High TDO and IDO1 expression in ovarian cancer-associated cells isolated from malignent ascites

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Summary

We have used malignant ascites collected from EOC patients to identify biomarkers of chemotherapy response and to develop assays to support drug discovery programs on small molecule inhibitors of the tryptophan (Trp) catabolizing enzymes TDO and IDO1 with relevant assays.

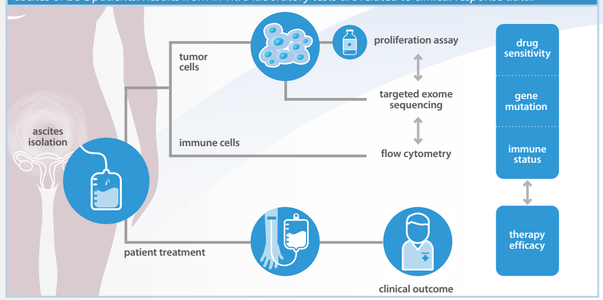
Introduction

- In epithelial ovarian cancer (EOC), 15-20% of tumors do not respond to first-line chemotherapy, consisting of platinum-based chemotherapy plus paclitaxel, and in recurrences the response rate is even lower.
- Clinical trials with immunotherapies, such as PD-1/PD-L1 blockade, have so far not been successful.
- There is a great need of novel therapies with improved long-term treatment outcome, as well as diagnostic tools and biomarkers to predict chemotherapy response in the clinic.

Experimental approach

Ascites was gathered from nineteen patients with advanced stage EOC (**Figure 1; Table 1**). Cells were isolated by centrifugation and tumor cells were separated from immune cells by overnight adherence to tissue culture plates. Both cell fractions were characterized for the expression of immune and cancer cell markers by flow cytometry and quantitative real-time PCR (qPCR). TDO and IDO1 activity was determined using a functional assay and small molecule inhibitors.

Figure 1. *Schematic representation of in vitro analyses performed on tumor and immune cells from ascites of EOC patients. Results from in vitro laboratory tests are related to clinical response data.*



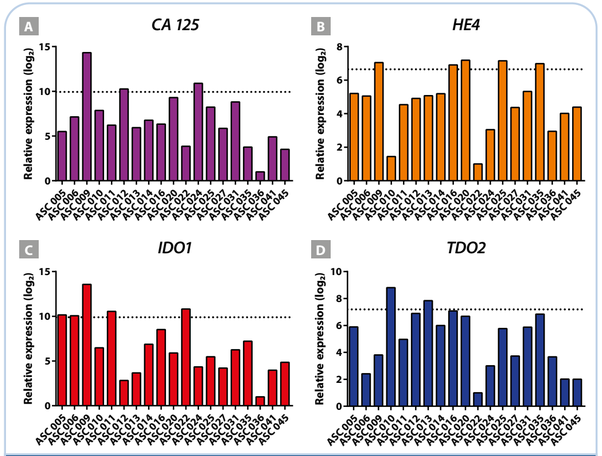


Figure 2. *Gene expression analysis by qPCR in ovarian cancer cell samples isolated from ascites. Expression of the ovarian cancer markers CA 125 (**A**) and HE4 (**B**), and the genes IDO1 (**C**) and TDO2 (**D**). IDO1 and TDO play a role in resistance of tumors against the body's immune response.[1] IDO1 has been correlated with poor prognosis in serous ovarian cancer.[2] Expression levels were normalized for the expression of β-actin (ACTB) and ribosomal protein S18 (RPS18), and scaled based on the lowest expressing sample. The dotted lines represent one standard deviation above the mean.*

Table 1. Histopathology, *BRCA* gene mutation status of tumor and clinical response data of ovarian cancer patients from whom proliferating adherent cell samples were characterized in Figures 2 to 4. High- and low-grade serous ovarian cancer are indicated as HGSOC and LGSOC, respectively. CA 125 > 35 E/ml after treatment is indicative of progressive disease (in red).

Sample	Tumor histo-pathology	*BRCA* mutant status	Chemotherapy treatment	CA 125 before (E/mL)	CA 125 after (E/mL)	*In vitro* cell doubling time (hours)
ASC 005	HGSOC		cis-, carboplatin + paclitaxel	1154	10	52
ASC 006	mucinous		cisplatin + paclitaxel	130	15	81
ASC 009	LGSOC		carboplatin + paclitaxel	174	153	69
ASC 010	HGSOC	*BRCA1* mutant	carbo + paclitaxel, AMG-900	1800	34	58
ASC 011	serous	no mutation	cis-, carboplatin + paclitaxel	1242	5	89
ASC 013	HGSOC		carboplatin + paclitaxel	1481	15	56
ASC 014	HGSOC		carboplatin + paclitaxel	2400	26	65
ASC 016	HGSOC	no mutation	carbo + paclitaxel, caelyx	980	12	64
ASC 020	HGSOC	no mutation	cisplatin + paclitaxel	67	11	54
ASC 022	serous	no mutation	carboplatin + paclitaxel	3600	258	96
ASC 023	HGSOC	*BRCA1* mutant	carboplatin + paclitaxel	25000	6895	>120
ASC 024	HGSOC	no mutation	carboplatin + paclitaxel	121	15	>120
ASC 025	HSCOC	*BRCA2* mutant	carboplatin + paclitaxel	1834	35	>120
ASC 027	HGSOC	no mutation	carboplatin + paclitaxel	1621	15	>120
ASC 028	HGSOC	*BRCA1* mutant	carboplatin + paclitaxel	2500	16	>120
ASC 031	HGSOC	no mutation	carboplatin + paclitaxel	351	14	75
ASC 035	serous	no mutation	carboplatin + paclitaxel	39	34	65
ASC 041	HGSOC		carboplatin + paclitaxel	3900	9	65

