**Abstracts**

**Introduction/Background** In our previous microarray study we identified the 96-gene signature related to differential survival of patients with high-grade serous ovarian cancer (OC).1 Top differentially expressed genes were e.g. POSTN, COL11A1, SFRP2, MFAP5, ITGBL1, LOX. Similar mesenchymal signature has been observed also by others, but it has been ascribed to cancer associated fibroblasts, not epithelial cells. However, we postulate that these genes can be also expressed by cancer cells themselves.

**Methodology** For survival analysis we used Kaplan-Meier Plotter and Microarray Gene Expression Database of OC Subtype (CSIOVDB). Proteins expression was assessed by Heterogeneity_Analysis Portal (lmdomics.org) [2]. Interaction networks were judged by STRING. Molecular cloning was performed using retroviral gene transfer; in vitro functional tests were done according to standard procedures; gene expression analyzed by PCR.

**Results** STRING algorithm applied to our prognostic signature showed interactions typical for proteins engaged in the function and structure of extracellular matrix. Our own qRT-PCR analysis, as well as Kaplan-Meier Plotter and CSIOVDB analysis confirmed that mRNA level of majority of genes from our negative prognostic signature is significantly related to survival of OC patients. Using Heterogeneity_Analysis Portal we analyzed 24 out of these genes and found that they are strongly expressed by tumor stromal cells, while weakly by epithelial cells. We analyzed ten of these genes in several OC cell lines by semi-quantitative RT-PCR, and we found that they are expressed by epithelial cells as well. By functional in vitro assays we observed that overexpression of these genes (ITGBL1, MFAP5, SFRP2) may affect OC cells phenotype (migration, invasiveness, proliferation, chemosensitivity).

**Conclusion** Mesenchymal signature with negative prognostic significance in OC is expressed mostly by stromal, but also by epithelial cells, and may affect phenotype of the latter. Exact role of these genes in OC cells remains to be assessed.

**REFERENCE**

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**EFFECT OF IMIQIMOD TREATMENT ON HLA-G EXPRESSION IN HIGH-GRADE CERVICAL LESIONS**

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**Introduction/Background** We showed that the topical application of the 5% imiquimod in treating cervical high-grade squamous intraepithelial lesions (HSIL) leads to a regression of about 50% of the lesions after 16 weeks of treatment (1). Tissue culture studies have attributed imiquimods antiviral and antitumor activity to the enhancement of the innate immune response. Knowing that the increase in soluble HLA-G (sHLA-G) expression is associated with disease progression and that the HLA-G molecule has an inhibitory effect on different immune cells, we further investigated whether imiquimod modulates the sHLA-G expression in the cervical lesions.

**Methodology** Cervical biopsies of 52 patients aged 18-40 with histological HSIL (CIN2p16+ and CIN3), who self-applied 250 mg cream containing 5% of imiquimod three times a week for 16 weeks, were collected at admission and after completion of the treatment. Treatment success was defined as the absence of HSIL after treatment. For immunohistochemistry, we used the monoclonal anti-HLA-G antibody (Exbio, 5A6G7) (figure 1). The intensity of pixels obtained from the images of the slides, using the program Gimp 2.10.18, defined the protein levels (figure 2). We used the T-test to compare two groups, the ROC curve to define the cut-off point for risk-predicting sHLA-G expression, and the Fisher’s exact test to calculate the risk.

**Results** High tissue sHLA-G levels before treatment were associated with unsuccessful imiquimod treatment (p=0.0025). Imiquimod down-modulated sHLA-G in the lesion of those successfully treated (p=0.0467) or not (p<0.0001). According to the area under the curve of 0.72, 95%CI 0.61-0.84, p=0.0012, sHLA-G expression over 870847 pixels represented a 4-fold risk for unsuccessful imiquimod treatment (p=0.0088).

**Conclusion** We showed that imiquimod modulates HLA-G in cervical lesions and that treatment success depends on sHLA-G levels at admission.

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**A SINGLE-CELL MAP OF RARE OVARIAN CANCER**

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**Introduction/Background** Non-epithelial ovarian tumours encompass a heterogeneous group of neoplasms that mainly include germ cell tumours (GCT) and sex-cord stromal tumours (SCST). These tumours are characterised by an extensive inter- and intratumoral heterogeneity. By applying single-cell RNA sequencing (scRNA-seq), we attempt to elucidate the complexity of the tumour microenvironment.

**Methodology** We performed scRNA-seq of 66 919 cells collected from 12 patients. Most fresh tissue samples were derived from SCST (n=9), including 7 adult granulosa cell...