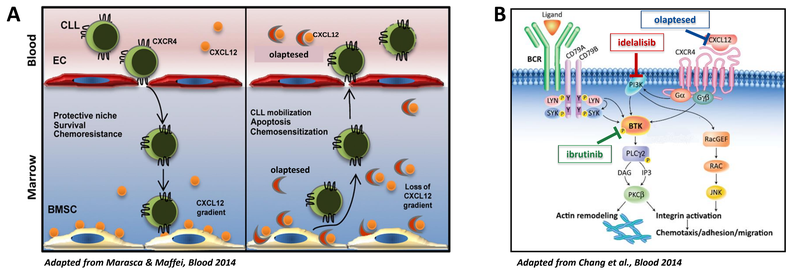
Comparison of Ibrutinib, Idelalisib and Olaptesed Pegol on the Immune Effector Function Mediated by Rituximab and Obinutuzumab

Dirk Zboralski, Axel Vater and Anna Kruschinski

NOXXON PHARMA AG

BACKGROUND & RATIONALE

Olaptesed pegol (NOX-A12), a CXCL12 binding Spiegelmer®, was found to detach CXCL12 from the surface of bone marrow stromal cells (Hoellenriegel & Zboralski *et al*., Blood 2014) and to long-term mobilize CXCR4 expressing, malignant cells from protective niches in the bone marrow or other secondary lymphoid tissues, thereby sensitizing them to standard therapy (Figure 1A) (Roccarro *et al*. 2014, Hinterseer *et al*. 2013). This therapeutic concept was corroborated in two Phase IIa trials in combination with bendamustine and rituximab in patients with Chronic Lymphocytic Leukemia (abstract #1996) and in combination with bortezomib and dexamethasone in patients with Multiple Myeloma (abstract #2111).



Adapted from Marasca & Maffei, Blood 2014 — *Adapted from Chang et al., Blood 2014*

Figure 1. Overlapping mechanisms of actions of olaptesed pegol compared to ibrutinib or idelalisib. A Olaptesed pegol (NOX-A12) binds CXCL12 and detaches it from the surface of bone marrow stromal cells (BMSCs), thereby neutralizing the chemokine gradient and inhibiting signaling via CXCR4 and CXCR7 receptors. As a consequence, olaptesed mobilizes CLL cells from their protective microenvironment, inducing apoptosis and chemo-sensitization of leukemic cells. **B** BTK and PI3Ks are involved in CXCR4 signaling. This cross-talk might be associated with the clinical response to BTK and PI3Kδ inhibitors such as ibrutinib and idelalisib, which is characterized by "mobilization" of tissue-resident CLL cells into the blood, analogous to the clinical response of olaptesed pegol.

Instead of targeting the genetically unstable tumor cells, olaptesed selectively targets the tumor microenvironment, thereby increasing the efficacy of anti-cancer therapy.

In addition to malignant cells, CXCR4 expressing immune cells are effectively mobilized by olaptesed. In a phase I clinical trial with healthy volunteers a long-term mobilization of CXCR4-expressing cells was observed, such as CD34+ stem cells, B cells, T cells, neutrophils and monocytes (Figure 2).

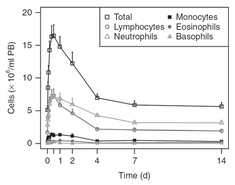


Figure 2. Olaptesed-mediated mobilization of various immune cells in a Phase I clinical trial with healthy volunteers (Vater *et al.* 2013).

The mechanism of action of olaptesed is partly overlapping with ibrutinib (BTK inhibitor) and idelalisib (PI3Kδ inhibitor). Ibrutinib and idelalisib induce a transient lymphocytosis, accompanied by a reduction of lymphoid organ size, suggesting that the mode of action of these drugs involves the mobilization of CLL cells from this microenvironment into the blood (Woyach *et al*. 2014, Brown *et al*. 2014). Mechanistically, BTK and PI3Kδ inhibition was observed to interfere with homing and adhesion of CLL cells, likely by the involvement of BTK and PI3Kδ in CXCL12/CXCR4 signaling in CLL cells (Figure 1B) (Ponader *et al.* 2012, Hoellenriegel *et al*. 2011). However, it has been shown that ibrutinib antagonizes rituximab-mediated antibody-dependent cellular cytotoxicity (ADCC) (Kohrt *et al*. 2014). A recent study showed that besides the inhibition of ADCC, also antibody-dependent cellular phagocytosis (ADCP) is inhibited by ibrutinib and that also idelalisib inhibits immune-cell functions (Da Roit *et al.* 2014).

Based on the mechanistic and pharmacodynamic similarities of olaptesed compared to ibrutinib or idelalisib, the aim was to analyze whether olaptesed influences the immune effector cell function mediated by rituximab or obinutuzumab as has been shown for the BTK or PI3Kδ inhibitors.

RESULTS

Antibody Dependent Cellular Cytotoxicity (ADCC)

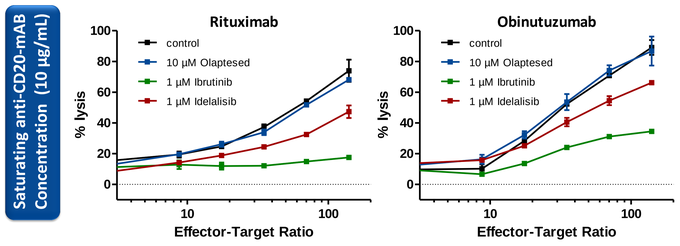


Figure 3 . PBMCs were used as effector cells and exposed to clinically relevant concentrations of olaptesed, ibrutinib or idelalisib overnight. Calcein stained Raji cells were used as target cells and were opsonized with rituximab or obinutuzumab before effector cells were added for 4 hours. Fluorescence intensity of supernatants was quantified and normalized to % lysis.

