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OPEN

Olaptesed pegol, an anti-CXCL12/SDF-1 Spiegelmer, alone and with bortezomib–dexamethasone in relapsed/refractory multiple myeloma: a Phase IIa Study

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Olaptesed pegol (olaptesed, NOX-A12) is a pegylated L-oligoribonucleotide that binds and neutralizes CXCL12, a chemokine which signals through CXCR4 and CXCR7 regulating a variety of processes during multiple myeloma (MM) development.[1] CXCL12 inhibition reduces the myeloma-supportive activity of the bone marrow microenvironment and mobilizes myeloma cells to the circulation.[2] In addition, CXCL12α levels were reported to correlate with osteolytic bone lesions and with increased bone marrow angiogenesis.[3] Targeting CXCL12, therefore, is a promising strategy for disrupting myeloma-stroma interactions and inhibiting myeloma growth and survival. Here, we report pharmacokinetic, pharmacodynamic, safety and efficacy data of olaptesed in patients with relapsed/refractory MM and present data on baseline CXCL12α levels in this patient cohort. This first-in-patient Phase IIa study aims to translate the novel concept of combining proteasome and CXCL12 inhibition into the clinic and builds on a preclinical proof-of-concept regarding the significance of CXCL12 blockade in MM[2] and on Phase I data in healthy subjects.[4] Combining CXCL12 inhibition with bortezomib and dexamethasone (VD) was investigated in 28 patients with relapsed/refractory MM, who were either bortezomib-naive or considered not refractory to bortezomib. The median number of prior lines of therapy was 2 (range: 1–5), 39 and 14% of the patients presented with $\geqslant 3$ or $\geqslant 4$ prior lines, respectively. Cytogenetics were tested in 21 patients and high risk features were found in 36% of them. 54% of patients had prior treatment with bortezomib, 39% had a prior stem cell transplant and 57% were refractory to prior treatment. Further details on patient characteristics (Supplementary Table S1) and inclusion and exclusion criteria are available in the Supplementary Information. In the pilot phase, three cohorts of three patients each (four patients in the 1 mg/kg cohort due to patient replacement) were administered single doses of 1, 2 or 4 mg/kg of olaptesed alone by slow intravenous bolus injection 2 weeks prior to starting the combination treatment. Combination treatment was administered for eight cycles of 21 days. An intra-patient escalation was applied for safety reasons as olaptesed was combined for the first time with VD in cancer patients. Olaptesed was given 1–2 h prior to bortezomib at doses of 1 mg/kg in cycle 1, 2 mg/kg in cycle 2 and

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4 mg/kg in cycles 3–8. Bortezomib was given on days 1, 4, 8 and 11 of each 21-day treatment cycle as intravenous injection of 1.3 mg/m^2. Oral dexamethasone (20 mg) was added on the day of and the day after bortezomib administration. An outline of the study and details of patients' flow are given in Supplementary Figures S1 and S2.

