies on L-R-CHOP in first-line have recorded SPMs around 5%.[34,35] These studies included somewhat younger patients at a median age of 56, 65 and 69 years respectively, why age-adjusted incidence would be valuable for comparison.

In summary, the NLG/MCL4 trial shows that LEN in combination with R-B is an active regimen in untreated elderly patients with MCL and molecular remission may be achieved, but associated with an unfavorable safety profile including a high infection rate as well as a notably high incidence of second primary malignancies. Despite the fact that all components are highly active in MCL, LEN may not be the optimal partner of R-B in untreated patients in favour of other combinations, including cytarabine or bortezomib. It is likely that the increased toxicity associated with LEN addition outweighs a possible benefit in efficacy. In this regard, non-chemotherapy combinations including LEN and rituximab, seem to be associated with a more favourable balance of activity and toxicity, and may also be given as a maintenance treatment following chemoimmunotherapy. Long term data on these patients as well as results from ongoing trials on chemotherapy-free combinations as well as randomized trials will bring further insight on how to improve outcome in elderly MCL patients.

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Authorship

Contribution

A Albertsson-Lindblad: collection and assembly of data, writing of manuscript draft

A Kolstad: concept and protocol design, collection and assembly of data

A Laurell: concept and protocol design; collection and assembly of data

R Räty: concept and protocol design; collection and assembly of data

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M Ehinger: collection and assembly of data

C Geisler: concept and protocol design, collection and assembly of data

M Jerkeman: concept and protocol design, collection and assembly of data

Conflicts-of-interest disclosures

A Albertsson-Lindblad: no conflicts of interest.

A Kolstad: no conflicts of interest.

A Laurell: no conflicts of interest.

R Räty: no conflicts of interest

K Grønbæk: no conflicts of interest

J Sundberg: no conflicts of interest

L B Pedesen; no conflicts of interest

E Ralfkiær: no conflicts of interest

ML Karjalainen-Lindsberg: no conflicts of interest

C Sundström: no conflicts of interest

M Ehinger: no conflicts of interest

C Geisler: no conflicts of interest

M Jerkeman:	Honoraria by Janssen-Cilag and Celgene Consulting or Advisory Role: Gilead Sciences Research funding to institution by Janssen-Cilag, Celgene and Mundipharma

Figure legends

Figure 1 a

Overall survival

Figure 1 b

Progression-free survival

Figure 2

Boxplots of CD4-count (109/L) during treatment. LLN: Lower limit of normal range.

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Table 1

Patients´ characteristics

Characteristic	**no of patients (%)**
Age median, (range)	71 (62-84)
Male/female	37/13 (73/27))
MIPI risk group	
Low	5 (10)
Intermediate	19 (38)
High	26 (52)
Extra nodal sites (number)	
0	9
1	24
2	10
3	3
4	3
missing data	2
Prior treatment (1 cycle)	4 (8%)
1 R-CHOP	2 (4%)
1 R-Bendamustine	1 (2%)
1 R-ARA-C	1 (2%)
WHO Performance status	
0	25 (50%)
1	22 (44%)
2	3 (6%)
Ann Arbor Stage	
II	2 (4%)
III	4 (8%)
IV	44 (88%)
Median Leucocyte count, (n x $10^9/mm^3$)	8.4 (1.7-135.9)

MIPI, Mantle Cell Lymphoma International Prognostic Index.

Table 2

Summary of adverse events in phase I+II, reported as number of patients, the highest grade per patient

		Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Hematological	Anemia	29	14	2	1	
	Neutropenia	4		11	27	
	Thrombocytopenia	15	8	9	1	
Non-Hematological						
	Infection	2	6	13	6	2
Cutanous	Rash	10	8	9		
Immune system disorders	Allergic reaction	1	6	6		
	Cytokine release syndrome		1			
Gastrointestinal	Abdominal pain	1				
	Abdominal distension	1				
	Constipation	3	4			
	Diarrhea		5	2		
	Hemorrhoids/rectal bleeding	4				
	Mucositis/esophagitis	2	7	3		
	Nausea/vomiting	9	4	2		
Respiratory tract	Cough	1				
	Dyspnoea	2	1			
Cardiac	Acute coronary syndrome				1	
	Arrhythmia/conduction disorder	1	4	1		
Neurological/psychiatric	Cerebral infarction				1	
	Confusion			1		
	Dizziness	3				
	Dysgeusia		1			
	Headache		3			
	Neuropathy	4		1		
	Syncope	1				
	Insomnia	1				
Muskuloskeletal	Gout		1			
	Joint effusion		1			
	Musculoskeletal pain	4	5	3		
Hepatobiliary disorders	Cholecystitis		1			
	Hepatic failure			1		
	Hypoalbuminemia	1	2	0		
	Alkaline phosphatase elevation	2	1	1		
	Aminotransferase elevation	2				
	Gamma-GT elevation	1		1		
Vascular	Flushing		1			
	Hypotension	1	1	2		
	Phlebitis		2			

	Thromboembolic event		3			
Renal and Urinary	Creatinine elevation	2				
	Hematuria	2				
	Urinary tract obstruction		1			
	Other renal and urinary symptoms	4	3	1		
General	Anorexia	4	2	3		
	Chills		4			
	Edoema	2	3	1		
	Fatigue	8	3	2		
	Fever	5	6	1		
	Weight loss	2	4		1	
	Weight gain		1			
	Hyperglycemia	1				
	Sweating	1				
	Visual disturbance	1				
	Dry eyes	1				
	Tumor lysis syndrome			2		

Table 3

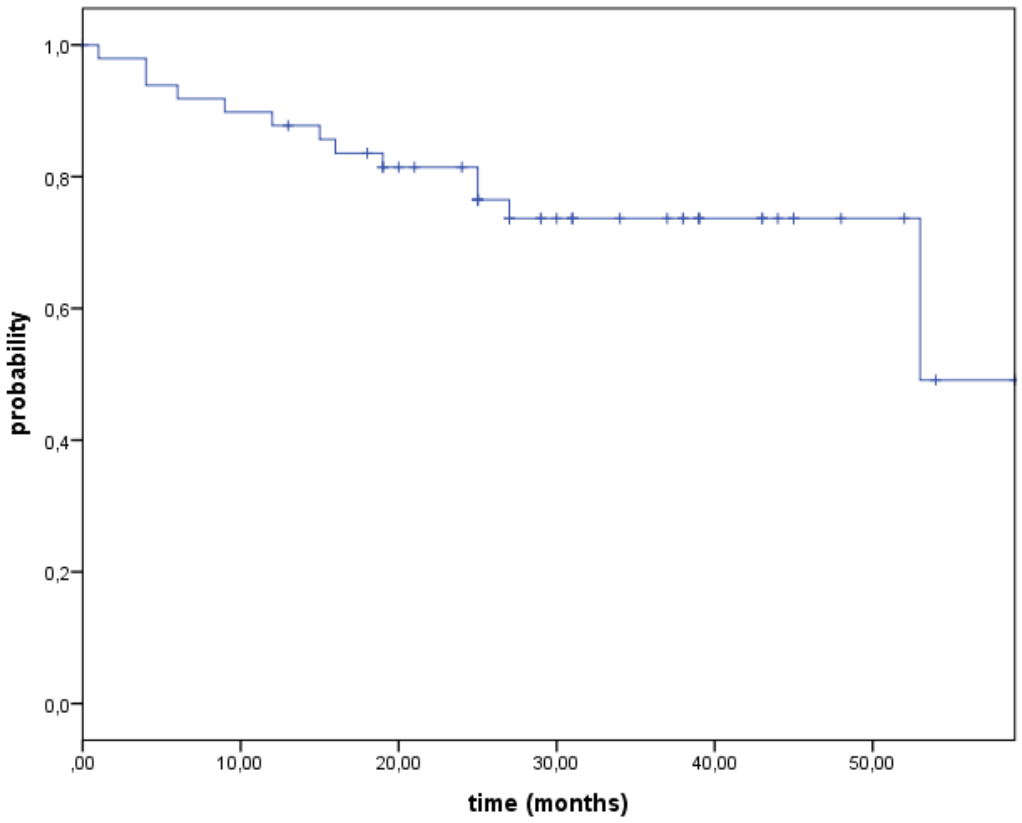
Response rates and minimal residual disease (MRD) according to CT scan and bone marrow examination