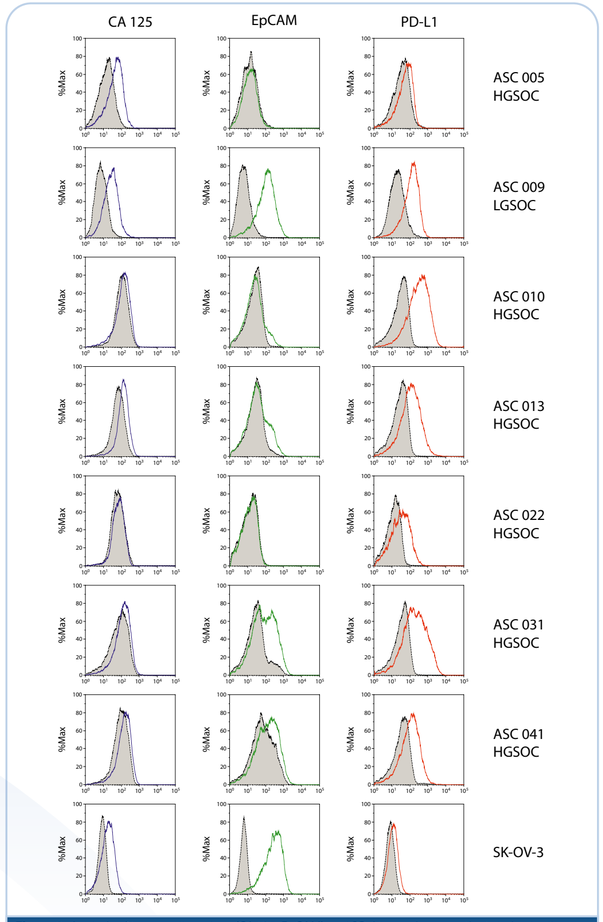


Figure 3. *Analysis of tumor cell markers (CA 125, EpCAM) and PD-L1 on primary patient-derived cells by flow cytometry. Grey-shaded peaks represent the staining with isotype control antibodies. The adenocarcinoma ovarian cancer cell line SK-OV-3 was analyzed for reference.*

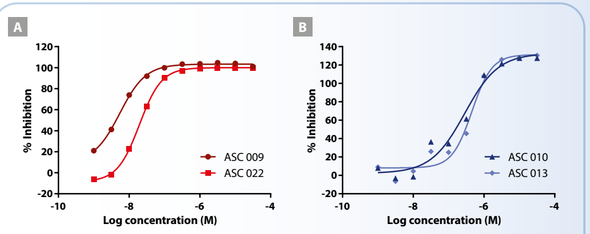


Figure 4. *Trp-catabolizing activity in ascites cell samples and modulation by small molecule inhibitors of IDO1 or TDO. (**A**) Inhibition of Trp-catabolizing activity in IFNγ-stimulated ASC 009 and ASC 022 with the selective IDO1 inhibitor epacadostat. (**B**) Inhibition of Trp-catabolizing activity in ASC 010 and ASC 013 with the selective TDO inhibitor NTRC 3531-0.[3]*

Results

- All samples expressed the ovarian cancer markers *CA 125*, *HE4*, or both (**Figures 2A and B**).
- Flow cytometry analysis of eleven adherent cell samples with anti-EpCAM or CA 125 antibodies confirmed their EOC origin. Six of seven analyzed samples showed high cell surface expression of PD-L1 (**Figure 3**).
- Adherent cell samples were characterized for *TDO2* and *IDO1* expression and Trp-catabolizing activity. Five samples out of nineteen showed relatively high *IDO1* gene expression (**Figure 2C**). However, only after stimulation with IFNγ, the Trp-catabolizing activity in these samples was detectable, and could be inhibited with selective IDO1 inhibitors (**Figure 4A**).
- In contrast, two samples expressed relatively high *TDO2* (**Figure 2D**) and detectable endogenous Trp-catabolizing activity, which could be inhibited with a selective TDO inhibitor (**Figure 4B**).

Conclusions

- High surface expression of PD-L1, and/or high expression of *IDO1* or *TDO2* was observed in several ovarian cancer-associated cell samples isolated from malignant ascites.
- In an ongoing study, in which hundred patients with high-grade serous ovarian cancer will be included, the immune status of the ascites, the *in vitro* drug response of tumor cells isolated from ascites, and the cancer gene mutation status are determined. Results are related to clinical response.

References: [1] Opitz *et al.* (2019) *Br J Cancer* 122: 30-49; [2] Takao *et al.* (2017) *Oncol Rep* 17:1333-1339; [3] manuscript under review.