ARTICLE

Olaptesed pegol (NOX-A12) with bendamustine and rituximab: a phase IIa study in patients with relapsed/refractory chronic lymphocytic leukemia

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‡This paper is dedicated to the memory of our colleague Prof. Michael Steurer, an extraordinary scientist and physician appreciated for his empathetic commitment to his patients, who recently passed away.

ABSTRACT

Olaptesed pegol (NOX-A12) is a pegylated structured L-oligoribonucleotide that binds and neutralizes CXCL12, a chemokine tightly regulating the life cycle of chronic lymphocytic leukemia cells. The resulting inhibition of CXCR4 and CXCR7 signaling reduces the protective activity of the bone marrow and lymph node microenvironment. CXCL12 inhibition mobilizes chronic lymphocytic leukemia cells into the circulation and prevents their homing into the protective niches. In this phase I/II study, 28 patients with relapsed/refractory chronic lymphocytic leukemia were treated with olaptesed pegol in combination with bendamustine and rituximab. Combination treatment was preceded by single escalating pilot doses of olaptesed pegol in the first ten patients for evaluation of safety and pharmacokinetics. Peak concentrations and systemic exposure of olaptesed pegol were dose-linear; plasma elimination was monophasic with a 53.2 h half-life. A rapid increase in circulating chronic lymphocytic leukemia cells was observed already 1 h after administration of olaptesed pegol and lasted for at least 72 h. Single-agent treatment was well tolerated and no dose-limiting toxicity was observed. The combination regimen yielded an overall response rate of 86%, with 11% of patients achieving a complete response and 75% a partial response. Notably, all ten high-risk patients, including four with a 17p deletion, responded to treatment. The median progression-free survival was 15.4 (95% confidence interval: 12.2, 26.2) months while the median overall survival was not reached with >80% of patients alive after a median follow-up of 28 months. Olaptesed pegol was well tolerated and did not result in additional toxicity when combined with bendamustine and rituximab (*ClinicalTrials.gov identifier: NCT01486797*). Further clinical development of this novel CXCL12 inhibitor is thus warranted.

Introduction

Olaptesed pegol (NOX-A12) is a novel, pegylated L-oligoribonucleotide, a so-called Spiegelmer®, which binds and neutralizes the chemokine CXCL12 (stromal cell-derived factor-1, SDF-1) with high affinity and specificity. As a result, it inhibits CXCL12 signaling through both of its receptors, CXCR4 and CXCR7.[1,2] In healthy

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volunteers, single doses of olaptesed pegol mobilized white blood cells into peripheral blood; the mobilization was long-lasting and increased dose-dependently to more than 4 days at the highest dose in a phase I study.[1] Further analyzing the mode of action, it could be shown that olaptesed pegol detaches CXCL12 from cell surfaces leading to a disruption of the existing chemokine gradient.[3] CXCL12 facilitates homing and retention as well as trafficking of hematopoietic and immune cells via CXCR4.[4] This feature makes CXCL12 one of the key factors known to support survival of chronic lymphocytic leukemia (CLL) cells in the protective niches of the bone marrow and lymph node microenvironment which is an essential part of the pathogenesis and progression of the disease.[5] The CLL dissemination inside tissue microenvironments is actively coordinated by a crosstalk between leukemic cells and stroma, where CXCL12 not only mediates CLL cell chemotaxis, actin polymerization, and migration beneath and underneath CXCL12-secreting stromal cells but also protects CLL cells from spontaneous and drug-induced apoptosis.[6] Although surface expression of CXCR7 was not observed on CLL cells,[7] CXCR7-dependent angiogenic mononuclear cell trafficking was shown to support bone marrow angiogenesis[8] which plays a pathophysiological role in the leukemic microenvironment.[9] Interference with CXCL12 signaling by olaptesed pegol was shown to inhibit CLL cell chemotaxis and induce chemosensitization *in vitro* using primary CLL cells[3] as well as to remove CLL cells from the nurturing and protective microenvironment, prevent homing and make them more vulnerable to conventional therapy *in vivo* in an Eμ-TCL1 transgenic mouse model.[10] A similar phenomenon was recently demonstrated preclinically and clinically in multiple myeloma, in which olaptesed pegol was combined with bortezomib and dexamethasone.[2,11] In relapsed/refractory CLL patients, disease control becomes increasingly difficult due to increased resistance to therapy. Olaptesed pegol represents a novel paradigm of therapy that moves away from cancer cells to microenvironmental elements as the primary treatment target.

We report here the findings of a phase IIa study, meant to translate the novel concept of combining chemoimmunotherapy and CXCL12 inhibition into the clinic (*Online Supplementary Figure S1* delineates the anticipated mode of action), in which we assessed the pharmacokinetic, pharmacodynamic, safety and first efficacy data of olaptesed pegol in patients with relapsed/refractory CLL. The main objectives of the study were to assess the safety and tolerability of olaptesed pegol alone and in combination with bendamustine and rituximab (BR) in CLL patients, as well as to determine the response rates and remission duration.