Olaptesed does not interfere with ADCC, whereas ibrutinib and idelalisib decrease ADCC activity of rituximab and obinutuzumab.

Antibody Dependent Cellular Phagocytosis (ADCP)

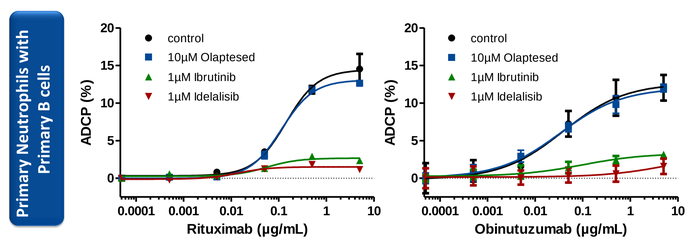


Figure 4. Neutrophils were isolated from fresh blood and monocytes from PBMCs. Phagocytic cells were incubated with 10 µM olaptesed, 1 µM ibrutinib or idelalisib for 6 hours before rituximab- or obinutuzumab-opsonized B cells were added in a ratio of 3:1. After 16 hours of incubation ADCP was quantified in a flow cytometer. Percentage ADCP was calculated by counting phagocytes with internalized B cells and normalizing to total phagocytes.

Olaptesed does not influence ADCP, whereas ibrutinib and idelalisib decrease ADCP activity of rituximab and obinutuzumab in neutrophils and monocytes.

B-Cell Depletion in Whole Blood

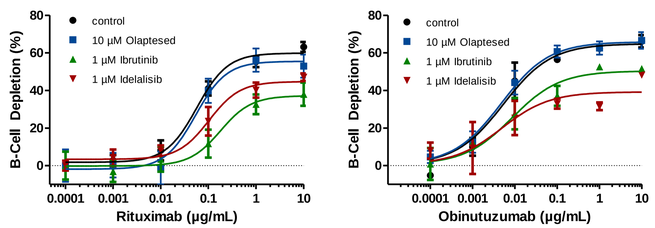


Figure 5. Heparin-treated whole blood was exposed to 10 µM olaptesed, 1 µM ibrutinib or idelalisib for 6 hours before different concentrations of rituximab or obinutuzumab were added. After 18 hours B-cell depletion is quantified by flow cytometry. Results are evaluated by gating CD45-positive cells and determining the CD19-positive B-cell and CD3-positive T-cell populations therein.

Olaptesed does not influence CD20-mAB activity in whole blood, whereas ibrutinib and idelalisib decrease rituximab- and obinutuzumab-mediated B-cell depletion in physiological conditions.

CONCLUSION & OUTLOOK

- Olaptesed pegol showed no negative interference with the immune effector function of anti-CD20-mABs (and potentially other mABs as well), whereas ibrutinib and idelalisib inhibited ADCC and ADCP.
- Instead, neutrophils and monocytes are effectively mobilized with olaptesed which may increase ADCC and ADCP, important mechanisms of action for glycoengineered obinutuzumab (Golay *et al.* 2013).
- Furthermore, olaptesed may enhance the immune system contribution by mobilization of T cells which are described to be exhausted in the peripheral blood of hematological malignancies like CLL (Riches *et al.* 2013).

Olaptesed represents an ideal combination partner for mABs, as it not only mobilizes malignant cells from protective tissues that may not be accessible to mABs, but also leads to effective mobilization of immune cells without impairing immune effector activity and adding toxicity.

56th ASH Annual Meeting, San Francisco, CA
Abstract #4499, Session Name: 625. Lymphoma: Pre-Clinical - Chemotherapy and Biologic Agents.
Monday, December 8, 2014

Conflict of Interest Disclosures & Acknowledgments
DZ, AV, AK: NOXXON Pharma AG: Employment.
We like to thank Kai Riecke for blood collection and Lisa Bauer for PBMC preparation.

References: Brown *et al.,* Blood. 2014 May 29;123(22):3390-7; Chang *et al.,* Blood. 2013 Oct 3;122(14):2412-24; Da Roit *et al.,* Haematologica. 2014 Oct 24; Golay *et al.,* Blood. 2013 Nov 14;122(20):3482-91; Hinterseer *et al.,* Blood 2013; 122(21):4111; Hoellenriegel *et al.,* Blood. 2011 Sep 29;118(13):3603-12; Hoellenriegel & Zboralski *et al.,* Blood. 2014 Feb 13;123(7):1032-9; Kohrt *et al.,* Blood. 2014 Mar 20;123(12):1957-60; Marasca & Maffei, Blood 2014;123:952-953; Ponader *et al.,* Blood. 2012 Feb 2;119(5):1182-9; Riches *et al.,* Blood. 2013 Feb 28;121(9):1612-21; Roccaro *et al.,* Cell Rep. 2014 Oct 9;9(1):118-28; Vater *et al.,* Clin Pharmacol Ther. 2013 Jul;94(1):150-7; Woyach *et al.,* Blood. 2014 Mar 20;123(12):1810-7

Dirk Zboralski
Senior Scientist - In Vitro Pharmacology
Tel.: + 49 - (0)30 - 726247 - 134
Email: dzboralski@noxxon.com