Rituximab plus bendamustine or chlorambucil for chronic lymphocytic leukemia: primary analysis of the randomized, open-label MABLE study

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Supplementary information

Contents

Patient inclusion and exclusion criteria, and protocol amendments affecting patient eligibility

Inclusion criteria

Patients who met all of the following inclusion criteria were included in this study:

1. Signed and dated written informed consent
2. Aged 18 years or older
3. Tumor cell phenotype consistent with chronic lymphocytic leukemia (CLL) by cell surface marker analyses: CD5+, CD19+, and CD23+ (as per local confirmation of diagnosis)
4. Patients with active CLL (Binet B and C) who required therapy per criteria according to the National Cancer Institute criteria 2008. Symptomatic Binet A patients and/or patients with low/intermediate Rai stages could also be included
5. Eastern Cooperative Oncology Group performance status ≤2
6. Ineligible for treatment with fludarabine

7. A negative serum pregnancy test within 1 week before the first cycle of treatment must have been available for women who were 2 years after the onset of menopause and not sterilized surgically

Exclusion criteria

Patients who met any of the following exclusion criteria were not included in this study:

1. From amendment No. 2, patients treated second-line (2L) were not allowed to be entered in the study
2. Any other concomitant anti-cancer therapy. Corticosteroids were allowed if they were given for reasons other than CLL and the dose was ≤20 mg of prednisolone equivalent per day
3. Patients with transformation to aggressive B-cell malignancy
4. Known or suspected central nervous system involvement of CLL
5. Any other malignancy within 5 years prior to enrollment except curatively treated carcinoma *in situ* of the cervix, squamous cell carcinoma of the skin, or basal cell skin cancer. Cervical carcinoma stage 1B or less, breast cancer *in situ*, or localized prostate cancer stage T1c or less was to be considered, provided that the patient was treated with curative intent and was relapse-free for at least 2 years prior to enrollment
6. Major surgery (excluding lymph node biopsy) within 28 days prior to first cycle of study treatment
7. Chronic or ongoing active infectious disease requiring systemic treatment
8. History of clinically significant cerebrovascular disease with residual sequelae
9. Patients who had known HIV, active hepatitis B virus, or hepatitis C virus infection
10. Serious underlying medical conditions that could have impaired the ability of the patient to participate in the study

11. Inadequate renal and hepatic function per the following laboratory values: creatinine clearance <30 mL/min, total bilirubin >1.5 × upper limit of normal (ULN), alanine aminotransferase and/or aspartate aminotransferase >2.5 × ULN, and alkaline phosphatase >2.5 × ULN
12. Inadequate hematologic function, defined as absolute neutrophil count <1.0 × 10^9/l (1000/µl), platelet count <50 × 10^9/l (50,000/µl), or hemoglobin <9.0 g/dl, unless due to involvement of bone marrow (BM) by CLL
13. Known or suspected hypersensitivity to components of investigational product
14. Life expectancy less than 6 months
15. Patients known or suspected of not being able to comply with a study protocol
16. Pregnant or breast-feeding patients
17. Male and female patients with reproductive potential who were not willing to use an effective method of contraception during the study and 1 year after last dose of study medication
18. Patients unable to provide informed consent
19. Patients with severe autoimmune cytopenia as assessed by the physician (Coombs positive patients without clinical signs of autoimmune hemolytic anemia were eligible for study entry)
20. Patients who had received any investigational treatment within 30 days before screening
21. Medical condition requiring chronic use of oral corticosteroids in doses >20 mg of prednisolone equivalent/day. Inhaled or topical steroids were permitted

Protocol amendments affecting patient eligibility

1. Inclusion of patients with Binet stage A disease that required treatment
2. Exclusion of 2L patients

These protocol changes were not considered to have had a meaningful impact on the data of the overall population throughout the study.