CT	**3 months**	**6 months**	**1,5 months after completed therapy**
ORR (%)	88,0	80,0	64,0
CR/CRU	24(48%)	32 (64%)	31 (62%)
PR	20	8	1
PD	1	3	8
not evaluated*	5	7	10
total	50	50	50
MRD-negativity			
	3 months	6 months	12 months
BM	18 (50%)	18 (56%)	16 (64%)
PB	23 (61%)	21 (68%)	19 (80%)
evaluated BM/PB	36/38	32/31	25/24
MRD-negativity (based on intention to treat)			
	3 months	6 months	12 months
BM	18 (36%)	18 (36%)	16 (32%)
PB	23 (46%)	21 (42%)	19 (38%)
total	50	50	50

CR – complete remission, CRu – complete remission undetermined, PR-partial remission, ORR-overall response rate, PD – progressive disease

*not evaluated: death of any cause, consent withdrawn, end of study due to other than PD ,end of treatment due to any cause and not evaluated at this time point, not done of other cause/missing data

Figure 1a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0,6
0,4
0,2
0,0
probability
,00
10,00
20,00
30,00
40,00
50,00
time (months)

Figure 1b

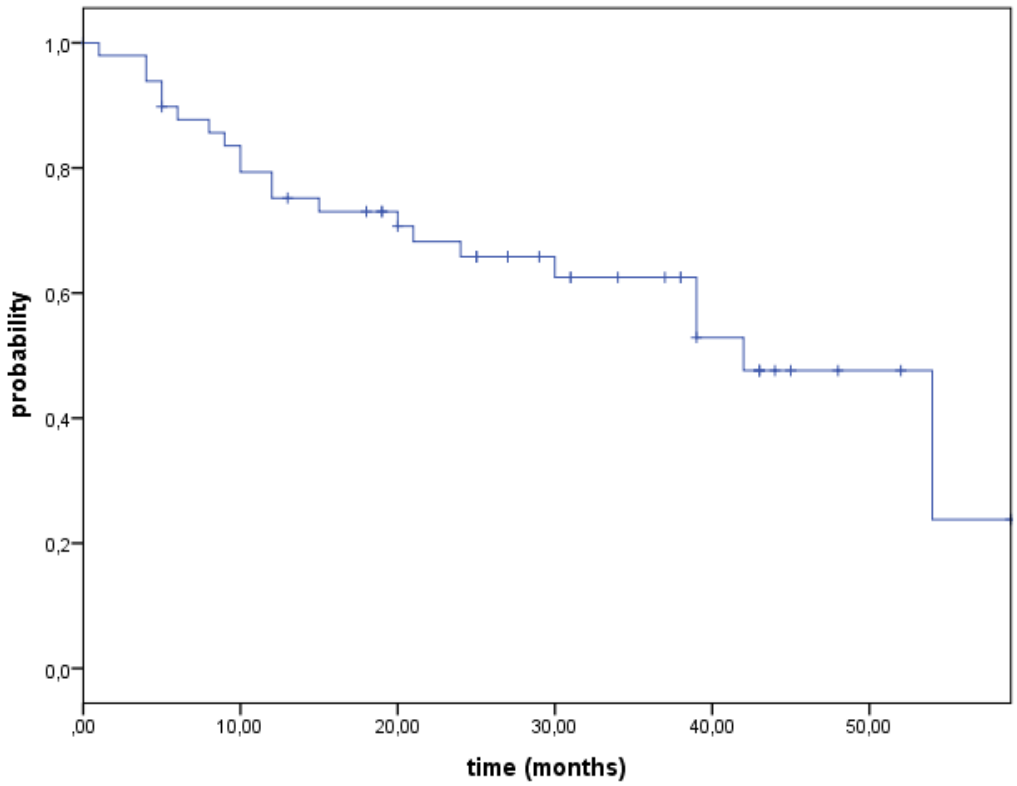
1,0
0,8
0,6
0,4
0,2
0,0
probability
,00
10,00
20,00
30,00
40,00
50,00
time (months)

