A precision medicine platform to predict the clinical response to chemo- and immunotherapy for epithelial ovarian cancer

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Introduction

- Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy.
- First-line therapy in advanced EOC is surgery in combination with platinum-based chemotherapy and paclitaxel.
- 15-20% of patients do not respond to this therapy, and in 80% of advanced cases, the disease recurs within three years.
- PARP inhibitors synergize with platinum therapy and have been approved for platinum-sensitive EOC.
- Clinical trials with immunotherapies, such as PD-1/PD-L1 blockade, have so far not been successful.
- Currently the only approved companion diagnostic is *BRCA* gene mutations for PARP inhibitors.

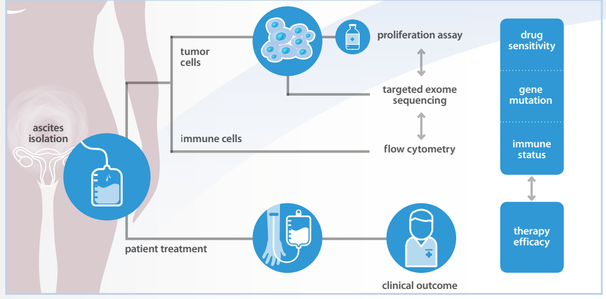
Aim

- More diagnostic assays to predict the clinical response to chemo- and immunotherapies are needed.
- We have developed a biomarker discovery platform using ascites of ovarian cancer patients.

Data are collected and combined from:

1. Tumor histopathology and clinical response of EOC patients;
2. Mutation status of cancer genes and homologous recombination repair genes;
3. *In vitro* tumor cell proliferation assays;
4. Immune cell characterization of ascites.

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.*



Experimental approach

Ascites was gathered from patients by ascites punction or during debulking surgery. Low passage adherent cell samples from eighteen patients were characterized for the expression of markers of EOC (CA 125, EpCAM, *HE4*) [1]. The expression of genes and markers implicated in resistance to chemotherapy (*ABCB1*, *CCNE1*) [2], or suppression of anti-tumor immune response (PD-L1, *TDO2*, *IDO1*) [3] were determined by qPCR and flow cytometry. Sensitivity of tumor cell samples to various cytotoxic agents and targeted anti-cancer agents was determined in proliferation assays using ATPlite™ 1Step (PerkinElmer) as an indirect read-out of cell number [4].

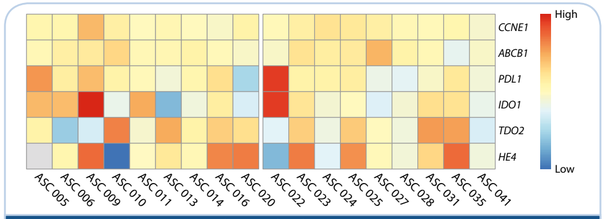


Figure 2. *Analysis of the expression of genes related to poor response to either chemotherapy (CCNE1, ABCB1) or anti-tumor immune response (PDL1, IDO1, TDO2) [2,3]. HE4 is a marker of EOC [1]. Expression levels were normalized to the expression of β-actin (ACTB) and scaled based on the root mean square of the individual gene.*

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

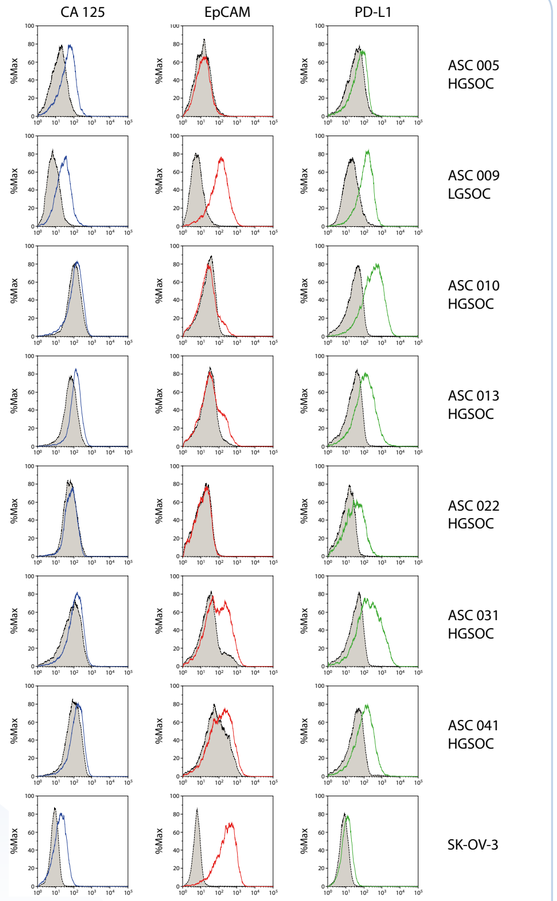


Figure 3. *Analysis of tumor cell markers (CA 125, EpCAM) and PD-L1 on primary patient-derived cells by flow cytometry. Grey-shaded peaks represent the staining with isotype control antibodies. The adenocarcinoma ovarian cancer cell line SK-OV-3 was analyzed for reference.*

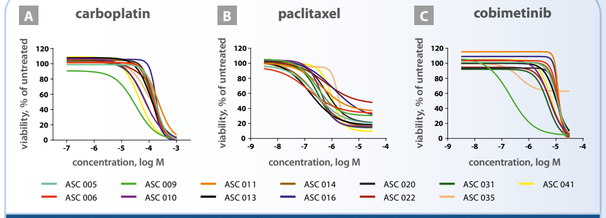


Figure 4. *In vitro drug sensitivity analysis of primary patient-derived tumor cells. Dose-response curves of the standard-of-care chemotherapeutic agents carboplatin (**A**) and paclitaxel (**B**) on proliferating cells (doubling time < 120 hour) from thirteen patients did not reveal more than 2-fold differences in potency. However, one sample (ASC 009) with an oncogenic NRAS mutation was more sensitive to MEK inhibitors, such as cobimetinib (**C**).*

Results

- Adherent cells were isolated from ascites of EOC patients and characterized.
- Flow cytometry surface staining of CA 125 and EpCAM, and qPCR analysis of *HE4* gene expression confirmed the EOC origin of the cells.
- Many samples showed high PD-L1 protein expression. Several samples showed high gene expression of *IDO1* or expression of *TDO2*.
- *In vitro* cell proliferation assays did not reveal clear differences in sensitivity to cytotoxic anti-cancer agents, such as carboplatin and paclitaxel. However, an *NRAS* mutant tumor cell sample showed much higher sensitivity to MEK inhibitors than samples not harboring this mutation.

Conclusions

- Primary patient-derived tumor cells from ascites can be used to determine *in vitro* drug response in cell proliferation assays.
- Remarkably high expression levels of PD-L1 and/or *IDO1* gene expression were observed in some samples. The relationship with therapy response will be determined.

Outlook

In an ongoing study, in which hundred patients with high-grade serous ovarian cancer will be included, the *in vitro* drug response of tumor cells from ascites is determined. Results are related to gene mutations, immune status and clinical response.

References: [1] Helström *et al.* (2003) *Cancer Res.* 63, 3695-3700; [2] Kanska *et al.* (2016) *Gynecol. Oncol.* 143, 152-158; [3] Okamoto *et al.* (2005) *Clin. Cancer Res.* 11, 6030-6039; [4] Uitdehaag *et al.* (2019) *Mol. Cancer Ther.* 18, 470-481.