Supplementary methods

Investigator assessment of ineligibility for fludarabine-based treatment

Assessment of ineligibility for fludarabine-based treatment was based on one or more of the following: a history of opportunistic infections; repeated grade 3/4 infections of other types; severe impairment of BM function other than underlying CLL (e.g., thrombocytopenia, anemia, or granulocytopenia); immunodeficiency; age >75 years (because data on the use of fludarabine in this age group are limited); a history of autoimmune processes or positive Coombs test status; autoimmune phenomena (e.g., autoimmune hemolytic anemia, autoimmune thrombocytopenia, thrombocytopenic purpura, pemphigus, or Evan's syndrome); and severe renal impairment.

Assessments

Genetic profiling

Baseline peripheral blood (PB) samples were assessed for CLL-related chromosomal abnormalities (fluorescent *in situ* hybridization), immunoglobulin variable-region heavy chain (IgVH) mutational status (sequencing), and ZAP-70 and CD38 levels (immunophenotyping/polymerase chain reaction [PCR]).

Tumor response

Response was assessed after Cycle (C)3 and C6 as per International Workshop on CLL (iwCLL) 2008 guidelines.[1] Patients with complete response (CR), CR unconfirmed, or partial response (PR) after C6 had a follow-up confirmatory assessment 12 weeks later (8 weeks for patients treated according to protocol v1.0). BM aspirate and biopsy assessment were required for CR confirmation. Response was also assessed in the rituximab plus chlorambucil (R-Clb) arm at C12, with treatment being discontinued for patients showing evidence of CR during C7-C12. A follow-up confirmatory assessment was performed 12 weeks later (8 weeks for patients treated according to protocol v1.0).

Progression-free survival (PFS) and overall survival (OS)

PFS was defined as the interval from the study treatment start to the date of site investigator-confirmed progressive disease/death by any other cause. OS was defined as the interval from the study treatment start to the date of death by any cause.

Minimal residual disease (MRD)

MRD was analyzed centrally according to international guidelines using an allele-specific oligonucleotide (ASO)-PCR assay.[2,3] MRD was assessed in PB at baseline. For patients with CR/PR after C6, MRD was analyzed in BM aspirates (or PB when BM was unavailable) at the confirmation-of-response visit. MRD negativity was defined as a ratio of malignant B-cells to white blood cells of $<10^{-4}$.

Safety

Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for AEs v4.0 and coded according to the Medical Dictionary for Regulatory Activities v17.0.

Statistical analysis

The null hypothesis was confirmed if the CR rate after C6 with rituximab plus bendamustine (R-B) was ≤ the CR rate with R-Clb. For first-line (1L) patients, the difference between arms was tested using a one-sided continuity-corrected chi-square test; the significance level for the primary endpoint was defined as α_2=0.048 using the O'Brien–Fleming approach. For 2L patients, the difference was tested using a two-sided Fisher's exact test. An interim analysis was performed when 100 patients completed C6 plus 12 weeks (8 weeks for patients treated according to protocol v1.0) for their confirmation assessment using a significance level of α_1=0.0052.

An exploratory logistic regression analysis assessed the influence of baseline covariates (Binet stage, IgVH mutational status, 17p/11q deletion, and Eastern Cooperative Oncology Group performance status) on treatment outcome. When adjusting for covariates, the odds ratio for treatment was estimated and the *P*-value of the Wald test was derived. Disease response and safety data were summarized using descriptive statistics.

A two-sided continuity-corrected chi-square test assessed differences between arms in overall response rates (ORRs) and molecular responses. PFS and OS were summarized by Kaplan–Meier estimates. Log-rank test *P*-values were used to compare treatment arms. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated based on the Cox proportional hazard model, with and without baseline Binet stage as a covariate.

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Efficacy in 2L patients

In 2L patients (n=116), the CR rate after C6 was higher in the R-B group (16% *vs.* 2%; *P*=0.008; Table S3). ORRs at the end of rituximab treatment were similar (R-B, 88%; R-Clb, 83%; *P*=0.654).