Figure 2

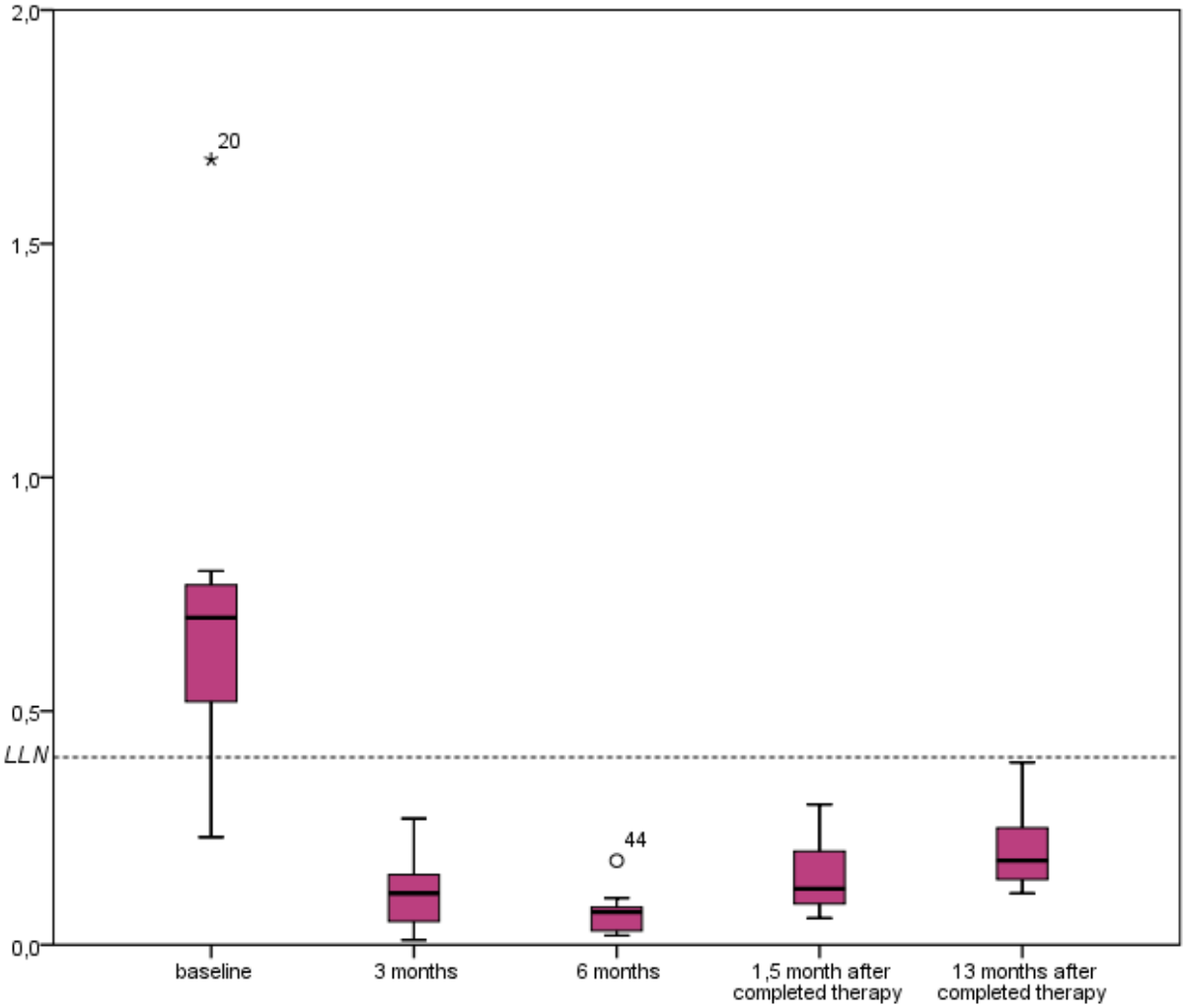
2,0
1,5
1,0
0,5
0,0
LLN
20
44
baseline
3 months
6 months
1,5 month after completed therapy
13 months after completed therapy

