

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

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Supplemental Information

Methods

Treatment

In the pilot phase, 3 cohorts of 3 patients each (4 patients in the 1 mg/kg cohort due to patient replacement) were administered one single dose of 1 mg/kg (cohort 1), 2 mg/kg (cohort 2) or 4 mg/kg (cohort 3) of olaptesed pegol alone by slow intravenous bolus injection over 15 minutes two weeks prior to starting the combination treatment. During combination treatment, olaptesed pegol was given once per cycle one hour prior to rituximab in intra-patient escalating doses of 1 mg/kg in cycle 1, 2 mg/kg in cycle 2 and 4 mg/kg in cycles 3 to 6. Rituximab was administered intravenously at doses of 375 mg/m² on day 1 of the first 28-day cycle and 500 mg/m² on day 1 of subsequent cycles. Bendamustine (70 - 100 mg/m² depending on the respective clinical practice) was given intravenously on days 2-3 (cycle 1) or days 1-2 (cycles 2-6) of each 28-day cycle following administration of rituximab.

Study assessments

Olaptesed pegol concentrations were measured in plasma at pre-dose (baseline), 1, 3, 6, 24 and 72 hours after the pilot dose, as well as in combination treatment cycles 1 and 4 on day 1 at pre-dose, 1 hour post dose and on days 2, 8 and 26-28 at pre-dose by a validated assay for pharmacokinetic analyses (see supplement to ¹). Pharmacokinetic parameters were derived by non-compartmental methods and comprised: C_{max} , peak concentration 1 hour after dosing; $C_{max(norm)}$, dose-normalized C_{max} ; C_{min} , plasma concentration 72 hours after dosing, $C_{min(norm)}$, dose-normalized C_{min} ; AUC_{∞} , area under the plasma concentration–time curve from 0 to infinity; $AUC_{\infty(norm)}$, dose-normalized AUC_{∞} ; $t_{1/2}$, terminal plasma elimination half-life; CL, apparent total body clearance; V_{ss} , apparent volume of distribution at steady state. AUC values were determined by log-linear trapezoidal rule.

The pharmacodynamic activity of olaptosed pegol was studied by 5-color flow cytometry analysis using a standard diagnostic panel based on CD5, CD19, CD45, 7-AAD plus beads was performed 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) at pre-dose (baseline), 1, 3, 6, 24 and 72 hours after the pilot dose of olaptosed pegol as well as in combination treatment cycles 1 and 4 at pre-dose, 1, 3, 6 and 24 hours.

Immunogenicity assessment

Biotinylated olaptosed pegol was immobilized to a streptavidin coupled Biacore sensor chip. Serum samples were analyzed at a serum dilution of 1 in 4 in running buffer. Acceptable performance of the assay was demonstrated by the analysis of normal individual human serum samples previously shown to have negative, low and high levels of anti-polyethylene glycol (PEG) antibodies. Each test sample was analyzed in duplicate, blank and following immunodepletion with 10 μ M olaptosed pegol. Due to the presence of antibody responses prior to treatment, standard statistical approaches to calculate a positive response cut-point could not be applied. Instead, a patient-specific floating cut point was used (Shankar et al. J Pharm Biomed Analysis 2008). The cut point of percent increase above baseline was set to the 95th percentile of the normal distribution of the individual percent differences in pre-treatment samples and was determined to be 35.9%. Samples above the cut-point and specific to olaptosed pegol (immunodepletion > 20%) were re-analyzed after immunodepletion with PEG alone in order to clarify whether the antibodies were specific to the PEG or the oligonucleotide part of olaptosed pegol.