Methods

The trial (EudraCT number 2011-004672-11, NCT01486797) was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practices Guidelines. The clinical study protocol and its amendments, informed consent documents, and any other study-related documents were reviewed and approved by the applicable regional review boards or ethics committees. All authors had access to the primary clinical data.

Patients

Twenty-eight patients with relapsed/refractory CLL were enrolled out of 32 patients screened. Patients were eligible for this study if they were bendamustine-sensitive (having achieved at least a partial response lasting at least 6 months) or bendamustine-naïve. Patients were required to present with a World Health Organization (WHO) Performance Status ≤2 and a modified Cumulative Incidence Rating Scale (CIRS) score <7, to have a serum creatinine level ≤1.5 x the upper limit of normal (ULN) and/or calculated creatinine clearance ≥50 mL/min/1.73 m^2, and appropriate hematologic (platelet count ≥75x10^9/L, absolute neutrophil count >0.75x10^9/L) and liver parameters (bilirubin ≤1.5 x ULN, aspartate transaminase and/or alanine transaminase ≤2.5 x ULN).

Trial design and treatment

Initially, a single dose of olaptesed pegol was administered intravenously to ten patients in the pilot study phase to study safety, pharmacokinetics and pharmacodynamics of olaptesed pegol alone. Subsequently, olaptesed pegol was administered intravenously once per cycle in combination with BR as six cycles of 28 days to all 28 eligible patients including the initial ten pilot patients to study safety and efficacy of this novel combination. Details on drug administration are provided in the *Online Supplementary Information*.

Study assessments

Responses were assessed at the end of cycle 6 according to the 1996 National Cancer Institute-Working Group (NCI-WG) criteria updated in 2008 by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL).[12] Adverse events were continuously monitored until 30 days after the last olaptesed pegol dose and were graded by National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Olaptesed pegol concentrations were measured in plasma at the indicated time points by a validated assay for pharmacokinetic analyses (see supplement to Vater *et al.*[1]). The pharmacodynamic activity of olaptesed pegol was studied by five-color flow cytometry analysis using a standard diagnostic panel based on CD5, CD19, CD45, 7-AAD plus beads on peripheral blood samples to study the mobilization of CLL cells. Additionally, CXCR4 expression was assessed on the detected CLL cells. Analyses were performed centrally by MLL GmbH (Munich, Germany). Minimal residual disease was not assessed.

A fluorescence *in situ* hybridization cytogenetics panel was used to investigate CLL cells unless this had been performed within the last 24 weeks prior to screening. Deletions of 11q22-q23, 13q14, 17p13 as well as a marker for trisomy 12 were assessed. IGHV status and *TP53* mutations were not assessed.

Serum for immunogenicity analyses was collected at screening, day -14, before first dosing at cycles 1 and 4 as well as at the final examination and 6 months thereafter. Further details can be found in the *Online Supplementary Information.*

Statistical analyses

Data management and biostatistics were performed by AMS Advanced Medical Services GmbH (Mannheim, Germany). All statistical analyses are descriptive and exploratory. Efficacy parameters were analyzed using SAS version 9.1.3 (SAS Institute Inc, Cary, NC, USA). Actuarial survival curves were estimated according to the Kaplan-Meier method.

Results

Patients' flow and characteristics

Thirty-two patients were screened. Ten patients were enrolled into the pilot group, which included a replacement for a patient withdrawn from the study after cycle 1. Eighteen more patients were enrolled after completion of the pilot group: these 18 patients started directly with combination treatment. Three patients discontinued therapy before completion of three treatment cycles because of chlamydial pneumonia, *E. coli* sepsis and one patient's decision and five more patients discontinued therapy before completion of six treatment cycles because of rash, multiple episodes of infection, start of a new therapy after progressive disease and personal decision by two patients (*Online Supplementary Figure S2*). The median number of olaptesed pegol + BR cycles administered was six (range, 1-6). All 28 enrolled patients constitute the intent-to-treat (ITT) population and all analyses presented further below were performed on the ITT population. One patient withdrew his consent for the study before any disease assessment, thus the remaining 27 patients constitute the full analysis set.

Table 1. Patients' demographics and baseline characteristics (n = 28).

