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BIOMARKERS OF FAVORABLE PROGNOSIS GUIDES THE IDENTIFICATION OF TUMOR REACTIVE CD4+ AND CD8+ TILS IN ENDOMETRIAL CANCER

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Background Endometrial cancer (EC) is the most prevalent gynecological cancer and it can be categorized into four molecular subtypes; ultramutated POLE, hypermutated MSI, CNL and CNH. To date, the prevalence, specificity and phenotype of tumor infiltrating lymphocytes (TILs), and their exact role in EC remain controversial. Thus, a thorough investigation deciphering the phenotypic landscape of EC-infiltrating T lymphocytes and their anti-tumor reactivity is still missing.

Methods In order to study the implications of the T-cells infiltrating EC for prognosis or susceptibility to immune checkpoint inhibitors, we analyzed the phenotypic traits of CD8 and CD4 EC-resident T cells from single-cell suspensions (n=47) by multicolor flow cytometry (19 biomarkers). Correlation analyses between the phenotype of the TILs, EC molecular subgroups and the survival of the patients were performed to identify biomarkers that could predict prognosis or survival. This identification guided the isolation of specific CD8 and CD4 subpopulations based on the differential expression of the selected biomarkers to evaluate their ability to recognize tumor (figure 1).

Results Our analysis evidenced the presence of CD8+ and CD4+ T cells with distinct levels of PD-1 expression: cells that did not express PD-1 (PD-1-), cells expressing intermediate (PD-1dim) or high (PD-1hi) levels of PD-1. We found that TIM-3+, CD39+, CXCL13+, BCL6+ or Ki67+ cells frequently co-expressed almost exclusively on the PD-1hi, but not on the PD-1- or dim CD8+ TILs. On the other hand, CD4+ TILs displayed co-expression of TIM-3, CD103, CD39, CD38, HLA-DR, CXCL13, BCL6, CXCR5 or Ki67 within the PD-1hi, but also PD-1dim cells. Importantly, our data shows that the frequency of PD-1hi or CD39+ CD8+ EC TILs, and of PD-1hi but not CD39+ CD4+ TILs was associated with improved survival. Of the 5 predominant CD8 + tumor-resident subpopulations observed (PD-1-CD39-, PD-1dimCD39-, PD-1dimCD39+, PD-1hiCD39- and PD-1hiCD39+) the PD-1hiCD39+, but not the PD-1hiCD39- lymphocytes, harbored the highest frequency of autologous tumor-reactive lymphocytes in all four EC tumors studied. However, both the CD4+ PD-1hiCD39+ and the PD-1hiCD39- contained autologous tumor-reactive lymphocytes in all four tumors screened; being the PD-1hiCD39+ cells the subpopulation containing the majority of tumor-reactive CD4+ cells.

Abstract 61 Figure 1 Schematic workflow of the study. A section of a primary EC tumor resection is digested and stained with specific antibodies to characterize the phenotype of CD8 and CD4 TILs by flow cytometry (upper panel). Targeted sequencing and IHC including typically altered genes in EC are performed in order to molecularly classify the patient's cohort into different disease subgroups. Combining the data generated by flow cytometry, targeted sequencing and IHC, specific biomarkers on CD8 and CD4 TILs are investigated for their potential role in predicting molecular subtypes or survival (middle panel). CD8 and CD4 TILs are isolated from the tumor digest based on the differential expression of PD-1 and CD39 and the specific isolated CD8 and CD4 subpopulations are cultured in vitro to expand them to large numbers. The expanded CD8 and CD4 subpopulations are subsequently screened for tumor recognition by co-culturing the isolated subpopulations with autologous tumor cell lines. Tumor recognition is assessed by measuring the up-regulation of the activation markers 4-1BB or OX40 on CD8+ or CD4+ T cells, respectively, by flow cytometry, and by measuring IFN- γ production by ELISPOT.

Conclusions Overall, our data suggest that CD39 expression on CD8+PD-1hi EC-resident T cells defines a tumor-reactive population that plays an important role in protecting patients from recurrence after surgery. However, PD-1 expression but not CD39 on CD4+ TILs, better guides the identification of lymphocyte subsets with enriched tumor-recognition potential that contribute to improved clinical benefit in EC.

Ethics Approval This study was approved by the 'Comité de Ética de Investigación con Medicamentos del Hospital Universitario Vall d'Hebron' institution's Ethics Board; approval number PR(AG)537/2019.

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