Supplemental Tables

Supplemental Table S1: Single dose pharmacokinetic parameters of olaptosed pegol

Dose Group	1 mg/kg	2 mg/kg	4 mg/kg
N	4	3	3
C _{max} [μmol/L]	1.76 ± 0.32	3.95 ± 1.43	7.20 ± 0.76
t _{max} [h]	1.50 ± 1.00	1.67 ± 1.15	2.33 ± 1.15
AUC _{0-72h} [μmol/L·h]	81.6 ± 28.8	133 ± 24	334 ± 85
AUC _{0-tz} [μmol/L·h]	177 ± 104	198 ± 30	561 ± 173
AUC [μmol/L·h]	200 ± 142	200 ± 31	569 ± 177
AUC _{extrap} [%]	6.4 ± 9.3	0.8 ± 0.3	1.3 ± 0.4
t _{1/2} [h]	78.7 ± 46.2	48.2 ± 3.5	53.2 ± 3.8
CL [mL/h]	27.3 ± 13.4	46.0 ± 9.1	36.1 ± 10.2
V _{ss} [L]	2.73 ± 0.57	3.32 ± 0.87	2.90 ± 0.63

All data are presented as arithmetic means and standard deviations.

C_{max}, peak plasma concentration; t_{max}, the amount of time olaptosed pegol is present at the maximum concentration; AUC, area under the plasma concentration–time curve; t_{1/2}, terminal plasma elimination half-life; CL, total body clearance; V_{ss}, volume of distribution at steady state.

Supplemental Table S2: Plasma concentration of olaptosed pegol after combined treatment

Sampling Time	Dose Level	N	Olaptosed Pegol [μmol/L]
Cycle 1 Day 1, pre-dose	1 mg/kg	28	-
Cycle 1 Day 1, 1 h post-dose		26	1.73 ± 0.45
Cycle 1 Day 2, 24 h post-dose		27	1.15 ± 0.38
Cycle 1 Day 8		28	0.174 ± 0.154
Cycle 1 Day 26-28		22	<0.002
Cycle 4 Day 1, pre-dose	4 mg/kg	22	0.0057 ± 0.0095
Cycle 4 Day 1, 1 h post-dose		21	6.44 ± 1.25
Cycle 4 Day 2, 24 h post-dose		21	4.74 ± 1.31
Cycle 4 Day 8		18	0.592 ± 0.411
Cycle 4 Day 26-28		17	0.0113 ± 0.0156

Data are presented as arithmetic means with standard deviation.

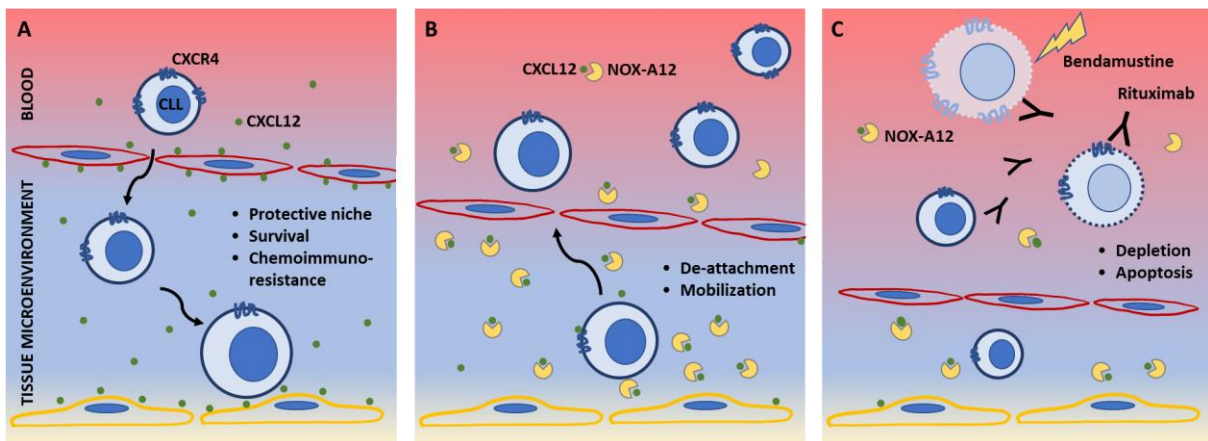
Supplemental Table S3: Response rates

Response	ITT (N=28) N (%)	FAS (N=27) N (%)	PPP (N=21) N (%)
CR	3 (10.7%)	3 (11.1%)	2 (9.5%)
PR	21 (75.0%)	21 (77.8%)	17 (81.0%)
SD	0	0	0
PD	3 (10.7%)	3 (11.1%)	2 (9.5%)
NE	1 (3.6%)	NA	NA
ORR (95%CI)	86% (67 – 96)	89% (71 – 98)	91% (70 – 99)

