Efficacy of bendamustine in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase I/II study of the German CLL Study Group

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Background and Objectives. Although bendamustine has been used for more than 30 years in the treatment of lymphoma, little is known about the optimal dosing schedule in relapsed or refractory B-cell chronic lymphocytic leukemia (CLL). Various dose and treatment schedules have been used empirically, and several phase II studies have shown impressive efficacy. To determine the maximal tolerated dose, dose-limiting toxicity and the optimal therapeutic dose of bendamustine for further phase III clinical trials the GCLLSG designed a phase I/II study for pre-treated CLL patients.

Design and Methods. Sixteen patients (median age 67 years) with relapsed or refractory CLL were enrolled. All patients had been pre-treated with a median of three different regimens. Bendamustine was given at a starting dose of 100 mg/m^2 on day 1 and 2, repeated every 3-4 weeks.

Results. Major toxicities were leukocytopenia (CTC grade 3+4) in 8/16 and infections (CTC grade 3+4) in 7/16 patients. Six patients had dose-limiting toxicity which led to dose de-escalation from 100 to 70 mg/m^2 in three partients. The maximum tolerated dose was 70 mg/m^2. According to NCI-WG criteria, 9/16 patients (56%) responded to therapy, seven to doses ≤80 mg/m^2. Two patients achieved complete remission. Five patients had a partial response and two patients stable disease. The median duration of response was 42.7 months. After a follow-up period of 53.2 months, five patients (31%) were still in remission. The median overall survival time for all patients was 45.6 months.

Interpretations and Conclusions. The study confirms the excellent efficacy of bendamustine in heavily pre-treated and treatment-refractory patients, even at reduced doses of 140 mg/m^2 per course. In pre-treated CLL patients, impaired bone marrow function is likely to enhance the myelotoxic side effects of bendamustine. Based on these results, the recommended optimal therapeutic dose of bendamustine in refractory CLL is 70 mg/m^2 on days 1 and 2 every 4 weeks.

Key words: fludarabine or chlorambucil-refractory B-CLL, bendamustine, phase I/II clinical trial.

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Despite the availability of new agents such as purine analogs in combination with alkylating agents[1] or monoclonal antibodies,[2,3] the treatment of symptomatic B-cell chronic lymphocytic leukemia (B-CLL) is generally palliative and does not have a curative potential.[4] The median survival time for patients with treatment-requiring B-CLL ranges from 18 months to 6 years, depending on the clinical stage.[5] Therefore, identification of new therapies remains a high priority. Bendamustine is an alternative agent, with both alkylating and purine-like qualities, which is able to induce a high number of remissions in CLL.[6] Bendamustine seems to have a low cross-resistance with alkylating substances and fludarabine. This has elicited recent interest in this drug in the treatment of CLL, either as monotherapy or in combination with other effective drugs.[7,8] A current phase I/II trial determining the efficacy of bendamustine in combination with mitoxantrone showed an overall response rate of 86% with tolerable toxicity.[9] Indeed, the marked efficacy of bendamustine in extensively pre-treated CLL patients has made this drug an attractive second-line or salvage treatment option, but the optimal therapeutic dose remains to be determined.

Doses of bendamustine of 100 mg/m^2 up to 120 mg/m^2 on two days every 3-4 weeks have been used in advanced CLL and non-Hodgkin's lymphoma.[10,11] Even higher doses (up to 160 mg/m^2 on days 1 and 8 every 4 weeks) were well tolerated in patients with solid tumors.[12,13] So far, no systematic phase I dose-finding study has been performed in CLL. Moreover, little information is available on bendamustine in heavily pre-treated CLL patients with a poor prognosis, who are resistant to chlorambucil and/or fludarabine. This phase I/II study was designed to determine the dose-limiting toxicity, the

maximum tolerated dose and the optimal therapeutic dose of bendamustine in pre-treated and treatment-refractory patients with CLL.

