CXCL12 Inhibition with NOX-A12 (Olaptesed Pegol) Increases T and NK Cell Infiltration and Synergizes with Immune Checkpoint Blockade and ADCC in Tumour-Stroma Spheroids

Dirk Zboralski, Anna Kruschinski, Dirk Eulberg and Axel Vater

NOXXON PHARMA AG

BACKGROUND & RATIONALE

T and NK cell-based cancer immunotherapy requires physical contact between immune effector cells and malignant cells, which is generally restricted by the tumour microenvironment (TME).[1] The chemokine CXCL12 has recently been described as a T cell exclusion factor in the TME-driven immune suppression.[2] The clinical stage L-aptamer (Spiegelmer®) NOX-A12 (olaptesed pegol) was found to detach CXCL12 from the surface of stromal cells[3] and to block CXCL12 binding to CXCR4 and CXCR7[4,5]. In this study we aimed to investigate whether NOX-A12 is able to enhance T and NK cell infiltration into tumour-stroma spheroids, thereby facilitating effective cancer immunotherapy.

Further known modes of action of the CXCL12 inhibitor NOX-A12:

- NOX-A12 mobilizes healthy immune cells [6, Figure 1]
- NOX-A12 exposes hidden tumour cells better to anti-cancer therapy by depriving their contact with the TME [3,4,7,8]
- NOX-A12 inhibits the formation and growth of metastases [4,9]

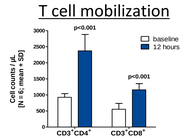


Figure 1. $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells increase in peripheral blood 12 hours after NOX-A12 treatment (2mg/kg) in a Phase I clinical trial with healthy volunteers (unpublished data).

METHODS & RESULTS

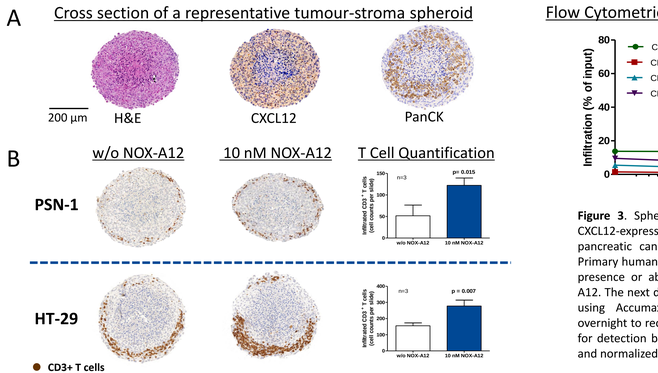


Figure 2. CXCL12-expressing murine stromal MS-5 cells were co-cultured with human cancer cell lines (pancreatic PSN-1 (A,B), colorectal HT-29 (B)) in the ratio 5:1 in ultra-low attachment plates for 3 days. Isolated primary human T cells were added to the spheroids in the presence or absence of 10 nM NOX-A12. After overnight incubation spheroids were washed, formalin-fixed (2h) and paraffin-embedded. 2 µm sections were prepared for H&E- or immuno-staining. (A) PanCK: Pan cytokeratin = tumor cell marker (B) NOX-A12 increases the amount of T cells in both tumor-stroma spheroid models. Brown spots = CD3+ T cells.

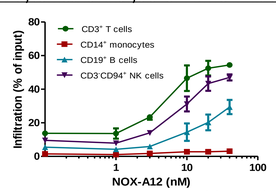
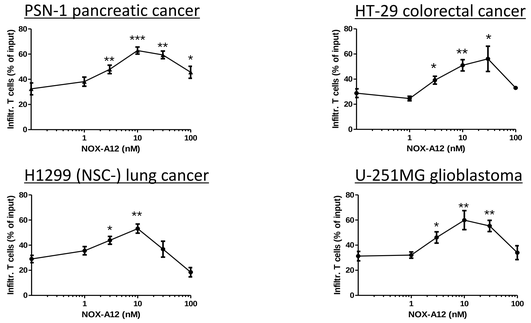


Figure 3. Spheroids were generated by co-culturing of CXCL12-expressing MS-5 cells with human PSN-1 pancreatic cancer cells in ultra-low attachment plates. Primary human PBMCs were added to the spheroids in the presence or absence of various concentrations of NOX-A12. The next day, spheroids were washed and dissociated using Accumax (eBiosciences). Cells were incubated overnight to recover Accumax-labile cell surface molecules for detection by flow cytometry. Cell types were counted and normalized to the input cell count of each cell type.

