Background: Granulosa cell tumors (GCTs) are rare tumor in ovaries accounting for 3-5% of all ovarian cancers. The main current treatment for GCT is surgery; however, a subset requires chemotherapy for residual and recurrent disease. GCT malignancies are often low-grade with a five-year survival rate up to 90%, however a critical characteristic of these tumors is a tendency for late recurrence and a high recurrence rate is the most critical factor for GCT death.

Methods: As GCTs are rare tumors and tissue availability is very limited, we used a dual multiplexing approach in order to maximize the data output from a total of 14 FFPE tumor samples (3 primary tumors, and 8 recurrent tumors). With this approach we used a single 4 µm section to detect 15 markers in an IF multiplexing assay, and an adjacent 10 µm section to analyse expression of 770 immune-related genes with the aim to qualitatively profile immune cell subsets, angiogenic vessels, as well as differentially expressed between primary and recurrent tumors.

Results: Of the 770 gene targets in the NanoString PanCancer Immune Profiling Panel, we detected a differential expression for 96 genes in recurrent tumors compared to primary tumors. Of these 96 genes IDO1 showed the highest differential with a significant 14-fold increase in recurrent tumors. In addition to promoting tumor angiogenesis, IDO1 is a key regulator of immune tolerance as an activator of immune-suppressive cell types such as Tregs and M2 TAMs, and thereby represents a therapeutic target within immune-oncology beyond checkpoint blockade.

When analyzing the presence of macromolecules on protein level in the tumor microenvironment, we found a 113-fold increase in TAM density in recurrent tumors compared to primary tumors, corresponding to an increase in the macrophage score on mRNA level. In a nearest neighbor analysis for TAMs and angiogenic markers, we found M6-type TAMs to be in closer proximity to vessels in recurrent versus primary tumors. TAMs are key cells in controlling tumor angiogenesis and can be recruited by tumors and upregulated to secrete factors mediating the angiogenic switch. One such recruitment factor is VEGF, the gene for which was found to be up-regulated more than 10-fold in the recurrent tumors samples.

Macrophage Increase in Recurrent Tumors – mRNA & Protein Correlation

We have used a dual multiplexing approach to immunoprofile the tumor microenvironment of rare ovarian Granulosa Cell Tumor (GCT) FFPE samples on both mRNA level (Nanostring nCounter assay), and protein level (MultiOmxa analysis).

Key Findings
- We have used a dual multiplexing approach to immunoprofile the tumor microenvironment of rare ovarian Granulosa Cell Tumor (GCT) FFPE samples on both mRNA level (Nanostring nCounter assay), and protein level (MultiOmxa analysis).
- IDO1 is one of several genes found to be significantly upregulated in recurrent GCTs compared to primary tumors.
- Concordance between the two assays was observed for tumor-associated macromolecules (TAMs), as both the gene signatures and protein levels were increased in recurrent tumors compared to primary tumors.
- In a spatial analysis of angiogenic vessels and TAMs, M2 type TAMs were found to be in closer proximity to vessels in recurrent tumors compared to primary tumors.