Design and Methods

Patients and inclusion criteria

This clinical trial was approved by the ethics committee of the medical faculty of the Ludwig-Maximilians University of Munich, and all patients signed an informed-consent form to their participation according to the Declaration of Helsinki.

The diagnosis of CLL was based on the National Cancer Institute-Sponsored Working Group (NCI-WG) guidelines.[14] The stage of the disease was assessed according to the Binet staging system.[15] Patients were eligible for enrollment if they were in Binet stage C or stage B with active disease as defined by the NCI-WG.[14] Patients were required to have received at least one prior therapy that included chlorambucil or fludarabine. Patients had to be refractory to the prior therapy (relapse within a period < 6 months after last therapy) or have progressive or relapsed disease. Other eligibility criteria included age older than 18 years, life expectancy of more than 3 months and an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2. Patients were excluded if they had severe organ dysfunction, a second malignancy or autoimmune cytopenias responsive to corticosteroid therapy. Patients had to have fully recovered from their last therapies.

Dose escalation schedule

The primary endpoint of this trial was to define the maximum tolerated dose of bendamustine. Given that several previous phase II studies with bendamustine had shown a good efficacy and tolerable toxicity using schedules with 100 mg/m^2 and 120 mg/m^2 on two days every 3-4 weeks,[6,10] the starting dose in this study was 100 mg/m^2 on days 1 and 2 every 3-4 weeks. If no dose-limiting toxicity occurred in the first three patients after the first treatment course, a dose escalation of 10 mg/m^2/day was planned for the next cohort. Dose-limiting toxicities were defined as non-hematologic toxicities of National Cancer Institute Common Toxicity Criteria (CTC) grade 3 or greater, or hematologic toxicity of CTC grade 4, which were experienced only during the first treatment cycle and which were not caused by bone marrow insufficiency due to CLL. Patients experiencing a dose-limiting toxicity were allowed to remain in the study provided the toxicity reversed completely. The dose level could not be changed within the same patient. It was planned that three patients would receive each dose level. If a dose-limiting toxicity occurred in the first three patients at a given dose level an additional three patients were accrued at that dose level. A second dose-limiting toxicity terminated accrual at that level and the dose had to be decreased by 10 mg/m^2/day for the next cohort. The dose level at which one or no dose-limiting toxicity was recorded was defined as maximum tolerated dose. Patients experiencing febrile leukocytopenia or thrombocytopenia of CTC grade 4 or bleeding were allowed to remain in the study provided the toxicity reversed completely. These patients had a 50% dose reduction. Patients experiencing grade 3 non-hematologic toxicity could remain in the study and continue with a 50% dose reduction after the toxicity had reversed completely. Patients experiencing grade 4 non-hematologic toxicity had to be withdrawn from study. If no toxicity occurred, the next course could start after a minimum of 3 weeks, if the leukocyte counts had recovered to more than 2.5×10^9/L and the platelet count had recovered to more than 100×10^9/L. If these values were not reached, the cell counts were controlled weekly. If no recovery had occurred by 6 weeks after the last dose of bendamustine, treatment had to be stopped.

The duration of treatment depended on the response. Those patients in whom disease progressed during the treatment were withdrawn from the study. Patients with partial remission or stable disease received up to a maximum of six courses. Patients who were in complete remission after six courses were planned to receive two additional courses, if no severe toxicity was observed.

Toxicity assessment and adverse events

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria, version 2.0 (NCI, Bethesda, MD, USA). Any adverse event or serious adverse event was recorded on the appropriate CRF page, specifying the dose of the study drug, date of occurrence, duration, frequency, treatment, changes in existing adverse events or severe adverse events and outcome.

Pre-therapeutic assessment and response assessment

Patients were screened for eligibility within 8 to 14 days before the initial administration of the study medication. Baseline data recording included the standard assessments established by the NCI-WG.[14]

Within 8 days prior to study entry, after the third course of bendamustine, and at the end of the last course a complete blood cell count including a differential count were performed. A Coombs' test was performed at baseline. Biochemistry and urine samples were analyzed at the same time points.

