CXCL12 Inhibition by NOX-A12 (Olaptesed Pegol) Synergizes With the ADCC Activity of CD20 Antibodies by Increasing NK Cell Infiltration in a 3D Lymphoma Model

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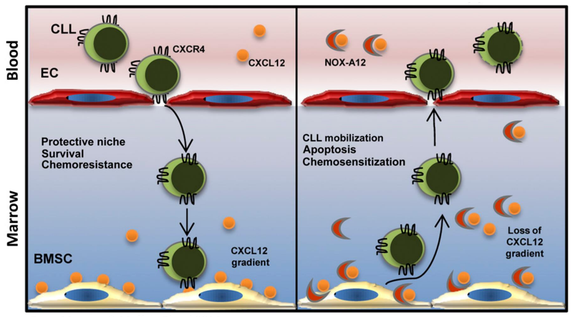
BACKGROUND & RATIONALE

NOX-A12 (olaptesed pegol), a CXCL12 binding Spiegelmer® (L-RNA aptamer), was found to detach CXCL12 from the surface of bone marrow stromal cells (Hoellenriegel *et al*., 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 1; Roccaro *et al*. 2014). This therapeutic concept was corroborated in two Phase IIa trials in combination with bendamustine and rituximab in patients with chronic lymphocytic leukemia (CLL; Steurer *et al*., 2014) and in combination with bortezomib and dexamethasone in patients with multiple myeloma (MM; Ludwig *et al.,* in press).



From Marasca & Maffei , Blood 2014

Figure 1. Mechanism of action of NOX-A12. NOX-A12 binds the chemokine 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, NOX-A12 mobilizes CLL cells from their protective microenvironment, inducing apoptosis and chemosensitization of leukemic cells.

Instead of targeting the genetically unstable tumor cells, NOX-A12 selectively targets the tumor microenvironment, thereby increasing the efficacy of anti-cancer therapy.

Hematological malignancies are characterized by the expansion of malignant cells in the peripheral blood and in stroma-rich niches such as the bone marrow or lymphoid tissues. Anti-CD20 monoclonal antibodies (mAbs) are highly effective in eliminating malignant cells in the peripheral blood with the help of immune effector cells, e.g. NK cells mediating antibody-dependent cellular cytotoxicity (ADCC). However, residual malignant cells often continue to persist in protective stromal niches. These compartments have similarities to the solid tumor microenvironment (TME) where mAb therapy is limited by poor tissue penetration and low effector cell infiltration. Recently we have shown that NOX-A12 increases lymphocyte infiltration into solid tumor-stroma spheroids, thereby synergizing with anti-PD-1 checkpoint blockade (Zboralski *et al.,* 2016). Here we established 3D lymphoid spheroidal microtissues mimicking the stroma-rich and CXCL12-abundant TME of lymphoid malignancies (Figure 2).

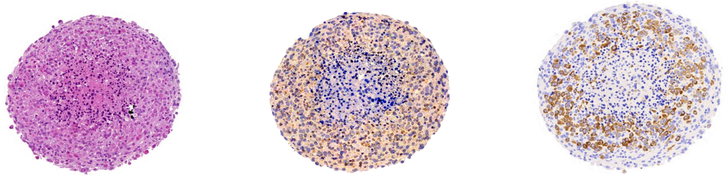


Figure 2. Cross section of a representative tumor-stroma spheroid. Spheroids were generated by co-culturing of CXCL12-expressing murine stromal MS-5 cells and tumor cells in ultra-low attachment plates for 3 days.

NOX-A12 was shown to facilitate lymphocyte infiltration into dense cellular compartments. The aim of this study was to investigate the effect of NOX-A12 on NK effector cell infiltration into lymphoma/stroma spheroids and to test the combination with anti-CD20 mAbs.

RESULTS

Primary human NK cells isolated from healthy donors were added to the lymphoma-stroma cell spheroids in the presence of various NOX-A12 concentrations. NOX-A12 increased the amount of NK cells in the spheroids up to 8-fold in a dose-dependent manner (Figure 3), likely by forming *de novo* CXCL12 gradients into the dense microtissue due to the particular penetration characteristic of Spiegelmers.

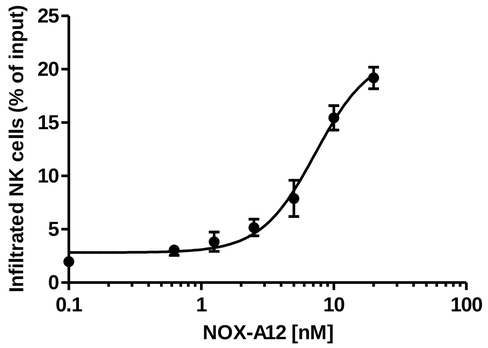


Figure 3. NK cell infiltration. Spheroids of MS-5 cells with CFSE-stained Raji lymphoma cells were generated and incubated overnight with primary human NK cells in the presence of various concentrations of NOX-A12. Spheroids were dissociated for NK cell quantification and determination of Raji cell viability by flow cytometry.