Age (years)		Mean	66
BMI (kg/m^2)		Mean	25
Sex	Male	N (%)	16 (57%)
	Female	N (%)	12 (43%)
Ethnic origin	Caucasian	N (%)	28 (100%)
Disease stage (Binet)	Stage A	N (%)	6 (21%)
	Stage B	N (%)	11 (39%)
	Stage C	N (%)	11 (39%)
WHO Performance Status	0	N (%)	23 (82%)
	1	N (%)	5 (18%)
CIRS Performance Score	0	N (%)	6 (21%)
	1	N (%)	3 (11%)
	2	N (%)	4 (14%)
	3	N (%)	1 (3.6%)
	4	N (%)	4 (14%)
	5	N (%)	5 (18%)
	6	N (%)	5 (18%)
Risk status	High risk*	N (%)	10 (36%)
	Non-high-risk	N (%)	17 (61%)
	Not known	N (%)	1 (3.6%)
Hemoglobin (g / dL)		Mean (± SD)	12.432 (1.890)
Absolute lymphocyte counts (x10^9/L)		Mean (± SD)	47.309 (50.620)
Platelets (x10^9/L)		Mean (± SD)	145.89 (72.426)
Neutrophils (x10^9/L)		Mean (± SD)	4.249 (3.074)
Prior treatment with fludarabine and/or bendamustine		N (%)	23 (82%)
Prior treatment with rituximab		N (%)	19 (68%)
Prior treatment lines		Median (range)	1 (1-3)
Response to previous line of therapy	CR	N (%)	10 (36%)
	PR	N (%)	17 (61%)
	PD	N (%)	-
	n.a.	N (%)	1 (3.6%)

*Defined as having a deletion of 17p13 or who relapsed within less than 24 months after previous fludarabine or bendamustine-containing treatment regimens according to Stilgenbauer & Zenz.[13] BMI: body mass index; WHO: World Health Organization; CIRS: Cumulative Illness Rating Scale; SD: standard deviation.

The patients' demographic details and baseline characteristics are shown in Table 1. The median age was 66 years (range, 41 – 79) and 78% of patients had Binet stage B or C disease. High-risk CLL, defined as having a deletion of 17p13 or relapse within less than 24 months after previous fludarabine- or bendamustine-containing treatment regimens,[13] was found in ten patients (36%), of whom four (14%) presented with a 17p13 deletion (Table 2). Eighty-two percent of the patients had received prior treatment with fludarabine and/or bendamustine and 68% had been previously treated with rituximab. The median number of prior lines of therapy was one (range, 1-3) and the majority of patients (61%) had responded to their last therapy line with a partial response whereas 36% achieved a complete response. Eight patients (29%) presented with a deletion of 11q22-q23 and 13 patients (46%) a deletion of 13q14. Three patients (11%) had trisomy 12 (Table 2).

Pharmacokinetics

In patients enrolled into the pilot phase, peak plasma concentrations of olaptesed pegol increased in an approximately dose-linear way with mean peak levels of 1.76, 3.95 and 7.20 μmol/L at doses of 1, 2 and 4 mg/kg, respectively (Figure 1 and *Online Supplementary Table S1*). The terminal elimination half-life in patients receiving 4 mg/kg olaptesed pegol was 53.2 h, total body clearance was 36.1 mL/h and the volume of distribution at steady state was 2.9 L; similar figures were obtained for patients receiving 1 and 2 mg/kg olaptesed pegol (*Online Supplementary Table S1*). Peak plasma concentrations at cycles 1 and 4, when olaptesed pegol was administered in combination with BR were similar to the values for the agent given as a single dose (*Online Supplementary Table S2*).

Pharmacodynamics

CLL cell mobilization in the pilot group was evident already 1 h after olaptesed pegol treatment with 10,196 CLL cells/μL on average above baseline detected in peripheral blood (mean baseline level: 41,318 CLL cells/μL). A peak of 22,939 CLL cells/μL on average above

Table 2. Detailed cytogenetic abnormalities.

Parameter	Number (%) of patients	
Deletion of 17p13	Abnormal	4 (14%)
	Normal	23 (82%)
	Missing	1 (3.6%)
Deletion of 11q22-q23	Abnormal	8 (29%)
	Normal	19 (68%)
	Missing	1 (3.6%)
Trisomy 12	Abnormal	3 (11%)
	Normal	22 (79%)
	Missing	3 (11%)
Deletion of 13q14	Abnormal	13 (46%)
	Normal	14 (50%)
	Missing	1 (3.6%)
Number of cytogenetic abnormalities	0	6 (21%)
	1	14 (50%)
	2	7 (25%)
	Missing	1 (3.6%)

The fluorescence *in situ* hybridization panel analysis identified 21 (75%) patients with one or more cytogenetic abnormalities. IGHV status and *TP53* mutations were not assessed.

baseline, corresponding to a 200% increase, occurred at 24 h and CLL cell mobilization was effectively maintained for at least 72 h (Figure 2A,B). Simultaneously, CXCR4 expression on CLL cells gradually increased during the intravascular circulation of CLL cells peaking at 24 h (Figure 2C). In cycle 4, mobilization was evaluated in 24 patients and, similarly to the pilot phase, CLL cells were mobilized already 1 h after olaptesed pegol treatment with 187 CLL cells/μL above baseline (mean baseline level: 224 CLL cells/μL). Mobilized CLL cell numbers gradually increased, peaking at 24 h with 679 CLL cells/μL on average above baseline, corresponding to a >300% increase (Figure 2D,E) accompanied by a steady CXCR4 increase on CLL cells (Figure 2F).