A 9-month extension in median PFS was observed for R-B *versus* R-Clb (26.0 months *vs.* 16.9 months; HR [adjusted for baseline Binet stage] 0.701, 95% CI 0.431-1.138; *P*=0.151; Figure S1A); median OS was not significantly different (not reached [NR] *vs.* 40.3 months; HR [adjusted for baseline Binet stage] 0.682, 95% CI 0.318-1.462; *P*=0.325; Figure S1B).

The MRD-negativity rate in 2L patients at the confirmation-of-response visit (intent-to-treat [ITT] population) was similar in the two arms (9% for both; Table S4), but patient numbers were low.

Table S1. Demographic characteristics for the pooled 1L and 2L patients.

	R-B (N=178)	R-Clb (N=179)
Age (years)		
Median, (min, max)	73 (41, 88)	72 (38, 91)
Gender, n (%)		
Male	105 (59)	118 (66)
Female	73 (41)	61 (34)
Patients receiving concomitant medication, n (%)	171 (96)	168 (94)
Number of active medical conditions		
Median (min, max)	3 (0, 12)	3 (0, 18)
Binet stage, n (%)		
A	10 (6)	14 (8)
B	102 (57)	95 (53)
C	61 (34)	66 (37)
Missing	5 (3)	4 (2)
ECOG PS, n (%)		
0	88 (49)	84 (47)
1	74 (42)	82 (46)
2	14 (8)	11 (6)
Missing	2 (1)	2 (1)
Body surface area, m^2		
Mean (SD)	1.802 (0.2365)	1.809 (0.1722)
Min, max	1.30, 2.48	1.41, 2.29
IgVH mutational status, n (%)		
Mutated	59 (33)	71 (40)
Unmutated	106 (60)	91 (51)
Other[a]	3 (2)	8 (5)
Not tested	10 (6)	9 (5)
11q status, n (%)		
Heterozygous deletion	33 (19)	27 (15)
Normal	144 (81)	149 (83)
Not tested	1 (1)	3 (2)

17p status, n (%)		
Heterozygous deletion	19 (11)	6 (3)
Normal	158 (89)	169 (94)
Not tested	1 (1)	4 (2)
11q/17p deletion, n (%)		
Heterozygous deletion	48 (27)	32 (18)
Normal	129 (73)	144 (80)
Not tested	1 (1)	3 (2)
13q deletion (S25 or S319 probe)[b], n (%)		
Homozygous deletion	7 (4)	8 (5)
Two clones (one heterozygote, one homozygote)	21 (12)	11 (6)
Heterozygote deletion	63 (35)	90 (50)
Normal	86 (48)	67 (37)
Not tested	1 (1)	3 (2)
Trisomy 12, n (%)		
Normal	135 (76)	144 (80)
Trisomy	41 (23)	32 (18)
Not tested	1 (1)	3 (2)

1L: first-line; 2L: second-line; ECOG PS: Eastern Cooperative Oncology Group performance status; IgVH: immunoglobulin variable-region heavy chain; R-B: rituximab plus bendamustine; R-Clb: rituximab plus chlorambucil; SD: standard deviation. [a]Other includes polyclonal and oligoclonal. [b]Deletion status according to at least one probe (NB, in the R-B group, one patient with two clones by S319 probe and heterozygous deletion by S25 probe is counted twice).

Table S2. Active medical conditions in 1L patients.

>5% patients by SOC, n (%)	R-B (N=121)	R-Clb (N=120)
Vascular disorders	62 (51)	56 (47)
Metabolism and nutrition disorders	42 (35)	47 (39)
Respiratory, thoracic and mediastinal disorders	25 (21)	28 (23)
Musculoskeletal and connective tissue disorders	25 (21)	26 (22)
Social circumstances	32 (26)	17 (14)
Cardiac disorders	27 (22)	21 (18)
Gastrointestinal disorders	23 (19)	23 (19)
Blood and lymphatic system disorders	24 (20)	17 (14)
Psychiatric disorders	19 (16)	18 (15)
Renal and urinary disorders	18 (15)	17 (14)
General disorders and administration site conditions	11 (9)	16 (13)
Nervous system disorders	14 (12)	12 (10)
Investigations	12 (10)	10 (8)
Reproductive system and breast disorders	7 (6)	15 (13)
Infections and infestations	12 (10)	9 (8)
Endocrine disorders	9 (7)	10 (8)
Neoplasms benign, malignant and unspecified	12 (10)	7 (6)
Skin and subcutaneous tissue disorders	6 (5)	12 (10)
Eye disorders	8 (7)	7 (6)
Ear and labyrinth disorders	6 (5)	7 (6)