Physical examinations were performed after each of the courses. Additional examinations in case of hematologic complete remission after any course (lympho-

Table 1. Patients' characteristics.

	Binet stage	Duration of disease (years)	Age	Sex	Unfavorable cytogenetics	No. of pretreatments	Resistant to chlorambucil	Duration of response	Resistant to fludarabine	Duration of response
#101	**C**	10	**83**	m	*Trisomy 12*	2	**yes**		N/A	
#102	B	10	**69**	m	Trisomy 12	**4**	no	17 months	no	45 months
#103	B	5	59	m	*11q-deletion*	**5**	**yes**		**yes**	
#104	**C**	8	**70**	m	none	**4**	**yes**		no	7 months
#105	**C**	0.5	**67**	f	*Trisomy 12*	1	**yes**		N/A	
#201	**C**	4	**65**	m	not done	**6**	**yes**		N/A	
#202	**C**	6	**72**	m	none	**4**	**yes**		**yes**	
#301	B	7	**76**	f	not done	2	no	36 months	N/A	
#302	**C**	3	**67**	m	not done	2	**yes**		N/A	
#303	**C**	4	57	m	not done	**3**	N/A		**yes**	
#304	B	7	64	f	not done	2	**yes**		N/A	
#401	**C**	11	60	m	not done	1	**yes**		N/A	
#402	**C**	5	64	m	not done	**3**	**yes**		no	15 months
#403	B	10	**65**	f	not done	**3**	**yes**		N/A	
#404	B	3	**71**	m	not done	**4**	**yes**		no	10 months
#601	**C**	5	**65**	m	not done	**5**	**yes**		**yes**	

All patients presented with relapsed disease and 15 out of 16 patients were refractory to chlorambucil or fludarabine or both agents. All poor prognosis parameters (Binet stage C, age 65+ years, unfavorable cytogenetics, more than three prior treatments, refractoriness to chlorambucil or fludarabine) are marked. N/A: not applicable as patient did not receive respective pretreatment.

cytes less than 4×10^9/L, neutrophils more than 1.5×10^9/L, platelets more than 100×10^9/L, hemoglobin (untransfused) more than 11.0 g/dL) included abdominal ultrasound (if enlarged lymph nodes present at baseline), bone marrow aspiration with analysis of the immune phenotype, bone marrow histology, and immunoglobulin assay.

Follow-up assessment

Patients who reached complete or partial remission or who had stable disease were followed at 3-month intervals after the last study visit until disease progression or death. After disease progression patients were followed until retreatment or death.

The primary end-points of the study were dose-limiting toxicity and the maximum tolerated dose. The response (complete or partial remission or stable disease) to treatment with bendamustine was the secondary end-point. The duration of response was another secondary end-point. The duration of complete remission was defined as time from achieving complete remission as the best response until progressive disease. The duration of partial remission and stable disease was defined as the time from the first day of treatment to progressive disease. Overall survival was measured from the time of entry into the clinical trial. Patients who received less than three courses of treatment were not evaluable for response as defined in the protocol.

Statistical methods

The time from start of therapy to treatment failure (progression of disease) or death and from date of best response to treatment failure or death was estimated according to the method of Kaplan and Meier.[16] The 95% confidence intervals (CI) were calculated with Greenwood's variances.[17] The analysis was performed with SPSS V12.0 statistical software.

Results

Patients' characteristics

The analysis reported here is based on data collected up to May 2005. A total of 16 patients (median age 67 years, range 57 to 83 years; 62,5% 65 + years; 12 male, 4 female) with relapsed or progressive, fludarabine- and/or chlorambucil-refractory B-CLL were enrolled in this open-label phase I/II study over a period of one year (October 30, 2000 to November 15, 2001).