Safety

Olaptesed pegol was safe and well tolerated as monotherapy with no serious adverse events reported in the pilot phase. All patients were escalated to the highest anticipated dose of 4 mg/kg olaptesed pegol, which was previously established in healthy volunteers as safe and efficacious in terms of lymphocyte mobilization.[1] Common adverse events (≥10%) observed in the trial are shown in Table 3. The most frequent adverse event observed on BR alone is neutropenia with grade 3/4 events recorded in up to 50% of the patients.[14-17] The triple regimen of olaptesed pegol in combination with BR does not seem to increase the incidence of grade 3/4 neutropenia, with a 50% incidence reported in this study. The incidences of grade 3/4 anemia and thrombocytopenia were low (14.3% each). There were very few non-hematologic grade 3/4 toxicities and these were mostly reported by single patients (nausea 3.6%, constipation 3.6%, abdominal pain 3.6%, pyrexia 3.6%, fatigue 3.6%, hyperuricemia 7.1% and cytokine release

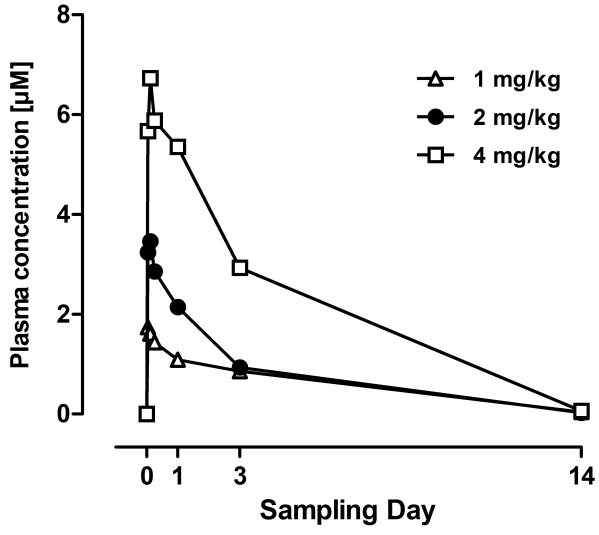

Figure 1. Plasma concentration-time curves of olaptesed pegol after administration of single intravenous doses (pilot phase, monotherapy). Data are shown as geometric means.