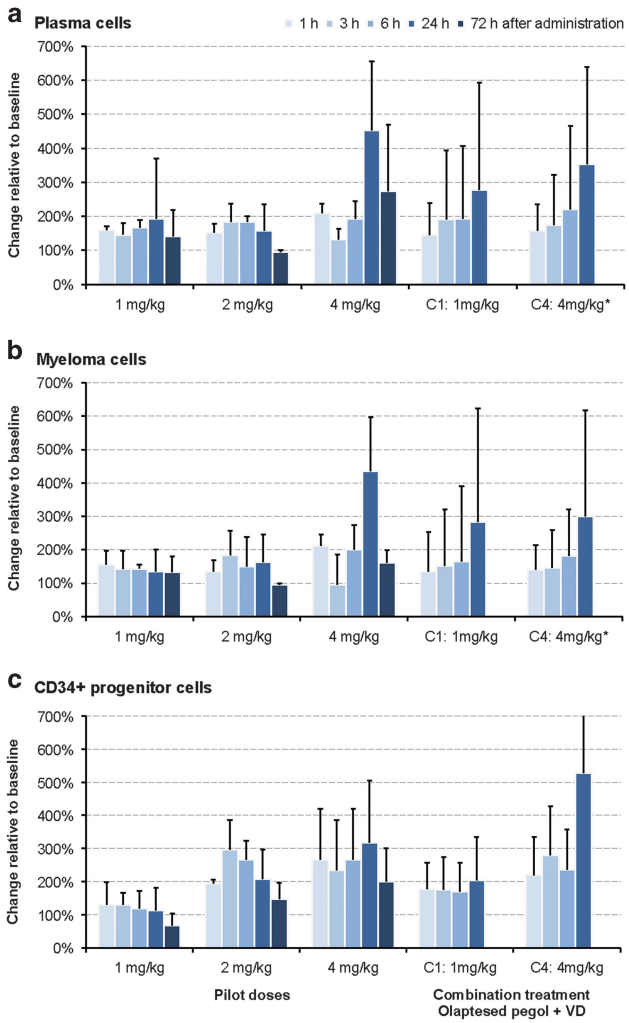

Figure 1. Mobilization kinetics of plasma cells (**a**), myeloma cells (**b**) and CD34$^+$ stem cells (**c**) after escalating doses of olaptesed pegol alone and in combination with VD. Data are depicted as change relative to baseline, which was set at 100%. Arithmetic means including s.d. are shown. C1, cycle 1; C4, cycle 4. * A single value for Cycle 4, 3 h was excluded as outlier.

The determination of the mean basal CXCL12α plasma concentrations revealed significantly higher levels in our patients (3232 (±608) pg/ml) compared with 20 healthy subjects (1664 (±264) pg/ml, $P < 0.0001$) (Supplementary Figure S3). The plasma pharmacokinetics of olaptesed were similar to those observed in healthy subjects.[4] Peak plasma concentrations increased in an approximately dose-linear way with mean peak levels of 2.12, 3.94 and 6.89 µmol/l at doses of 1, 2 and 4 mg/kg, respectively (Supplementary Figure S4 and Supplementary Table S2). The terminal elimination half-lives were in the range of the mean plasma elimination half-life of 38.5 h observed in healthy subjects at a dose of 2.7 mg/kg.[4] Peak plasma concentrations at cycles 1 and 4 when olaptesed was administered in combination with VD were similar to single-dose agent values (Supplementary Table S3).

The pharmacodynamic effects were evident 1 h after administration of olaptesed with mobilization of CD38+ CD138+ plasma cells and CD38+ CD138+ CD56+ CD19− myeloma cells resulting in up to three-fold increases compared with baseline values in the peripheral blood. Mobilization of plasma cells (Figure 1a) and myeloma cells (Figure 1b) remained increased for at least 72 h without returning to baseline levels. Administration of olaptesed in combination with VD during treatment cycles 1 and 4 resulted in mobilization profiles comparable to those observed after administration of olaptesed alone (Figures 1a and b). CD34+ stem cells, which were assessed as an internal control, were similarly mobilized by olaptesed (Figure 1c). A trend to higher cell mobilization with higher drug exposure was observed for all three cell types namely plasma, myeloma and CD34+ stem cells (Supplementary Figure S5). These data extend our previous observation[4] of a significant and clinically relevant mobilizing capacity of olaptesed pegol.

Response rates for all patients (intent-to-treat (ITT) population) are shown in Table 1. A partial response (PR) or better was obtained in 19 of the 28 patients (68%). Complete response (CR) was noted in 2 (7%), a very good PR (VGPR) in 5 (18%), a PR in 12 (43%) patients, whereas 2 (7%) patients achieved a minor response. Hence, the clinical benefit rate added up to 75%. The overall response rate (ORR) was similar in patients with and without high-risk cytogenetics (70% and 73%, respectively), but was slightly lower (60%) in patients previously exposed to bortezomib; in the latter subgroup no patient achieved a VGPR and 1 (7%) patient achieved a CR. A VGPR was observed in 5 (39%) bortezomib-naive patients and 1 (8%) patient achieved a CR. Patients without a prior stem cell transplant had a higher ORR

Table 1. Response rates in the ITT population and response by subgroups

Parameter	*ITT*	*Cytogenetic risk*		*Prior bortezomib treatment*		*Prior stem cell transplant*		*Refractory*	
		High	*Standard*	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*
# of patients	28	10 (36%)	11 (40%)	15 (54%)	13 (46%)	11 (39%)	17 (61%)	16 (57%)	12 (43%)
ORR	19 (68%)	7 (70%)	8 (73%)	9 (60%)	10 (77%)	6 (55%)	13 (77%)	11 (69%)	8 (67%)
CR	2 (7%)	0	1 (9%)	1 (7%)	1 (8%)	1 (9%)	1 (6%)	1 (6%)	1 (8%)
VGPR	5 (18%)	3 (30%)	1 (9%)	0	5 (39%)	1 (9%)	4 (24%)	3 (19%)	2 (17%)
PR	12 (43%)	4 (40%)	6 (55%)	8 (53%)	4 (31%)	4 (36%)	8 (47%)	7 (44%)	5 (42%)
MR	2 (7%)	1 (10%)	1 (9%)	1 (7%)	1 (8%)	1 (9%)	1 (6%)	1 (6%)	1 (8%)
SD	5 (18%)	1 (10%)	1 (9%)	4 (27%)	1 (8%)	3 (27%)	2 (12%)	3 (19%)	2 (17%)
PD	1 (4%)	1 (10%)	0	1 (7%)	0	1 (9%)	0	1 (6%)	0
Not evaluable	1 (4%)	0	1 (9%)	0	1 (8%)	0	1 (6%)	0	1 (8%)

Abbreviations: CR, complete response; ITT, intent-to-treat; MR, minor response; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

(77%) than the autografted patients (55%). The ORR in refractory patients (69%) was comparable to that observed in non-refractory patients (67%). Notably, the median time from the end of the last treatment line to first treatment with olaptesed was only 1.8 months (range 0–22.2) for refractory patients and 17.5 months (range 2.7–119.7) for non-refractory patients.