Figure 3 (supplement): CONSORT diagram for patients in phase 1+2

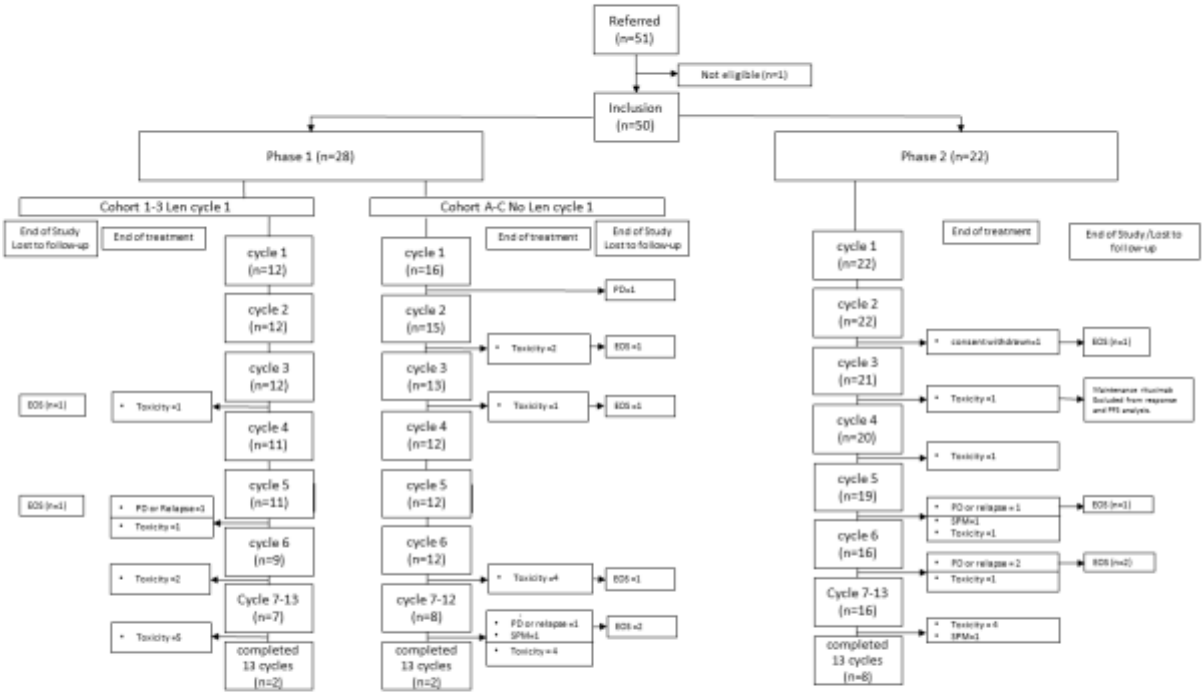

Table 4 (supplement)

Cohort 1-3 and A-C in phase I. Adverse events and Dose-limiting toxicity

AE: adverse event, DLT (Dose-Limiting Toxicity): Any non-hematological grade 3-5 adverse event with relation to treatment according to the Data Monitoring Committee.

		Cycle 1				Cycle 2			
Cohort	**UPN**	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT
1	**1**	5	90			5	90	0	
1	**2**	5	90	Cerebral infarction (4)	no DLT	5	90	Infection (3)	No DLT
1	**3**	5	90			5	90	Allergic reaction (3)*	No DLT
Cohort	**UPN**	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	
2	**4**	10	90			10	90	0	
2	**5**	10	90	Infection(3) + Allergic reaction (3)	no DLT	10	90	0	
2	**6**	10	90	Infection(3)+ Rash (3)	no DLT	10	90	0	
Cohort	**UPN**	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	
3	**7**	15	90	Urticaria(3)		10	90	0	
3	**8**	15	90			15	90	0	
3	**9**	15	90	Sensory neuropathy (3) + Hypotension (3)	DLT	0	90	0	
3	**10**	15		Allergic reaction (3) + Hypotension (3)	DLT	0		0	
3	**11**	15				15		fever (3)	no DLT
3	**12**	15				15		15	