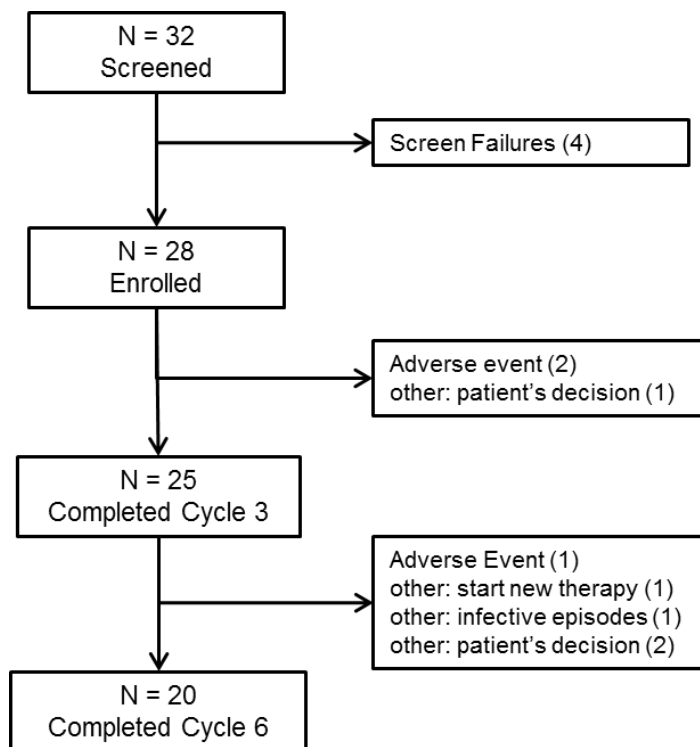
ITT: intent-to-treat population; FAS: full analysis set; PPP: per-protocol population. CR = complete remission, PR = partial response, SD = stable disease, PD = progressive disease, NE = not evaluable, ORR = overall response rate (\geq PR)

Supplemental Figures

Supplemental Figure S1: Mode of action of the combination of chemo-immunotherapy and olaptosed pegol. (A) CXCR4 positive CLL cells home towards the CXCL12 gradient into their protective niches within the bone marrow. (B) CXCL12 inhibition by olaptosed pegol (NOX-A12) releases the CLL cells from the protective niches and maintains them in circulation. (C) CLL cells in the peripheral blood are easily targeted by bendamustine and rituximab.



Supplemental Figure S2: Patient flow.

