Evaluation of tumor biomarkers in patients with microsatellite-stable, metastatic colorectal or pancreatic cancer treated with the CXCL12 inhibitor NOX-A12 and preliminary safety in combination with PD-1 checkpoint inhibitor pembrolizumab

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BACKGROUND

NOX-A12 (olaptesed pegol) is a novel inhibitor of the chemokine CXCL12 (SDF-1), ligand for CXCR4 and CXCR7, for treatment of solid tumors. Binding of the CXCL12 ligand by NOX-A12 prevents receptor engagement and also blocks the ability of CXCL12 to create a chemotactic concentration gradient by neutralization of the anchor domain.

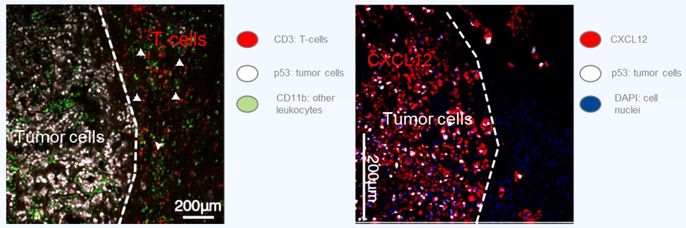

Figure 1: T cell exclusion in solid tumors is mediated by local concentrations of CXCL12 (Feig et al., PNAS 2013)

Immune checkpoint inhibitors promote T cell–mediated killing of cancer cells; however, only a subset of patients benefit from the treatment. A possible reason for this limitation may be that the tumor microenvironment (TME) is immune privileged and excludes cytotoxic T cells from the vicinity of cancer cells by high CXCL12 expression (**Fig. 1**). CXCL12 inhibition by NOX-A12 can break the immune privileged status of the TME by paving the way for immune effector cells to enter into the tumor, thereby broadening the applicability of checkpoint inhibitors in cancer patients.

METHODS

The OPERA study (NCT03168139) is a Phase 1/2 open label clinical study to evaluate pharmacodynamic effects and safety of monotherapy with NOX-A12 and safety and efficacy of a combination of NOX-A12 with pembrolizumab in advanced microsatellite-stable (MSS), metastatic colorectal and pancreatic cancer with liver metastasis. The study comprises two weeks of NOX-A12 monotherapy followed by repeated 21-day cycles of NOX-A12 plus pembrolizumab (**Fig. 2**). Here we present pharmacodynamic biomarker data from for monotherapy phase with NOX-A12 as well as safety for the combination with pembrolizumab.

Patients received 300 mg NOX-A12 by i.v. infusion on days 1, 4, 8, and 11 of the monotherapy phase. Needle biopsies were taken from suitable liver metastases before treatment and on day 14, peripheral blood was drawn at the same time points (**Fig. 2**). Collected tumor samples were assessed for immune cell infiltration by IHC and cytokine signature using multiplex protein analysis.

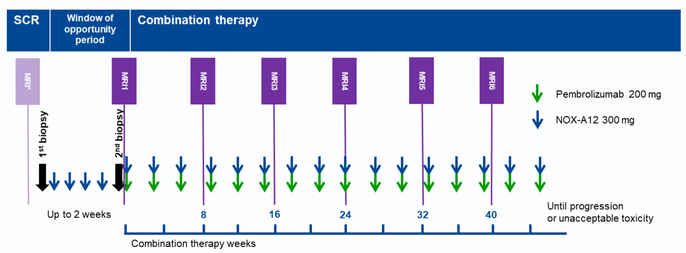

Figure 2: OPERA study design

RESULTS

Twenty patients were recruited, thereof 11 with metastatic colorectal and 9 with metastatic pancreatic cancer (**Table 1**). Fifteen of the patients included (75%) are male, with a median age of 62 (colorectal) and 68 years (pancreatic). All patients were heavily pretreated with a median of 5 lines (colorectal) and 3 lines (pancreatic) of prior systemic treatment, 7 (colorectal) and 3 (pancreatic) patients had prior surgery. Known best responses to the last prior treatment was progressive disease for 16 out of 20 patients. Microsatellite analysis of the tissue biopsies revealed that all patients had microsatellite-stable (MSS) cancer.

Table 1: Demographics

	Colorectal Cancer	Pancreatic Cancer
N	11	9
Male/Female	7 / 4	8 / 1
Age, mean (range)	63 (55 – 73)	67 (48 - 82)
Disease stage at diagnosis: I II III IV	 1 0 4 6	 0 2 2 5
Prior lines of systemic treatment (mean)*	5 (2 – 9)	3 (1 – 5)
Patients with prior surgery (# of surgeries)	7 (1 – 4)	3 (1 – 2)
Best response to last treatment	PD (7), SD (1), UNK (3)	PD (9)
Time since last systemic prior treatment (mean)	2.0 months	1.5 months

