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

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). Top-differentially expressed genes were e.g. POSTN, COL11A1, SFRP2, MFAP5, ITGB1L, 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 (ITGB1L, 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

IMIQIUMOD MODULATES THE sHLA-G EXPRESSION IN THE CERVICAL LESIONS

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 imiquimod’s antiviral and antitumor activity to the enhancement of the innate immune response. Knowing that the increase in soluble HLA-G levels at admission.

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=0.0023). 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=0.012, 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.

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 imiquimod’s 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
tumours, 1 primary juvenile granulosa cell tumour and 1 primary Sertoli-Leydig cell tumour. Three samples were obtained from treatment-naïve GCT (2 immature teratomas and one dysgerminoma). For each phenotype of tumour cells, immune cells, endothelial cells and cancer-associated fibroblasts, we identified specific transcriptomic markers.

**Results** Based on differential expression analysis and expression of transcriptomic markers, we identified 27 clusters consisting of 9 tumour cell and 18 stromal cell clusters. The first results of subcluster analysis revealed nearly absence of B cells in all granulosa cell tumours. In addition, the immune cell subcluster mainly consists of T cells derived from the dysgerminoma (58%) and Sertoli-Leydig cell (20%) samples. Further characterisation and differentiation of distinct subclusters is currently ongoing and will be presented.

**Conclusion** With this analysis we aim to generate a publicly accessible comprehensive blueprint of the tumour micro-environment, aiding other researchers to gain high-resolution insights in the heterogeneity and complexity of these rare ovarian cancers.

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**Abstract 2022-RA-1194-ESGO**

**EFFICACY OF DOSTARLIMAB IN ENDOMETRIAL CANCER BY MOLECULAR SUBTYPE: A POST HOC ANALYSIS OF THE GARNET STUDY**


10 Department of Medicine, British Columbia Cancer, Vancouver, Centre, University of British Columbia, Vancouver, BC, Canada; 12 Gynecologic Oncology Group (GOG) and Department of Obstetrics/Gynecology, Laura and Isaac Perlmutter Cancer Center, NYU Langone Health, New York, NY, USA; 13 Division of Gynecologic Oncology, McGill University Health Centre, Montreal, QC, Canada; 14 Department of Medical Oncology, Institut Paoli Calmettes, Aix-Marseille University, Marseille, France; 5 Division of Gynecologic Oncology, Levine Cancer Institute, Carolinas HealthCare System, Charlotte, NC, USA; 6 Department of Obstetrics and Gynecology, Georgia Cancer Center, Augusta University, Augusta, GA, USA; 7 Women and Infants Hospital of Rhode Island, Providence, RI, USA; 8 Division of Gynecologic Oncology and Gynecologic Oncology Phase I Program, The Ohio State University and the James Cancer Center, Columbus, OH, USA; 9 NEXT Oncology Hospital Univeristario Quinziotal Madrid, Madrid, Spain; 10 Clinical Trial Unit, Istituto Nazionale Tumor Fondazione G. Pascale, Naples, Italy; 11 Gynaecology Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, UK; 12 University College London, St. Bartholomew's Hospitals London, London, UK; 13 Department of Hematology, Regional Center of Oncology, Gdansk, Poland; 14 Department of Oncology, Righ Shafto Hospital, Copenhagen University Hospital, Copenhagen, Denmark; 15 GSK, Pennington, NJ, USA; 16 GSK, Waltham, MA, USA; 17 GSK, London, UK; 18 Gynecologic Cancer Programme, Vall d’Hebron Institute of Oncology (VHIO), Hospital Universitari Vall d’Hebron, Vall d’Hebron Barcelona Hospital Campus, Barcelona, Spain ed. 1 Employed by GSK at the time the study was conducted, study was funded by GSK.

**Abstract 2022-RA-1194-ESGO Table 1**

<table>
<thead>
<tr>
<th>Molecular subtype</th>
<th>Overall</th>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMRp/MSS</td>
<td>69/143</td>
<td>52/90</td>
<td>17/53</td>
</tr>
<tr>
<td>TP53mut</td>
<td>20/103</td>
<td>15/36</td>
<td>5/67</td>
</tr>
<tr>
<td>ER</td>
<td>25/100</td>
<td>17/50</td>
<td>8/50</td>
</tr>
<tr>
<td>IRS</td>
<td>47/150</td>
<td>38/76</td>
<td>9/74</td>
</tr>
<tr>
<td>NSEMP</td>
<td>4/47</td>
<td>2/33</td>
<td>2/14</td>
</tr>
</tbody>
</table>

**Conclusion** The observed ORRs in each molecular subgroup were consistent with the overall ORR in each cohort. Differences by ER expression status were not observed. These findings support the importance of testing patients with EC for MMR/MSI biomarker status as a predictor of response. Additionally, data suggest that TP53 mutation or ER expression should not modify treatment approach. The data are of interest for hypothesis generation.

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**Abstract 2022-RA-1195-ESGO**

**LONGITUDINAL STUDY OF VAGINAL MICROBIOME PRE- AND POST-TREATMENT IDENTIFIES BIOMARKERS FOR CERVICAL INTRAEPITHELIAL NEOPLASIA 3 (CIN3)**


**Introduction/Background** Increasing evidence suggests vaginal dysbiosis is associated with persistence of human papillomavirus (HPV) infection and cervical intraepithelial neoplasia (CIN1–3) development. In this pilot study we aimed to investigate the potential of vaginal microbiome biomarkers to predict CIN3 development in high risk HPV positive (hr-HPV+) women.

**Methodology** 59 women with normal cytology at initial screening and follow-up over six years were enrolled from ARTISTIC trial. The cohort included 14 hr-HPV negative (hr-HPV-) and 15 hr-HPV+ women through whole follow-up.