The median (95% confidence interval) progression-free survival (PFS) was 7.2 months (4.7–8.3) in the full analysis set. PFS was only slightly lower in refractory patients, in those previously exposed to bortezomib, and in patients with high-risk cytogenetics (6.7, 6.8 and 6.7 months, respectively). The median overall survival (OS) in the ITT population was 28.3 months. Further details on PFS and OS in subgroups of patients are given in Supplementary Figure S6. In general, treatment with olaptesed was well tolerated and did not result in relevant additional toxicity when combined with VD. Thrombocytopenia and anemia were the most frequent hematologic adverse events. Five (17.9%) patients experienced grade 1–2, 4 (14.3%) patients grade 3 and 2 (7.1%) patients grade 4 thrombocytopenia. Grade 1–2 anemia was noted in 7 (25.0%) and grade 3 in 4 (14.3%) patients. Diarrhea was the most frequent non-hematologic toxicity, which was defined as grade 1–2 in 11 (39.3%) patients and grade 3 in 3 (10.7%) patients. Grade 1–2 constipation was noted in 7 (25%) patients, grade 3 was observed in 2 (7.1%) patients. Any kind of neuropathy was reported in 15 (53.6%) patients, but all respective adverse events were of grade 1–2, with no higher grades reported. Details of the safety profile are presented in Supplementary Figure S7. Consistent with previous assessment of immunogenicity of olaptesed in healthy volunteers,[4] no relevant pre-existing or drug-induced antibodies neither against polyethylenglycol nor the oligonucleotide moiety were detected.

Although we acknowledge limitations of cross trial comparisons, we note that the ORR of 68% compares favorably with early bortezomib studies, such as the Apex,[5] the subcutaneous versus intravenous bortezomib[6] or the BoMER trial,[7] which reported response rates of 43%, 42% and 53%, respectively, but dexamethasone was only added in the latter trial. In recently conducted Phase III studies, the VD control arms of the Panorama,[8] Endeavor[9] and Castor[10] trials reported response rates of 55%, 63% and 63%, respectively. Notably, patients in these trials either had a lower number of previous therapy lines and/or a better International Staging System stage. Our results are comparable to other bortezomib-based combination treatments for relapsed/refractory MM, for example, 66% for VD plus either cyclophosphamide or lenalidomide[11] and 60.8% for VD plus bendamustine and dexamethasone.[12] The CXCR4 inhibitors ulocuplumab and plerixafor in combination with VD yielded an ORR of 40%[13] and 51%,[14] respectively. These lower efficacy rates compared with our results may be due to the different modes of action of targeting the CXCR4 receptor in contrast to olaptesed, which neutralizes the CXCL12 ligand. The inhibitory activity of plerixafor is overcome by high concentrations of CXCL12 as shown in Supplementary Figure S8, whereas the activity of olaptesed was independent from the concentration of the ligand. Furthermore, due to the role of CXCR7 signaling in MM progression,[15] the complete blockade of the CXCL12/CXCR4/CXCR7 axis achieved by olaptesed may be superior to blockade of CXCR4 receptor signaling only.

In conclusion, the data from our study clearly demonstrate that treatment with olaptesed results in effective mobilization of myeloma cells for at least 72 h and seems to enhance the clinical activity of VD with ORR of 68% in the ITT population. Olaptesed alone was safe and well-tolerated, and when combined with VD did not result in relevant additional toxicity. These data warrant further clinical development of this novel inhibitor of CXCL12 in combination with established and new anti-myeloma drugs in randomized studies.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

HL, KR, AK and MB designed the research. HL, KW, MTP, XL, AMC, LG, CL, RF, RG, ME and IYA performed clinical research. DZ, SV, TD and DB performed preclinical research and/or analysis, and interpretation of data. HL, KR, AK and MB wrote the initial draft of the manuscript and edited the final draft. All authors reviewed the manuscript in several rounds and approved the final version for submission.