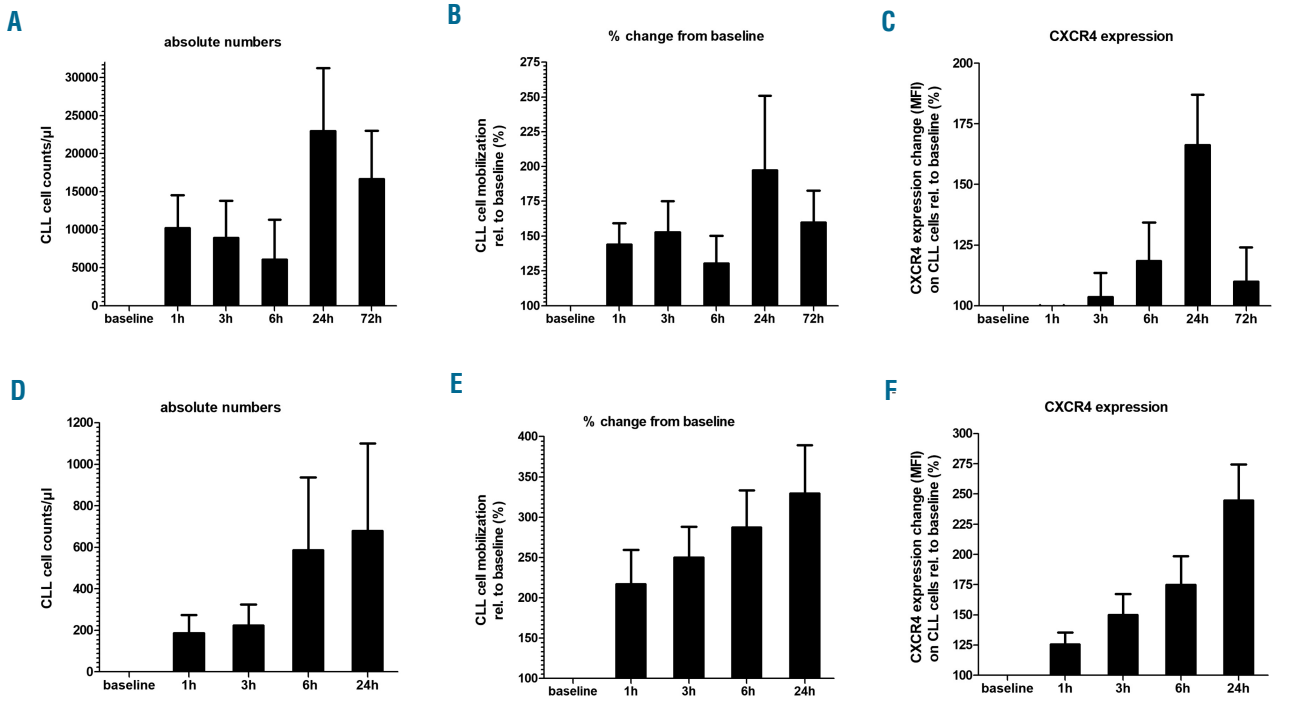

Figure 2. Mobilization kinetics of chronic lymphocytic leukemia cells and their CXCR4 expression levels after administration of olaptesed pegol. (A) Baseline values were set to 0 and chronic lymphocytic leukemia (CLL) cell counts above baseline are depicted as mean and standard error of mean (SEM) after administration of olaptesed pegol alone to ten patients in the pilot phase. (B) Baseline values were set to 100% and CLL cell mobilization above baseline is depicted in percent after administration of olaptesed pegol alone to ten patients in the pilot phase. (C) Mean fluorescence intensity (MFI) of CXCR4 expression on CLL cells was set to 100% at baseline and changes in MFI are depicted in percent for ten patients in the pilot group. (D) Baseline values were set to 0 and CLL cell counts above baseline are depicted as mean and SEM after administration of olaptesed pegol in combination with bendamustine and rituximab (BR) for 24 patients in cycle 4. (E) Baseline values were set to 100% and CLL cell mobilization above baseline is depicted in percent after administration of olaptesed pegol in combination with BR for 24 patients in cycle 4. (F) MFI of CXCR4 expression on CLL cells was set to 100% at baseline and changes in MFI are depicted in percent (whisker plots for 24 patients in cycle 4.

syndrome 3.6%). Grade 3/4 infections occurring during treatment were reported by single patients only (pneumonia 3.6%, cystitis 3.6%, infection 3.6%, lung infection 3.6%, chlamydial pneumonia 3.6% and sinusitis 3.6%) (*Online Supplementary Figure S3*). Of note, two (7.1%) patients experienced tumor lysis syndrome during the first treatment cycle, one laboratory (grade 1) and one clinical (grade 3) (*Online Supplementary Figure S3*).

Treatment response and survival

The overall response rate (ORR) defined as partial response or better, was assessed at the end of cycle 6. The ORR was 86% (24/28) in the ITT patient population, in which 75% (21/28) of the patients achieved a partial response and 10.7% (3/28) a complete response (Table 4). Similar response rates were observed in the full analysis set (ORR 89%; 24/27) and per-protocol population (ORR 91%; 19/21) (*Online Supplementary Table T3*). Notably, all ten patients who had a high-risk status responded to the treatment with a partial response (Table 4). Patients who had received two or more previous treatment lines had an ORR of 82% (9/11) and patients who had been pre-treated with fludarabine or bendamustine had an ORR of 83% (19/23) (Table 4). After an increase of lymphocytosis in the pilot group during olaptesed pegol monotherapy, a rapid reduction of lymphocytosis in peripheral blood, with normalization by treatment cycle 2 – 3, was observed (Figure 3A). The reduction of circulating CLL cells during the first treatment cycles also became evident by the significant improvement of the CLL to leukocyte ratio from cycle 1 to cycle 4 in the majority of the patients (Figure 3B). At screening, 24 out of 28 patients reported enlarged lymph nodes. A reduction of lymph node size by ≥50% at the end of treatment was achieved in 18 out of these 24 patients (Figure 3C). Hematologic parameters such as hemoglobin concentration and platelet count improved after an initial expected drop; neutrophil values stabilized throughout the treatment course (*Online Supplementary Figure S4*). After a median follow-up of 28 months, the median progression-free survival was 15.4 (95% confidence interval: 12.2, 26.2) months (Figure 4A) while the median overall survival was not reached: the 3-year survival rate was > 80% (Figure 4B) in the ITT population.

Table 3. Common adverse events (incidence ≥10%) observed in the trial.

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic toxicity				
Neutropenia		10.7	32.1	17.9
Anemia		7.1	14.3	
Thrombocytopenia	3.6		7.1	7.1
Febrile neutropenia		3.6	7.1	
Leukopenia		10.7		
Non-hematologic toxicity				
Nausea	21.4	14.3	3.6	
Constipation	17.9	3.6	3.6	
Diarrhea	14.3	10.7		
Vomiting	10.7	7.1		
Abdominal pain upper	7.1		3.6	
Pyrexia	17.9	14.3	3.6	
Fatigue	10.7		3.6	
Asthenia	14.3			
Chills	10.7	3.6		
General physical health deterioration	7.1	3.6		
Mucosal inflammation	10.7			
Edema peripheral	7.1	3.6		
Hypokalemia	14.3	7.1		
Hyperuricemia	7.1		7.1	
Cough	10.7	7.1		
Pleural effusion		10.7		
Rash	3.6	7.1		
Cytokine release syndrome		10.7	3.6	