We found that the ADCC efficacy of anti-CD20 mAbs is lower in 3D spheroids compared to conventional 2D assays due to low NK cell infiltration into the microtissues. However, we found that adding NOX-A12 significantly enhanced the ADCC activity of obinutuzumab and rituximab (Figure 4). This is likely due to the fact that by increasing the infiltration rate of NK cells, NOX-A12 facilitates direct contact of the effector cells with their malignant targets, thereby increasing the efficacy of anti-CD20 mAbs. The NOX-A12-mediated increase of NK cells in the spheroids synergized with both anti-CD20 mAbs tested in terms of NK cell-mediated killing of lymphoma cells (Figure 4).

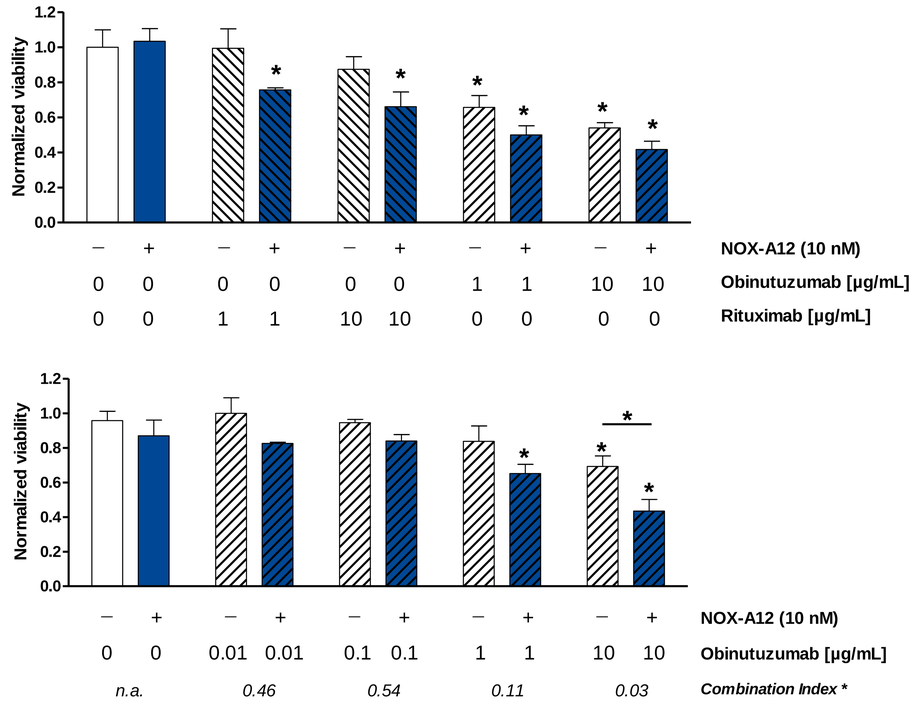


Figure 4. NOX-A12 acts synergistically with anti-CD20 mAbs. Spheroids were generated by co-culturing of MS-5 stromal cells and CFSE-stained Raji lymphoma cells in ultra-low attachment plates for 24 hours. Various concentrations of NOX-A12 and either obinutuzumab or rituximab were added to the spheroids and incubated with primary human NK cells overnight. Spheroids were dissociated for determination of Raji cell viability by flow cytometry (*Combination Index < 1 indicates synergy).

CONCLUSION & OUTLOOK

- In an effort to mimic the complexity of the lymphoid tumor microenvironment, tumor-stroma spheroids were established
- The CXCL12 inhibitor NOX-A12 increases the infiltration of NK cells into these tumor-stroma spheroids
- By facilitating physical contact of NK cells with tumor cells, NOX-A12 synergizes with NK cell-mediated ADCC

The present work complements the mode of action data of NOX-A12 by adding enhancement of NK cell infiltration into stroma-rich tumor compartments to the already established effects of mobilizing malignant and immune effector cells into the peripheral blood (Vater *et al.,* 2013). These data as well as the good toxicity profile and the promising data in phase IIa clinical trials in patients with CLL and MM justify further clinical trials in patients with hematological malignancies to verify the synergy of combination treatment employing ADCC-inducing mAbs and NOX-A12.

58th ASH Annual Meeting, San Diego, CA
Abstract #3021, Session Name: 625. Lymphoma: Pre-Clinical – Chemotherapy and Biologic Agents: Poster II. Sunday, December 4, 2016

Conflict of Interest Disclosure:
DZ, AK, DE and AV: NOXXON Pharma AG: Employment.

References:
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