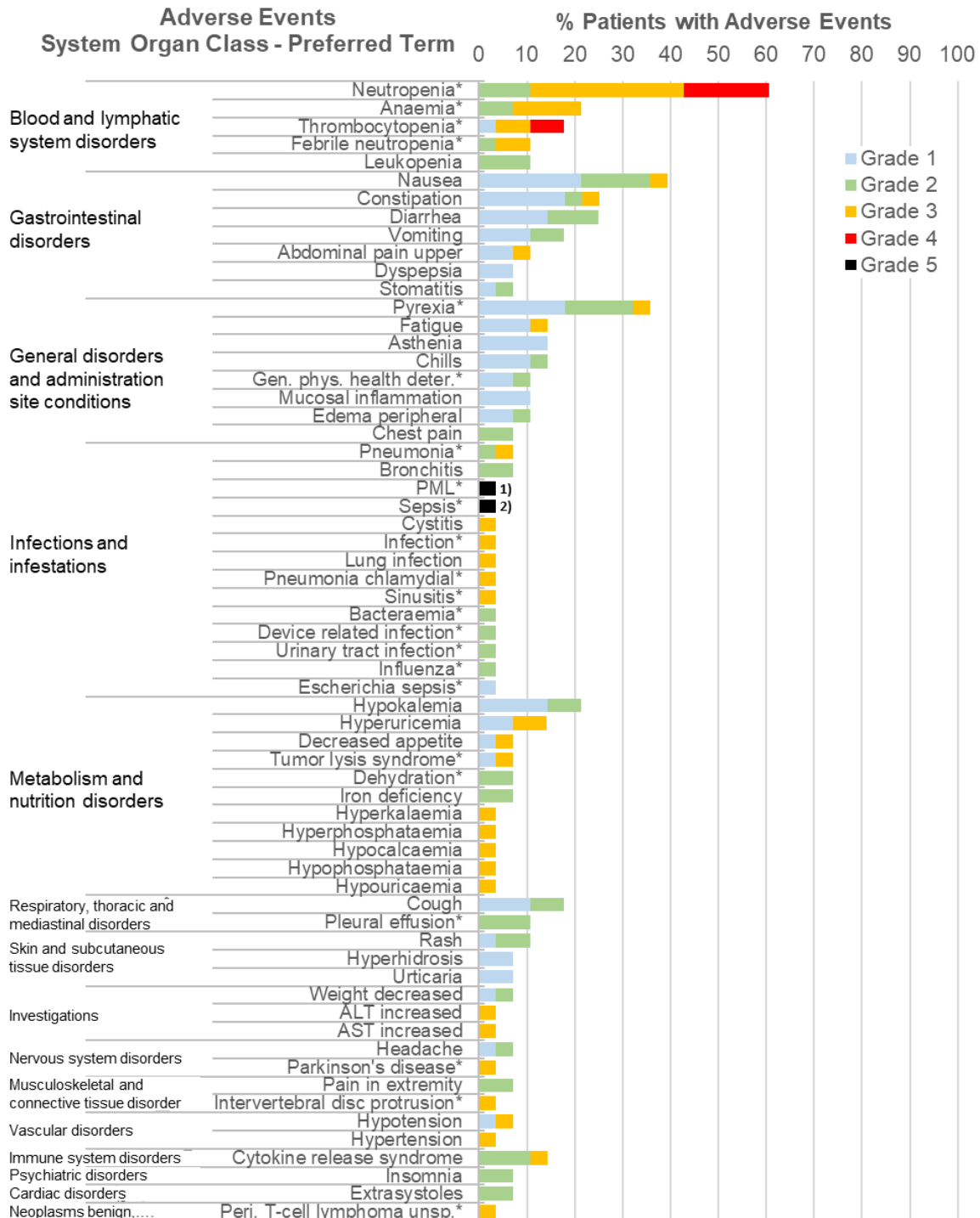


SCREEN FAILURES:

1. Not match exclusion criteria No. 3
2. Withdrawal of informed consent
3. Not match inclusion criteria No. 9
4. Patient has a special guardian for medical attentions

Supplemental Figure S3: Patients reporting adverse events (AEs) by highest CTCAE Grade.