Discussion

The survival of CLL cells depends on periodic CXCL12-mediated migration into the bone marrow and other lymphoid tissue in order to establish an interactive network of cellular contacts within the microenvironment.[18] This interaction represents a novel and vulnerable therapeutic target which can be utilized in combination therapies.[19] The here-presented phase IIa study was meant to investigate the concept of simultaneously targeting CLL and its microenvironment. The study builds on the preclinical proof-of-concept regarding the significance of CXCL12 blockade in CLL[3,10] and on clinical phase I data in healthy subjects[1] as well as phase II data relating to targeting the multiple myeloma microenvironment.[11]

The pharmacokinetic data of olaptesed pegol observed in this study were very similar to those from healthy subjects and patients with multiple myeloma studied previously with regard to peak concentrations and terminal elimination half-life.[1,11] This indicates that the uptake, dis-

Table 4. Response rates in the intention-to-treat population (n=28) including subgroups.

	ITT	High risk#			Prior treatment lines		Fludarabine OR bendamustine pre-treatment	
*		yes	no	missing	1	2 or more	pre-treated	naïve
N (%)	28 (100%)	10 (36%)	17 (61%)	1 (3.6%)	17 (61%)	11 (39%)	23 (82%)	5 (18%)
CR	3 (10.7%)	0	3 (17.6%)	0	3 (17.6%)	0 (0%)	1 (4.3%)	2 (40%)
PR	21 (75%)	10 (100%)	11 (64.7%)	0	12 (70.6%)	9 (81.8%)	18 (78.3%)	3 (60%)
PD	3 (10.7%)	0	2 (11.8%)	1 (100%)	2 (11.8%)	1 (9.1%)	3 (13%)	0
NE	1 (3.6%)	0	1 (5.9%)	0	0	1 (9.1%)	1 (4.3%)	0
ORR	86%	100%	82%	0%	88%	82%	83%	100%

defined as having a deletion of 17p13 or who relapsed within less than 24 months after previous fludarabine or bendamustine-containing treatment regimens according to Stilgenbauer & Zenz.[13] *Response percentages are calculated based on the number of patients in the respective subgroup. CR: complete remission; PR: partial response; PD: progressive disease; NE: not evaluable; ORR: overall response rate (≥PR); ITT: intention-to-treat.

tribution and metabolism of olaptesed pegol were independent from disease and combination partner. Rapid increases in CLL cell numbers were observed, with a peak at 24 h, which were maintained for at least 72h. Interestingly, CXCR4 expression on CLL cells gradually increased during the intravascular circulation of CLL cells, also peaking at 24 h. The mobilization efficiency was maintained throughout the cycles with an up to 3-fold increase in CLL cell numbers above mean baseline level in cycle 4. The observed CXCR4 increase that accompanied mobilization reflects the extended circulation of CLL cells in the periphery as described by Calissano *et al.*[5] and represents a pharmacodynamic biomarker for the sustained blockade of CXCL12 by olaptesed pegol. Of note, continuous circulation of CLL cells ultimately leads to apoptosis, a phenomenon also called 'death by neglect'[20] due to the fact that CLL cells need to regularly receive their survival signals from the protective niches.[5]