* excluding surgery

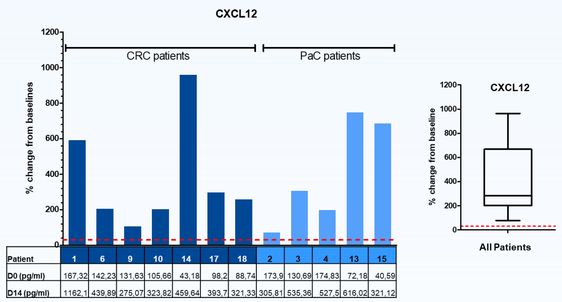

Figure 3: Increase of CXCL12 in tumor tissue

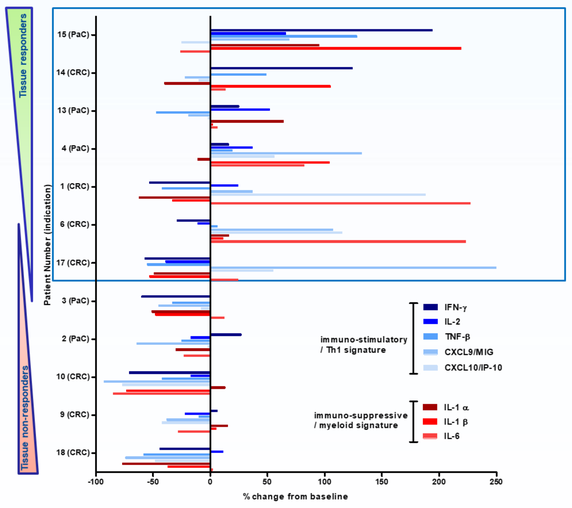

Figure 4: Cytokine tissue response

Serial biopsies at baseline and end of monotherapy suitable for immuno-histochemistry (IHC) and cytokine analysis were analyzed from 12 out of 20 patients.

CXCL12 levels were found to be increased in blood, but also in post-treatment tumor biopsies, suggesting penetration of NOX-A12 into the tumor (**Fig. 3**). The comparison of cytokine levels in the currently available baseline tumor biopsies with those taken after two weeks of NOX-A12 monotherapy revealed changes in markers that are consistent with a Th1 like immune response in multiple patients (**Fig. 4**). In particular IFN-γ, IL-2 and TNF-β were increased, accompanied by an increase of CXCL9 and/or CXCL10 indicating simultaneous T cell attraction which indicate a tissue response to treatment with NOX-A12. However, there were also increased levels of IL-1α, IL-1β and IL-6 in a number of patients indicating potential myeloid counter regulation (**Fig. 4**). A similar cluster of tissue responder patients was observed when all analyzed cytokines were compared in a heatmap analysis (**Fig. 5**). Interestingly, higher changes of CXCL12 levels in tissue observed upon monotherapy – indicating more complete CXCL12 neutralization – correlated with a favorable cytokine profile (**Fig. 6**).

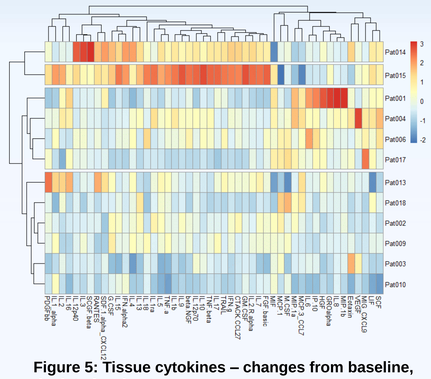

Figure 5: Tissue cytokines – changes from baseline, depicted as heatmap

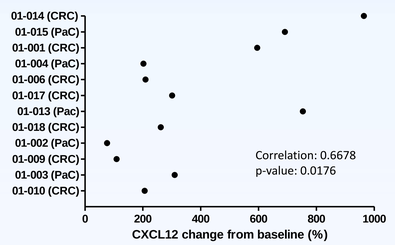

Figure 6: Correlation between heatmap cytokine analysis and CXCL12 changes

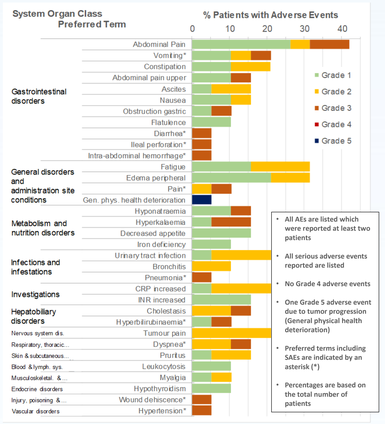

Figure 7: AE profile

In order to better understand the inflammatory context, a program of whole slide immune cell quantification has been performed for both T cell and myeloid cell markers, and further refining immunofluorescence analysis is ongoing to characterize the cell populations in more detail. One cell population of interest which might be associated with a tissue response are CD14+CD15+ double positive cells.

Treatment with NOX-A12 monotherapy and in combination with pembrolizumab was safe and well tolerated, with 141 AEs in total, thereof 47% grade 1; 36% grade 2; 16% grade 3; no grade 4 and 1 % grade 5, see **Fig. 7**.

The AE profile in the study was comparable with the safety profile for pembrolizumab or typical for the underlying diseases colorectal and pancreatic cancer.

CONCLUSION

NOX-A12 penetrates into the tumor where it binds and neutralizes CXCL12. Changes in the cytokine signature suggest that NOX-A12 modulates the tumor microenvironment and induces an immune-stimulatory Th1-like signature in multiple patients and that this phenomenon is linked to more complete inhibition of the target in tumors. Signatures indicate a myeloid cytokine pattern might be associated with resistance and the presence of CD14+CD15+ cells could possibly serve as a biomarker for response. Complementary mechanisms of action and dose-response merit further investigation. The safety profile of NOX-A12 combined with pembrolizumab is consistent with that of pembrolizumab in advanced cancer patients.

ACKNOWLEDGEMENTS & DISCLOSURES

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