At study entry ten patients were classified as having Binet stage C disease and six patients as having stage B. The median duration of disease was more than 6 years (range 0.5 to 11 years). Unfavorable cytogenetics (del (11)(q23), trisomy 12) were found in four patients, two patients had a normal karyotype, and in the other patients cytogenetic status was not analyzed. Patients had been pre-treated with a median of three treatments, usually chlorambucil, fludarabine and corticosteroids. Thirteen patients were resistant to chlorambucil, four patients to fludarabine, and three patients to both agents (Table 1). All patients had pre-existing concomitant diseases: the clinically significant diseases which caused notable side effects or even dose-limiting toxicity are summarized in Table 2. The ECOG performance status prior to enrollment was 0 (13 patients), 1 (2 patients), and 2 (1 patient).

Table 2. Significant concomitant diseases leading to severe toxicities, which were considered as dose-limiting toixicities.

No. pat.	*Dose level (mg/m²)*	*Significant concomitant diseases (ongoing/active at baseline)*	*Toxicities (all cycles) related to pre-existing concomitant diseases*	*DLT (after the first cycle)*
101	100	chronic renal impairment gr 3 hyperuricemia gr 1	renal impairment gr 4 hyperuricemia gr 4	hyperuricemia
102	80	hypacusis	severe worsening of hypacusis	
103	80	Sinusitis cough	pneumonia gr 4 diarrhea (due to antibiotics) gr 2	pneumonia
105	70	hypertension, atrial fibrillation gr 3	cardiac edema gr 1	
201	100	cryoglobulinemia	sensory (paresthesia) gr 1	
301	100	arrhythmia gr 3		leukocytopenia
302	90	thrombocytopenia gr 4	allergy (after platelet transfusion) gr 2 infection left eye gr 3	infection
304	80	hypertension gr 3	hypotension gr 4, dyspnea gr 4	
402	70	prostate hyperplasia, renal cyst	urinary tract infection gr 2	
403	70	granulocytopenia gr 4	infection (pneumonia and perianal abscess) gr 3 anemia gr4	anemia
601	80	severe antibody deficiency syndrome thrombocytopenia gr 3 granulocytopenia gr 4	sepsis resulting in renal failure gr4 elevated liver enzymes gr3 thrombocytopenia gr 4 leukocytopenia gr 4	sepsis elevated liver enzymes thrombocytopenia leukocytopenia

A dose-limiting toxicity is defined as any non-hematologic toxicity of CTC grade 3 or greater, or a hematologic toxicity CTC grade 4, which was experienced during the first treatment cycle and was not caused by CLL.

Toxicity of bendamustine requiring dose reduction

All observed toxicities per number of cycles at the different dose levels are summarized in Tables 3 and 4.

First dose level 100mg/m²: the first cohort of three patients was treated with the starting dose of 100 mg/m² bendamustine intravenously over 30 minutes on days 1 and 2: patient 201 received three courses, patient 101 received two courses and patient 301 received one course of treatment. Patient 201 had no dose-limiting toxicity after the first course. The treatment was discontinued after the third course because of severe pneumonia, CTC grade 3, which resolved after treatment with intravenous antibiotics. The two other patients (101, 301) treated at this dose level experienced dose-limiting toxic effects after the first course of treatment. Patient 101 had pre-existing renal dysfunction (CTC grade 3) and hyperuricemia (CTC grade 1). After the first course of bendamustine, the hyperuricemia worsened to CTC grade 4 due to massive tumor lysis. This was considered a dose-limiting toxicity. After the second treatment course, treatment was stopped due to severe hematologic and non-hematologic toxicities (leukocytopenia CTC grade 4, local cutaneous infection, erysipelas). The patient died 6 months later from multiorgan failure in consequence of septicemia following an elective surgical intervention.

Table 3. Toxicities of CTC grade 3 or 4 per number of cycles at the different dose levels.