RESULTS

A NOX-A12 Enhances T Cell Infiltration into Tumour-Stroma Spheroids



B NOX-A12 Enhances NK Cell Infiltration into Tumour-Stroma Spheroids

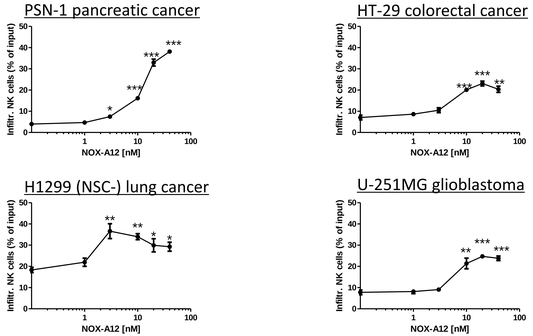


Figure 4. Spheroids of MS-5 cells with various tumor cell lines (PSN-1, HT-29, H1299 and U251MG) were generated as described in figure 2 and treated overnight with isolated primary T (A) or NK (B) cells from healthy donors in the presence of various concentrations of NOX-A12. The next day, spheroids were washed and dissociated for T or NK cell quantification by flow cytometry. *p<0.05; **p<0.01; ***p<0.001.

NOX-A12 Synergizes with T Cell-Based Checkpoint Inhibition (anti-PD-1 as Case Example)

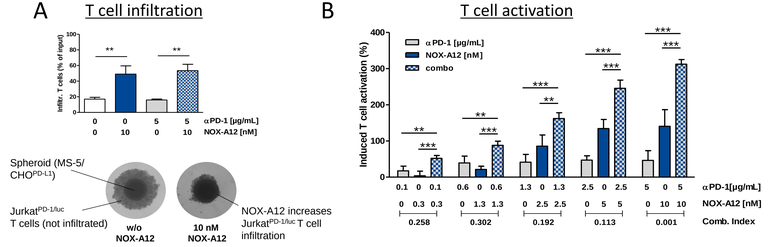


Figure 5. The reporter-based PD-1/PD-L1 blockade bioassay from Promega was adapted to the 3D format: $Jurkat^{PD\text{-}1/luc}$ T cells were incubated with anti-PD-1 (clone PD1.3.1.3) and added to NOX-A12-treated tumor-stroma spheroids ($CHO^{PD\text{-}L1}$ + MS-5). The next day, T cell infiltration was quantified by flow cytometry and T cell activation by incubating the spheroids with BioGlo substrate (Promega). (A) NOX-A12, but not anti-PD-1, increases T cell infiltration which is also visible under the microscope. (B) T cell activation using anti-PD-1 is restricted by limited T cell - tumor cell contacts in the 3D spheroid structure. NOX-A12 dose-dependently increases T cell activation by facilitating T cell infiltration. NOX-A12 acts synergistically with anti-PD-1 (Comb. Index < 1).[10] *p<0.05; **p<0.01; ***p<0.001

NOX-A12 Synergizes with NK Cell-Mediated ADCC (Obinutuzumab as Case Example)

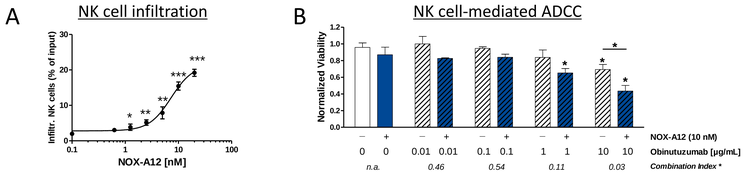


Figure 6. Spheroids of MS-5 cells with CFSE-stained Raji lymphoma cells were generated as described in figure 2 and treated overnight with primary human NK cells in the presence of various concentrations of NOX-A12. Spheroids were dissociated for NK cell quantification and determination of Raji cell viability by flow cytometry. NOX-A12 acts synergistically with obinutuzumab (Combination Index < 1).[10].

CONCLUSIONS & OUTLOOK

- We established tumour-stroma spheroids that mimic the complexity of the tumour microenvironment, in which the CXCL12 inhibitor NOX-A12 increases T and NK cell infiltration.
- By facilitating physical contact of both T and NK cells with tumour cells, NOX-A12 synergizes with T cell-based checkpoint inhibition and NK cell-mediated ADCC.
- This data suggests to combine NOX-A12 with T and NK cell-based cancer immunotherapy.

ESMO Congress 2016, Copenhagen , Denmark, 7-11 October 2016
Session Immunotherapy of cancer: Abstract #1083P
Location: Hall E, Sunday, Oct 9, 2016, 13 PM -14 PM

References: (1): Joyce and Fearon, Science. 2015 Apr 3;348(6230):74-80; (2): Fearon, Cancer Immunol Res. 2014 Mar;2(3):187-93, (3): Hoellenriegel & Zboralski *et al.*, Blood. 2014 Feb 13;123(7):1032-9; (4): Roccaro *et al.*, Cell Rep. 2014 Oct 9;9(1):118-28; (5): Liu *et al.*, Neuro Oncol. 2014 Jan;16(1):21-8; (6): Vater *et al.*, Clin Pharmacol Ther. 2013 Jul;94(1):150-7; (7): Ludwig *et al.*, Blood. 2014 124:2111; (8): Steurer, *et al*. Blood. 2014 124:1996; (9): Chernikova *et al.* Mol Cancer Ther. 2013;12(11 Suppl):C291; (10): Chou, Cancer Res. 2010 Jan 15;70(2):440-6

Dirk Zboralski - Senior Scientist
NOXXON Pharma AG - In Vitro Pharmacology
Email: zbo@gmx.net