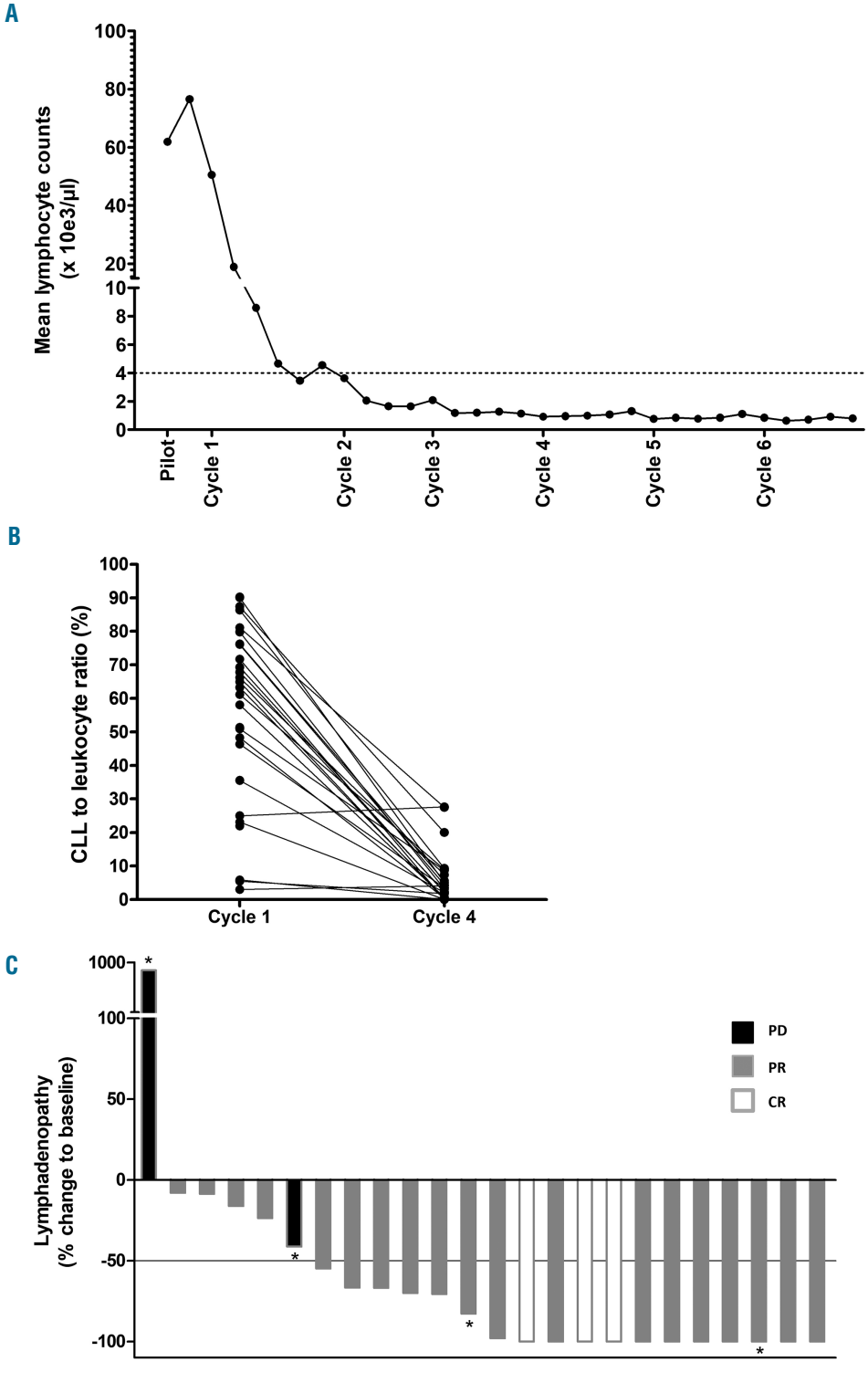

Figure 3. Mean lymphocyte counts, chronic lymphocytic leukemia to leukocyte ratio in cycle 1 *versus* cycle 4 and lymphadenopathy evaluation. (A) Mean lymphocyte counts (x 10^3/µL peripheral blood) evaluated at different time points during the pilot phase for ten patients (Pilot) and cycle 1 to cycle 6 for all 28 patients are depicted. (B) The chronic lymphocytic leukemia (CLL) cell to leukocyte ratio evaluated at cycle 1 and cycle 4 is depicted for each individual patient. (C) Lymphadenopathy at the end of treatment was assessed in 24 patients who presented with enlarged lymph nodes at screening. *For patients who discontinued treatment before cycle 6 the value at the end of cycle 3 is depicted. PD: progressive disease; PR: partial response; CR: complete response.

The triple regimen of olaptesed pegol in combination with BR was generally well tolerated with neutropenia recorded as the most frequent adverse event. The incidences of grade 3/4 anemia and thrombocytopenia were low. Non-hematologic toxicities, mostly nausea and pyrexia followed by mild diarrhea and constipation, were generally manageable. Altogether, the reported frequency and severity of adverse events were to be expected in a population of relapsed/refractory CLL patients during standard BR therapy.[14-17] Therefore, it does not appear that treatment with olaptesed pegol results in significant additional toxicities on top of BR.

Treatment with olaptesed pegol in combination with BR resulted in an ORR of 86% with 11% of patients achieving a complete response. Acknowledging the limitations of cross-trial comparisons due to different inclusion criteria of patients as well as the limited sample size of 28 patients in this study, the ORR of 86% compares favorably with responses to BR alone reported earlier with ORR ranging from 45-72%.[14-17] Although limited by the low numbers of patients, subgroup analyses showed that all ten high-risk patients, including four with a 17p deletion, achieved a partial response with the combination of olaptesed pegol and BR, even though 17p-deleted patients have been reported to respond poorly to BR alone.[15,17]

Other combinations with BR in relapsed/refractory CLL patients resulted in ORR of 84% for cytarabine + BR,[21] 82.7% for the BTK inhibitor ibrutinib + BR,[14] 70% for the PI3K inhibitor idelalisib + BR,[17] 67% for fludarabine + BR,[22] and 47% for lenalidomide + BR.[23] Of note, higher toxicity rates were reported for the addition of kinase inhibitors to BR regarding neutropenia, diarrhea and pneumonia[14,17] and very high incidences of grade 3/4 neutropenia and thrombocytopenia were reported if another chemotherapeutic drug was combined with BR.[21,22]