CTC grade 3 and 4 toxic effects	*100 mg/m²*	*90 mg/m²*	*80 mg/m²*	*70 mg/m²*	*Total*
Number of cycles	6	6	9	32	53
Number of patients	3	1	5	7	16
Leukocytopenia	3	1	3	1	8
Thrombocytopenia	1		2	1	4
Anemia	1		2	3	6
Elevated liver enzymes			1	1	2
Renal impairment	1		1		2
Infection	2	1	2	2	7
Diarrhea			1		1
Skin effects	1		1		2
Hypotension			1		1
Edema	1				1
Dyspnea			2		2
Body temperature			1		1
Dehydration			1		1
Total	10	2	18	8	38

Patient 301 developed severe leukocytopenia (CTC grade 4) after the first course of treatment. This dose-limiting toxicity persisted for more than six weeks; therefore, the patient was withdrawn from the study.

Second dose level 90 mg/m²: after two dose-limiting

Table 4. Toxicities of CTC grade 1 or 2 per number of cycles at the different dose levels.

CTC grade 1 and 2 toxic effects	*100 mg/m²*	*90 mg/m²*	*80 mg/m²*	*70 mg/m²*	*Total*
Number of cycles	6	6	9	32	53
Number of patients	3	1	5	7	16
Hemorrhage			1	1	2
Renal impairment			1	1	2
Infection			2	2	4
Nausea	1				1
Vomiting				1	1
Taste			1		1
Diarrhea			1		1
Skin effects				1	1
Weight loss				1	1
Allergic reaction		1			1
Stomatitis	1				1
Body temperature		1	2		3
Cough			2	2	4
Sensory system	1				1
Consciousness			1		1
Sweating		1			1
Total	3	3	11	9	26

toxicities in the first cohort the dose was reduced to 90 mg/m² according to the protocol. Patient 302 in the second cohort had finished his previous chemotherapy only 10 days before inclusion in the study, but required immediate treatment because of disease progression as manifested by severe anemia and thrombocytopenia due to bone marrow infiltration. This patient experienced a CTC grade 3 infection after the first course of bendamustine. This was considered a dose-limiting toxicity and further courses were given with a 50% dose reduction, which were well tolerated. In total, this patient received six courses of bendamustine.

Third dose level 80 mg/m²: due to the severe toxicities seen in the first patients treated at 90 mg/m² and 100 mg/m² the protocol committee decided to open the next dose level of 80 mg/m² prematurely. At this dose level, two (103, 601) of five patients experienced a dose-limiting toxicity. Patient 102 received four courses of bendamustine without any complication. Patient 304 received two courses of bendamustine. After the second course this patient developed CTC grade 3 leukocytopenia, anemia and thrombocytopenia, as well as diarrhea causing CTC grade 4 dehydration followed by acute renal failure. Further treatment was stopped and the patient was withdrawn from the study. Patient 303 presented with rapidly progressing, generalized lymphadenopathy. After the first course of bendamustine, he still showed evidence of progression and received another salvage therapy three weeks after bendamustine. In this patient CLL could not be controlled with any of the subsequent therapies. The patient died within three months after the bendamustine treatment because of septicemia.

Patient 103 treated at the 80 mg/m² dose level developed atypical pneumonia of CTC grade 3 during the first treatment course as a dose-limiting toxicity, when leukocytes began to decrease. Thereafter, bendamustine was discontinued. The leukocytopenia did not improve in the meantime. The pneumonia was reversible but recurred 2 weeks later and, being refractory to anti-bacterial, anti-fungal and anti-viral treatment, resulted in the patient's death. Unfortunately the patient did not undergo a post-mortem examination. Therefore, the possibility of extranodal pulmonary CLL could not be ruled out.

Patient 601 treated in this cohort had impaired bone marrow function with thrombocytopenia and leukocytopenia prior to treatment. After the first course of bendamustine, he suffered from prolonged thrombocytopenia and leukocytopenia CTC grade 4 over weeks, both were irreversible until the death of the patient. As a further complication after the first course the patient acquired a viral pneumonia (*Herpes simplex*), followed by acute renal failure and septicemia. The patient died four weeks after the start of chemotherapy because of a septic multiorgan failure.