		Cycle 1				Cycle 2			
Cohort	**UPN**	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT	Len (mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT
A	**13**	0	90	0		10	90		
A	**14**	0	90	0		10	90		
A	**15**	0	90	0		10	90		
A	**16**	0	90	0		10	90		
A	**17**	0	90	rash with pruritus (3)†		10	90		
A	**18**	0	90	0		10	90		
Cohort	**UPN**	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT	Len (mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT
B	**19**	0	70	tumor lysis syndrome (3)		10	70	Acute coronary syndrome + atrial fibrillation (4)	no DLT
B	**20**	0	70			10	70	rash with pruritus (3)	DLT
B	**21**	0	70			10	70		
B	**22**	0	70			10	70		
B	**23**	0	70			10	70	rash (3)	DLT
B	**24**	0	70			10	70	rash(3)+ mucositis (3)	DLT
Cohort	**UPN**	Len(mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT	Len (mg)	Bendamustine (mg/m^2)	Grade 3-5 AE (grade)	DLT
C	**25**	0	70			5	70	infection (3)	no DLT
C	**26**	0	70			5	70		
C	**27**	0	70	infection (3)		5	70	infection (4)	DLT
C	**28**	0	70	PD (End of study)		0	0		

*Assessed related to rituximab.
†remaining in cycle 2

Figure 4a (supplement): Overall survival according to MIPI risk group

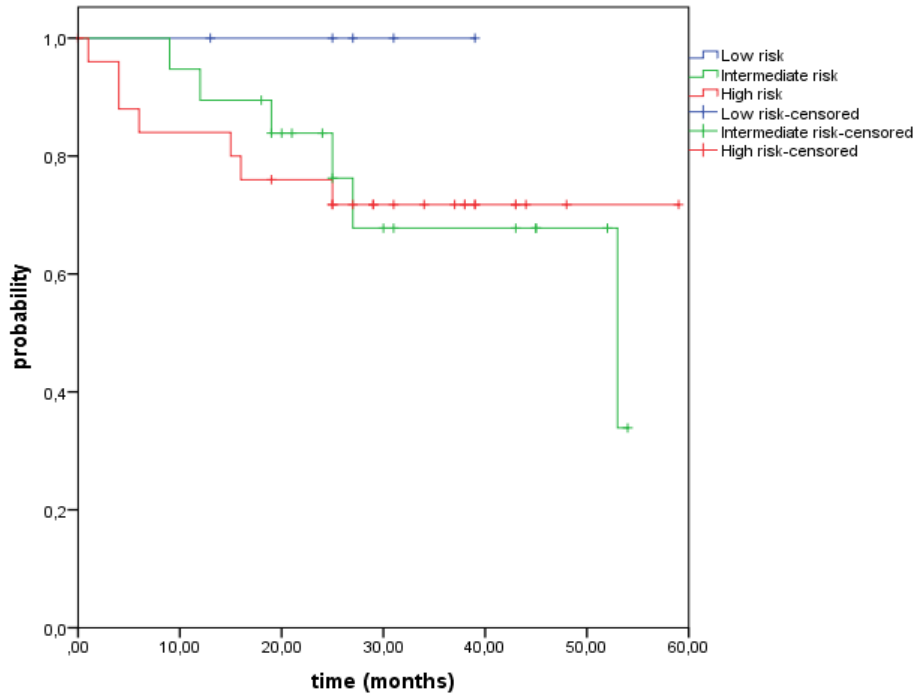

Figure 4b (supplement): Progression-free survival according to MIPI risk group