The median progression-free survival of 15.4 months in the ITT population is in the range of what is expected after BR treatment alone (11.1, 13.3, 14.7, and 17 months).[14-17] Seymour *et al.* reported a progression-free survival of 16.6 months for patients treated with BR only after one prior therapy line, which is slightly longer than the progression-free survival of 15.4 months in our study; however only 12.9% of the patients in the MURANO study had Rai stage III-IV CLL whereas 39% of patients in our study had Binet stage C disease.[16] Comparable progression-free survival values were achieved by the addition of another chemotherapy to BR such as fludarabine + BR or cytarabine + BR (19 and 16 months, respectively),[21,22] however much better progression-free survival results were reported for the combination of kinase inhibitors and BR (20.8 months for idelalisib[17] and not reached for ibrutinib[14]) . Notably, kinase inhibitors strongly interfere with CXCR4 downstream signaling and the resulting CLL cell displacement from their supportive microenvironment, followed by leukemia cell death due to 'death by neglect', is a central mechanism of action of kinase inhibitors.[20] In contrast to olaptesed pegol which was given once per 28-day cycle, however, the kinase inhibitors were administered daily or twice daily. Thus, the authors suggest studying whether longer progression-free survival could be achieved if olaptesed pegol were to be administered more often to fully exploit the 'death by neglect' mechanism indicated by the transient CXCR4-increase on peripheral CLL cells following olaptesed pegol treatment. Furthermore, kinase inhibitor treatment was sustained after completion of six BR cycles and could, therefore, further prolong the time to progression, whereas olaptesed pegol treatment was stopped after six BR-combination cycles. The median overall survival was not reached, resulting in a 3-year survival rate of >80% in the ITT population which compares favorably with other effective BR combinations (60% - 75%).[17,21,22]

A recent combination therapy utilizing bendamustine for initial debulking followed by obinutuzumab and venetoclax resulted in an ORR of 90% in a subgroup of 29 relapsed/refractory CLL patients.[24] A comparable ORR of 92% was achieved in 12 relapsed/refractory CLL patients when chemotherapy was omitted and obinutuzumab, ibrutinib and venetoclax were administered sequentially.[25] Another chemotherapy-free combination of rituximab and venetoclax produced an ORR of 92.3% in 194 relapsed/refractory CLL patients.[16] Interestingly, olaptesed pegol was reported to synergize with anti-CD20 antibodies such as rituximab or obinutuzumab by enhancing immune cell infiltration and antibody-dependent cellular

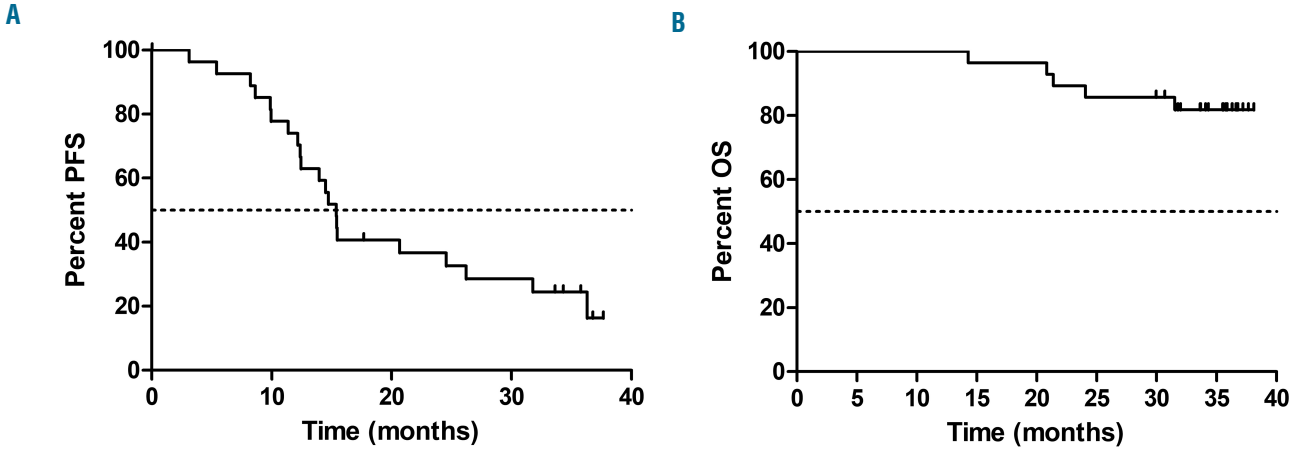

Figure 4. Progression-free survival and overall survival of patients. Kaplan-Meier analyses of (A) progression-free survival and (B) overall survival in the intent-to-treat population (n=28) are depicted. PFS: progression-free survival; OS: overall survival.

cytotoxicity.[26] Thus, combination therapies including anti-CD20 antibodies and novel agents, such as venetoclax together with olaptesed pegol, could be a viable option based on their complementary mechanisms of action in future clinical trials.

In conclusion, the data from our study demonstrate that treatment with olaptesed pegol results in the intended pharmacodynamic effect by effectively mobilizing CLL cells. The high response rate of 86% as well as 3-year overall survival rate of >80% compare favorably with those achieved by BR alone and in recent BR combination trials. These data together with the benign safety profile warrant further clinical development of this novel CXCL12 inhibitor in combination with targeted anti-CLL drugs in randomized studies.

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