Fourth dose level 70 mg/m²: after two dose-limiting toxicities in the cohort treated with 80 mg/m² bendamustine, the daily dose of bendamustine had again to be lowered by 10 mg/m² to 70 mg/m²/day according to the protocol.

One dose-limiting toxicity was observed in this cohort (403). Five of seven patients treated at the 70 mg/m² dose level experienced no major toxicity. Four of seven patients (104, 105, 202, 404) received six treatment courses, while the other patients received four courses (401) (decision of physician, due to good response), three courses (402) (decision of physician, due to good response) and one course (403) (due to toxicity). Two patients experienced major side effects. Patient 403 had granulocytopenia CTC grade 4 at baseline. This patient developed an infection (pneumonia, CTC grade 3) and a perianal abscess (CTC grade 3) as dose-limiting toxicities after the first course of bendamustine. Additionally, CTC grade 4 anemia was observed. Hematologic and non-hematologic side effects occurred three weeks after the first administration of bendamustine resulting in interruption of the treatment. After recovery, the patient was retreated with bendamustine off study and achieved a complete remission. Patient 105 started the study treatment with severe CTC grade 3 leukocytopenia and CTC grade 2 thrombocytopenia due to bone marrow infiltration. Two weeks after administration of bendamustine, the leukocytes and platelets decreased to CTC grade 4 and grade 3, respectively. The decrease of the leukocytes was not to be considered as dose-limiting toxicity, since a decrease in the leukocyte count was a desired therapeutic aim. Despite those difficulties after the first

Table 5. Ten patients (nine of whom received ≥3 courses of therapy, one patient had progressive disease after one course) were evaluated for response, duration of response, time to re-treatment and overall survival.

Patient No.	Dose level mg/m²	DLT	No. of cycles	Best response	Duration of response (months)	Time to re-treatment (months)	Overall survival (months)	Death	Current stage
102	80	no	4	CR	41.0	not reached	49.5 +	no	PD
402	70	no	3	CR	41.0	not reached	43.6 +	no	PD
201	100	no	3	PR	42.7 +	not reached	42.8 +	no	PR
401	70	no	4	PR	43.6 +	not reached	43.9	no	PR
404	70	no	6	PR	26.6	40.0	42.0 +	no	PR
202	70	no	6	SD	7.0	8.0	46.1+	no	SD
105	70	no	6	nPR	39.9	not reached	40.0	January 2005 Secondary malignancy	
104	70	no	6	PR	8.8	10.8	33.4	February 2004 progressive CLL	–
302	90	yes	6	SD	8.8	8.8	45.6	December 2004 progressive CLL	
303	80	no	1	PD	–	1.2	1.2	March 2001 progressive CLL	–

DLT: dose-limiting toxicity; CR: complete remission; PR: partial remission; nPR: nodular partial remission; SD: stable disease; PD: progressive disease.

Table 6. Six patients were withdrawn from study because of toxicity. They could not complete three courses of therapy and were not evaluable for response.

Patient No.	Dose level mg/m²	DLT	No. of cycles	Response after withdrawal	Duration of response	Time to re-treatment	Overall survival	Death	Current stage
301	100	yes	1	CR	50.0	not reached	53.2 +	no	CR
403	70	yes	1	PR	16.7	18.0	43.2 +	no	PR
101	100	yes	2	PR	8.0	not reached	8.1	July 2001 multiorgan failure	–
304	80	no	2	SD	15.7	15.7	22.8	December 2002 progressive CLL	–
601	80	yes	1	PD	–	–	1.3	May 2001 multiorgan failure	–
103	80	yes	1	PD	–	–	2.3	June 2001 Infection/CLL	–

DLT: dose-limiting toxicity; CR: complete remission; PR: partial remission; nPR: nodular partial remission; SD: stable disease; PD: progressive disease.

course, the patient completed six courses of bendamustine. Five more patients were treated on this dose level without complications. Patient 104, 202 and 404 completed six treatment cycles, patient 401 completed four cycles and patient 402 completed three cycles. All of them responded to therapy (Table 5).