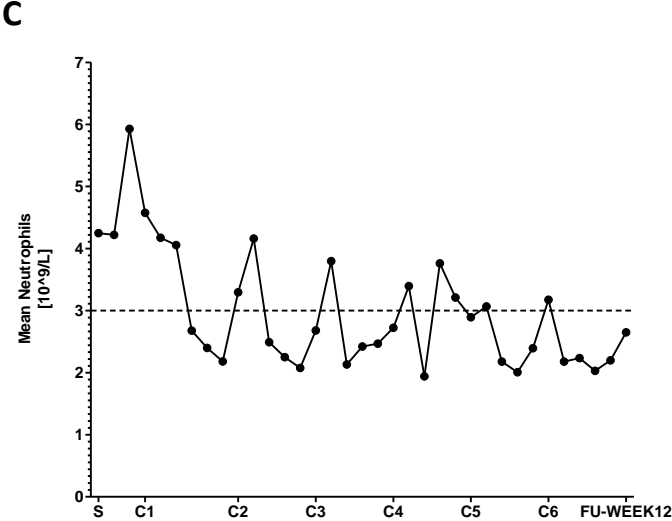
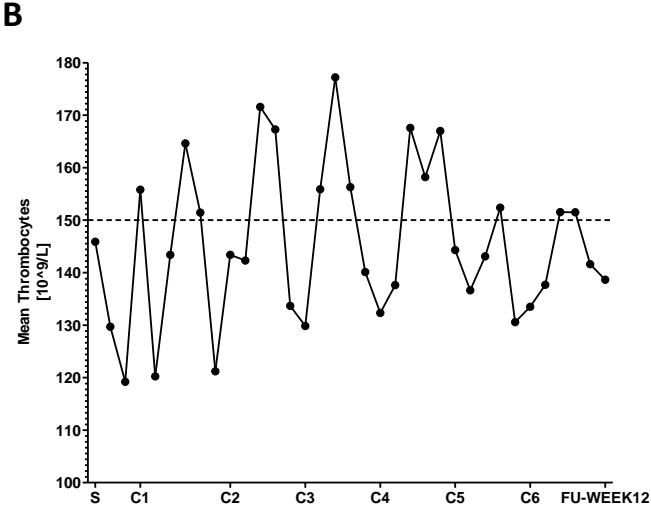
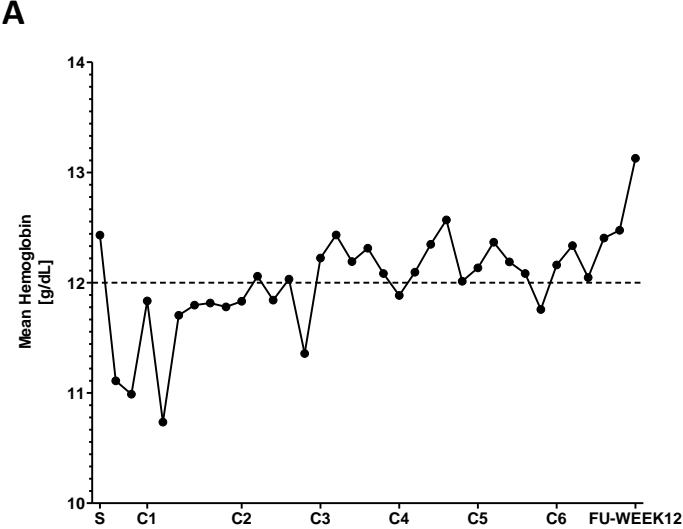
AEs which were reported in at least 2 patients with grade 1 or grade 2, or any patient with AE grade 3 or higher, are listed. AEs given as % of 28 patients are shown. Preferred terms including SAEs are indicated by an asterisk (*). Only the adverse event of highest severity grade (CTCAE) was counted in each patient.



- 1) Fatal progressive multifocal leukoencephalopathy (PML) occurred 9 months after the last treatment (during follow-up) and was assessed as not related to treatment with olaptesed pegol considering the long post-treatment interval.
- 2) Sepsis occurred 21 months after the last administration of study medication (during follow-up) and was assessed as not related to treatment with olaptesed pegol considering the long post-treatment interval.

Supplemental Figure S4: Hematological parameters throughout the treatment.

Development of mean A) haemoglobin values (g/dL), B) thrombocyte numbers (10⁹/L) and C) neutrophil numbers (10⁹/L) in the ITT patient population throughout the treatment course which were evaluated at different time points during screening (S) and cycle 1 (C 1) to cycle 6 (C 6) as well as the follow-up (FU) period.



References

- 1 Vater, A. *et al.* Hematopoietic stem and progenitor cell mobilization in mice and humans by a first-in-class mirror-image oligonucleotide inhibitor of CXCL12. *Clin Pharmacol Ther* **94**, 150-157, doi:clpt201358 10.1038/clpt.2013.58 (2013).