1L: first-line; R-B: rituximab plus bendamustine; R-Clb: rituximab plus chlorambucil; SOC: System Organ Class.

Table S3. CRs and PRs at C6 in 2L patients and for the pooled 1L and 2L patients.

Assessment	Analysis		2L patients		All study patients	
			R-B	R-Clb	R-B	R-Clb
CR confirmed by BM biopsy[a]	CR	N	57	59	178	179
		n (%)	9 (16)	1 (2)	38 (21)	12 (7)
		P-value[b]	0.008		<0.001	
	Logistic regression[c]	N	49	56	162	159
		OR (95% CI)	14.00 (1.67-117.58)		5.27 (2.42-11.48)	
		P-value[d]	0.015		<0.001	
PR based on the investigator's assessment	PR	N	57	59	178	179
		n (%)	22 (39)	30 (51)	88 (49)	109 (61)

1L: first-line; 2L: second-line; BM: bone marrow; C: cycle; CI: confidence interval; CR: complete response; ECOG PS: Eastern Cooperative Oncology Group performance status; IgVH: immunoglobulin variable-region heavy chain; OR: odds ratio; PR: partial response; R-B: rituximab plus bendamustine; R-Clb: rituximab plus chlorambucil. [a]CRs confirmed by BM biopsy only were included. [b]*P*-value is based on a one-sided continuity corrected chi-square test for all patients and two-sided Fisher's exact test for 2L patients. [c]The following covariates were included in the logistic regression: Binet stage (A and B *vs.* C); IgVH mutational status (mutated *vs.* unmutated); 17p/11q deletion (heterozygote deletion *vs.* normal); ECOG PS (0 *vs.* ≥1). [d]*P*-value is based on the Wald test.

Table S4. MRD at the confirmation-of-response visit[a] in 2L patients.

	R-B	R-Clb
Overall ITT population	(n=57)	(n=59)
MRD-negative patients, n (%)	5 (9)	5 (9)
Patients with CR	(n=9)	(n=1)
MRD-negative patients, n (%)	2 (22)	0 (0)
Patients with CR or PR based on the investigator's assessment[b]	(n=31)	(n=31)
MRD-negative patients, n (%)	5 (16)	4 (13)

2L: second-line; ASO-RQ-PCR: allele-specific oligonucleotide real-time quantitative polymerase chain reaction assay; BM: bone marrow; C: cycle; CR: complete response; CR: complete response ITT: intent-to-treat; MRD: minimal residual disease; PB: peripheral blood; PR: partial response; R-B: rituximab plus bendamustine; R-Clb: rituximab plus chlorambucil. Negative MRD was defined as proportion of malignant B-cells to white blood cells of $<10^{-4}$, as assessed by ASO-RQ-PCR measured in BM aspirate (or PB if BM unavailable). MRD data were available for 45/50 patients with a CR based on the investigator's assessment (BM, n=42; PB, n=1; unknown=2) and 182/241 patients overall (BM=145; PB, n=32; unknown=5). [a]Performed 12 weeks after the end of C6 disease response assessment. [b]Includes patients with CR (with or without BM confirmation) or PR by investigator assessment.

Figure S1. Efficacy in 2L patients, (A) PFS and (B) OS. 2L: second-line; CI: confidence interval; HR: hazard ratio; NR: not reached; OS: overall survival; PFS: progression-free survival; R-B: rituximab plus bendamustine; R-Clb: rituximab plus chlorambucil.