Treatment outcome

Only patients who received three or more treatment cycles were evaluable for response according to the protocol. The duration of complete remission was defined as time from complete response, as best response, to progressive disease. The duration of partial remission and stable disease was defined as time from the first day of treatment to progressive disease. Follow-up data were collected until May 2005 (53.2 months) and are summarized in Tables 5 and 6.

100 mg/m² dose level: patient 201, who was resistant to chlorambucil, received three treatment courses and achieved a partial remission that is ongoing for 42.7

months.

90 mg/m² dose level: patient 302, who was resistant to chlorambucil, completed study treatment after one course with a 100% dose followed by five cycles at a dose reduced by 50%. This patient's spleen reduced to normal size and the platelet count rose from 24×10⁹/L to 77×10⁹/L. This patient achieved stable disease according to the NCI-WG guidelines. After 8.8 months of stable disease the CLL became progressive and the patient was retreated. The patient died after 45.6 months from progessive disease.

80 mg/m² dose level: patient 102 entered the study after four other treatments including fludarabine and COP (cyclophosphamide, vincristine, and prednisolone). This patient withdrew from the study after completion of four treatment courses by his own wish. The patient achieved a complete remission as best response and remained in hematologic complete remission for 41.0 months with a slight increase of lymph nodes until March 2005. Up to May 2005 retreatment was not necessary.

70 mg/m² dose level: patient 202, who was resistant to fludarabine and chlorambucil, achieved stable disease after six courses of bendamustine for 7.0 months. After 8.0 months he had a successful splenectomy to resolve persisting thrombocytopenia caused by hypersplenism. No further treatment was necessary up to March 2005. Patient 104, resistant to chlorambucil and pre-treated with fludarabine and cyclophosphamide, required study treatment only 20 days after his previous treatment with CHOP. He achieved a partial remission after three courses of bendamustine. Treatment was continued up to six courses. The patient relapsed after 8.8 months but was successfully retreated with bendamustine off study. Patient 404, resistant to chlorambucil and pre-treated with fludarabine, also received six cycles of treatment and achieved a partial remission that lasted for 26.6 months. Only after 40.0 months did he have to be retreated because of progressive disease.

Patient 401, resistant to chlorambucil, achieved a very good partial remission after four courses and treatment was discontinued. During follow-up the partial remission improved to a complete remission. The patient has now been in complete remission for 43.6 months. Patient 402, resistant to chlorambucil and pretreated with fludarabine, achieved a complete response after three courses, after which treatment was stopped. The response lasted 41.0 months. Patient 105 achieved a nodular partial remission after six cycles of bendamustine; the remission remained stable for 39.9 months, then the patient developed a secondary malignancy and died in January 2005.

Discussion

This is the first phase I/II study designed to determine the maximum tolerated dose and dose-limiting toxicities of bendamustine in relapsed, progressive or treatment-refractory CLL. Many different dose regimens have gained popularity and have been used empirically, ranging from daily or weekly low dose applications to monthly doses of 200 mg/m² on two days. However, no single schedule has been formally established or evaluated by a phase I trial so far. Therefore, the GCLLSG conducted a phase I/II trial, which resulted in an unexpectedly high rate of toxicities occurring after the first course at the initial doses of 100 mg/m² to 80 mg/m² on days 1 and 2. Given these dose-limiting toxicities, above all the myelotoxicity, the dose was decreased three times in 10 mg/m² steps to 70 mg/m², which is the recommended dose determined by this study. The maximum tolerated dose of bendamustine in refractory CLL is 70 mg/m² on days 1 and 2. All toxicities per number of cycles at the different dose levels are summarized in Tables 3 and 4.