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Gain of function in Jak2^{V617F}-positive T-cells

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Myeloproliferative neoplasms (MPNs) are clonal disorders of aging hematopoietic stem cells and early myeloid progenitors. A somatic activating point mutation in the Jak2 tyrosine kinase (Jak2^{V617F}) is the most prevalent genetic aberration in BCR-ABL-negative MPNs. Janus kinases (JAKs) are essential for cytokine-induced intracellular signaling and their inactivation leads to impaired immune cell function. Therapeutic inhibition of JAKs in MPN patients causes decreased number and function of immune cells,[1,2] and therefore may contribute to increased incidence and re-activation of viral infections.[3] Jak2^{V617F} mutation is detectable in hematopoietic stem and progenitor cells of MPN patients and has also been described in lymphoid progenitors and more differentiated lymphocytes,[4–7] including Jak2^{V617F} mutated T-cells.[4] However, depending on the allelic burden, incidence and clone size, the number of Jak2^{V617F} mutated T-cells may have been underestimated, and it is currently unclear whether both CD4$^+$ and CD8$^+$ T-lymphocytes may be equally affected by the Jak2^{V617F} mutation.

Therefore, we aimed to assess for the frequency of Jak2^{V617F} mutation in CD3$^+$ T-lymphocytes of patients with high-allelic-burden MPNs and investigated the impact of Jak2^{V617F} mutation on T-cell function *in vivo*. A model with T-cell-specific Jak2^{V617F} expression was analyzed under physiological conditions and upon infection with an intracellular pathogen.

To address the frequency of JAK2^{V617F} mutations in human T-cells, we selected 13 MPN patients from our institutional database diagnosed with Jak2^{V617F}-positive polycythemia vera, essential thrombocythemia or myelofibrosis (PMF) that exhibited an allelic burden of more than 50% in peripheral blood granulocytes. CD3$^+$ T-cells and granulocytes were FACS-sorted and analyzed for quantitative expression of Jak2^{V617F} (Figure 1a). Six out of thirteen patients tested positive for Jak2^{V617F} in sorted CD3$^+$ cells, with an allelic burden between 2 and 47.8% within the T-cell compartment (Figure 1a). When analyzing sorted CD3/CD4 and CD3/CD8 T-cells of three additional patients with a high allelic burden separately, Jak2^{V617F} burden was comparable in both T-cell subsets (Figure 1b). These data suggest that JAK2^{V617F} mutations may be more frequent in patients with high allelic burden, especially in those diagnosed with polycythemia vera and PMF. The presence of JAK2^{V617F} in both CD4$^+$ and CD8$^+$ T-cells is consistent with a genetic event that arises at the stem- and progenitor-cell level.

To explore the T-cell-specific function of Jak2^{V617F} mutated clones *in vivo*, we crossed C57BL/6 Jak2^{V617F} [8] with CD4-Cre mice. The resulting CD4-Cre Jak2^{V617F} mice (henceforth designated as Jak2$^{+/VF}$) exhibited heterozygous expression of Jak2^{V617F} in CD4$^+$ and CD8$^+$ T-cells but not in the myeloid lineage (for example, granulocytes, Figure 1c). Those animals were compared to wild-type and Cre-negative littermate controls (henceforth designated as Jak2$^{+/+}$).

Activation of Jak2^{V617F} in T-cells could potentially lead to alteration of thymic development and composition of T-cell subsets. However, T-cell development in the thymus (Supplementary Figure 1a) of Jak2$^{+/VF}$ animals remained unchanged and comparable to Jak2$^{+/+}$ littermate controls. Moreover, numbers of splenic naive (CD62L$^+$ CD44$^-$), memory-like (CD62L$^+$ CD44$^+$) and effector (CD62L$^-$ CD44$^+$) CD4$^+$ and CD8$^+$ T-cells (Supplementary Figure 1b), numbers of Foxp3$^+$ CD25$^+$ CD4$^+$ regulatory T-cells (Supplementary Figure 1c), and expression of the T-cell activation marker CD69 (Supplementary Figure 1d) were equal in both strains of mice. Importantly, Jak2$^{+/VF}$ mice did not develop any clinical signs or symptoms that could be attributed to immunedefects or dysregulation of autoimmunity.

Activated T-cells play an important role in protecting the organism from infections with intracellular pathogens, such as intracellular bacteria and viruses. To investigate the function of Jak2^{V617F} T-cell clones in a relevant model, we analyzed T-cell responses upon infection with *Listeria monocytogenes* (Lm), a Gram-positive facultative intracellular bacterium (Figure 1d). In animals and humans, Lm causes severe brain, intestine, liver and bloodstream infections. While T-cell function is crucial for the elimination of Lm, experimental studies in mice revealed that innate immune cells (dendritic cells, macrophages, inflammatory monocytes and natural killer (NK) cells) additionally contribute to

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