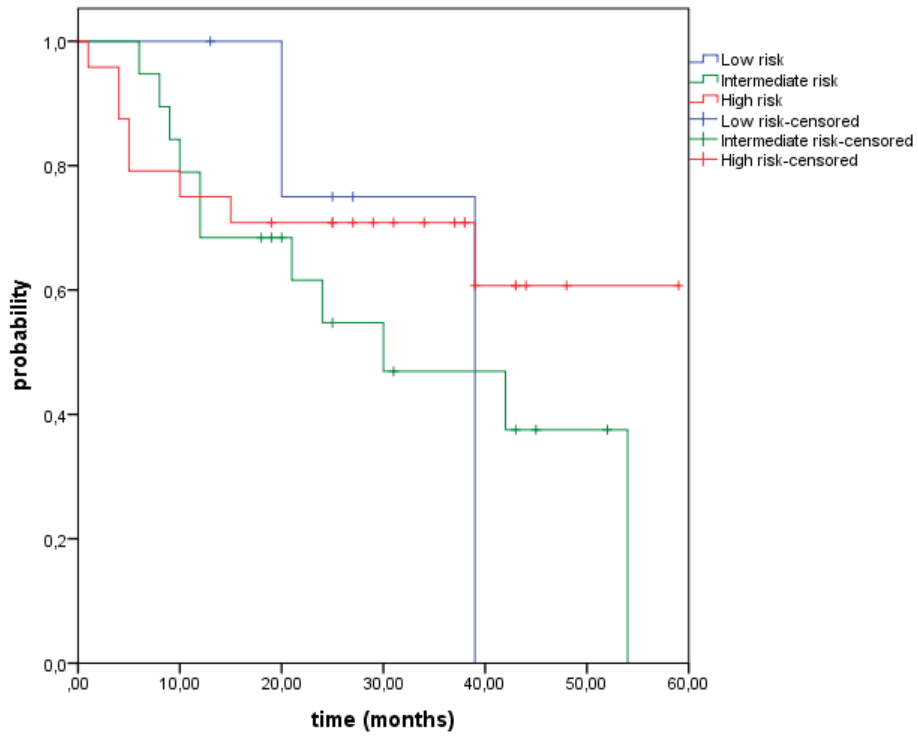

Table 5 (supplement)

Median levels (IQR) of lymphocyte subpopulations and immunoglobulins

CD: cluster of differentiation, IQR: interquartile range.

Reference values: CD counts (10^9/L): CD3: 0,55-2,0 (58 – 82%); CD3+/CD4: 0,37-1,45 (32 – 59%); CD3+/CD8+:0,12-1,07 (12 – 44%); CD19: 0,06-0,52 (5,9 – 21%); CD16+CD56: 0,02-0,55 (2,4 – 22%); CD4/CD8 ratio: 0,84-3,8; Immunoglobulins (g/L) IgG: 6,7 - 14,5; IgA: 0,88 - 4,5; IgM: 0,27 – 2,10

CD counts (10^9/L)	Baseline	3 months	6 months	1.5 months after completed therapy	13 months after completed therapy
CD3+	1.14 (0.76)	0.43 (0.71)	0.37 (0.41)	0.42 (0.56)	0.51 (0.64)
CD3+/CD4+	0.60 (0.34)	0.12 (0.09)	0.08 (0.06)	0.11 (0.07)	0.17 (0.14)
CD3+/CD8 +	0.54 (0.63)	0.31 (0.90)	0.26 (0.36)	0.26 (0.44)	0.35 (0.25)
CD19+	0.58 (4.09)	0.00 (0.00)	0.00 (0.00)	0.04 (0.07)	0.19 (0.22)
CD16+/CD56+	0.26 (0.39)	0.11 (0.14)	0.10 (0.11)	0.15 (0.12)	0.23 (0.21)
CD4/CD8 ratio	1.08 (1.05)	0.30 (0.43)	0.34 (0.53)	0.40 (0.49)	0.60 (0.52)
Immunoglobulins (g/L)					
IgG	11.2 (5.60)	7.80 (5.98)	8.30 (5.70)	8.06 (5.00)	9.60 (6.00)
IgA	2.08 (2.40)	0.98 (1.38)	0.90 (1.20)	1.00 (1.44)	1.58 (1.20)
IgM	0.85 (1.20)	0.29 (0.39)	0.28 (0.24)	0.40 (0.33)	0.60 (0.37)