The efficacy of bendamustine was investigated as a secondary end-point of the protocol. Interestingly, the study indicated that bendamustine was a very effective compound, at a relatively low dose, even in this heavily pre-treated population. The majority of patients were resistant to chlorambucil and/or fludarabine (14 out of 16 patients). Six patients who had to be withdrawn from treatment due to toxicity before completing three courses were not evaluable for response. Of the remaining ten patients, nine (56%) responded to therapy (seven of them to 80 mg/m² or less): two (12.5%) with a complete response, one (6%) with a nodal partial remission, four (25%) with a partial response and two (12.5%) with stable disease. One patient had progressive disease. Early death within 3 months occurred in three (19%) patients and life-threatening infections in two (12.5%) patients. The median duration of response in the patients evaluable for response was 42.7 months, the range being from 7.0 to 43.6 months (Table 5). The median duration of response for all patients who responded was 43.6 months (95%CI, 42.2-45.0) (Tables 5 and 6). After a follow-up period of 53.2 months five patients (31%) were still in remission. The median overall survival for all patients was 45.6 months (95% CI, 33.6-57.5). This is an impressively high and durable response rate in patients with pre-treated and fludarabine- and/or chlorambucil-resistant CLL.

The phase II studies so far conducted with bendamustine used doses of 100 mg/m² and 120 mg/m² on two days every 3 to 4 weeks. However, these studies

included patients with low grade non-Hodgkin's lymphoma. It is possible that the side effects of bendamustine, in particular during the first course of treatment, show some degree of disease specificity. Unlike patients with other non-Hodgkin's lymphoma, patients with advanced stages of CLL almost uniformly have a bone marrow failure due to leukemic marrow infiltration. In addition, severe immune defects are a hallmark of CLL. Therefore, infectious complications often occur. It is possible that the safety of bendamustine at higher doses could be improved by antibiotic prophylaxis,[18] leading to a lower incidence of infectious complications. It should be emphasized, however, that the high incidence of complications can be explained by the selection of particularly heavily pre-treated patients with a poor prognosis in this trial. Most patients had an advanced stage of disease, were elderly and were resistant to at least one of the two standard drugs in CLL: chlorambucil and fludarabine. Overall these patients had been pre-treated with a median of three chemotherapy regimens, commonly including chlorambucil. Eight patients (50%) had also received one or two fludarabine-containing regimens. One patient had also been treated with rituximab. Five patients presented with impaired bone marrow function due to CLL or prior treatment. Three of these patients had been pre-treated with fludarabine 4 weeks, 6 weeks and 6 months prior to the study. This might explain why four patients had to stop bendamustine treatment during or after the first course because of myelotoxicity or related side effects.

The main hematologic toxicity of CTC grade 3 or greater at all dose levels and in all courses was leukocytopenia, followed by anemia (Table 3). The main non-hematologic toxicity was infection as a consequence of the myelosuppression. Two patients died because of atypical pneumonia after the first course of treatment; one of these two patients also suffered from septicemia and renal insufficiency. Other toxic effects were renal impairment, dehydration, diarrhea, elevated concentrations of liver enzymes, hypotension, edema, dyspnea and fever. Cutaneous side effects were erysipelas.

In contrast to earlier reports on bendamustine in low grade non-Hodgkin's lymphoma, we found that high doses were associated with marked toxicity in pre-treated patients with CLL. A reduced dose schedule of 70 mg/m^2 given on day 1 and 2 every 4 weeks appears to have an acceptable toxicity profile with a high efficacy for pre-treated CLL patients and is the recommended dose for future studies.

MB: participating physician and co-ordinating investigator of the trial (responsible for defining the drug level for each cohort and rating of the dose-limiting toxicities that occurred), responsible for the design and conception of the trial, the analyis and interpretation of data, drafting the article, revising it critically for important intellectual content, final approval of the article; MG, MH, CR, LB, BE: participating physician, responsible for collecting and analyzing data, revising the article critically for important intellectual content, final approval of the article; MH: principal inverstigator of the trial, participating physician responsible for the design and conception of the trial, analyis and interpretation of data, drafting the article, revising it critically for important intellectual content, and final approval of the article. All physicians were chosen for treating patients with bendamustine because of their pre-existing experience with the drug. The authors declare that they have no potential conflicts of interest.

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