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Biology and Treatment of Small Cell Carcinoma of the Ovary, Hypercalcemic Type

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The main focus of our research is elucidating the biology of gynecologic cancers with the goal of improving prevention and identifying effective treatment options for women with these malignancies. The efforts in our laboratory are towards translating basic research findings into clinical practice. More precisely, our ongoing studies aim to identify molecular features (e.g. gene mutations, aberrant protein expression) of each gynecologic cancer subtype and tailor therapeutic approaches according to genetic landscape of cancer. This precision medicine approach allows us to identify therapeutic options that could ultimately increase the odds of a patient's successful response.

Studying rare gynecologic cancers, particularly small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) is one of our main research undertakings. SCCOHT is very aggressive malignancy with poor prognosis that affects women at a very young age (average age at diagnosis is 24) and new effective treatments are urgently needed. Our group and others spearheaded the series of studies in the past several years that unraveled the biology and molecular features of SCCOHT. Our studies, in part supported by the Katie Oppo Research Fund, identified SCCOHT as a monogenic disease (a disease that have only one gene mutated) with mutations in SMARCA4 gene. We also identified the non-mutational loss of SMARCA2 (protein mutually exclusive to SMARCA4) as another molecular feature that is typical for nearly all SCCOHTs. This dual loss of SMARCA2 and SMARCA4 is now considered a signature SCCOHT diagnostic tool and it has been implemented by gynecologic oncology pathologists in their practice. Although these findings opened doors for exploring some promising targeted therapies, effective treatment options for the patients with SCCOHT have not been identified yet.

Our most recent work described SCCOHT as an immunogenic disease, suggesting that patients with this malignancy could benefit from immunotherapies. We identified four SCCOHT cases where women benefitted from the treatment with a specific type of immunotherapy utilizing immune checkpoint blockade approach. This particular immunotherapy is very effective in patients with tumors that are expressing an immune-marker called PD-L1 (programmed death ligand 1). Tumors expressing PD-L1 have the ability to evade the immune attack by activating immune checkpoints and signaling to immune T cells to shut down and die. The immune checkpoint blockade immunotherapy takes advantage of antibodies that specifically block PD-L1 positive cells from shutting down the

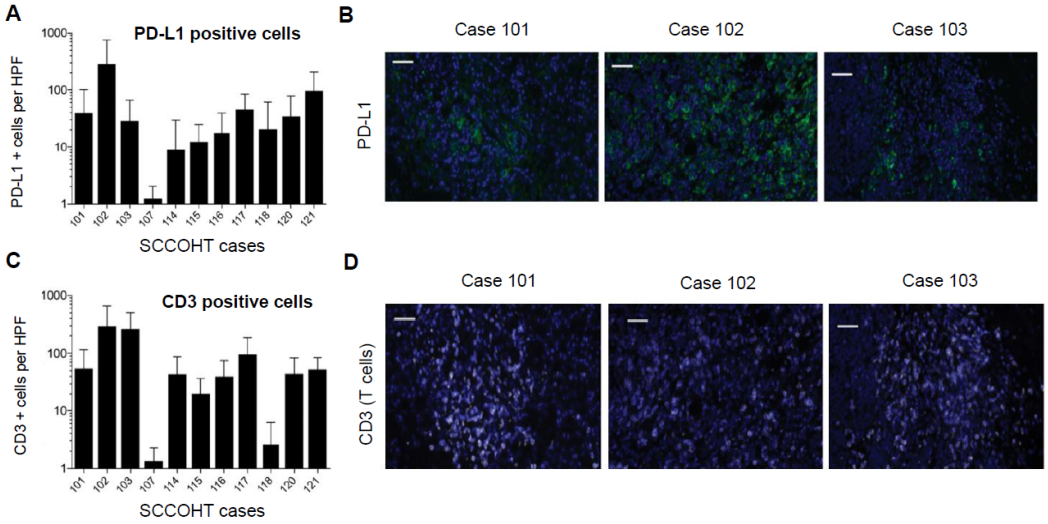

Figure 1. SCCOHT exhibit immune-active tumor microenvironment. A) PD-L1 positive C) CD3 (T cells) positive cell counts per high-power field (HPF). The y-axis indicates number of marker-positive cells, and the x-axis indicates SCCOHT cases. Error bars represent one SD per 10 HPFs. B) anti-PD-L1 D) anti-CD3 immunohistochemistry staining of three representative SCCOHT cases. Scale bars = 50 μm.

immune system allowing persistence of T cell activity that are now able to kill cancer cells. We identified PD-L1 expression in ten out of eleven SCCOHT cases providing a rationale for why these four women with SCCOHT responded well to immunotherapies (Figure1 A and B). In addition, we identified the presence of tumor infiltrating T cells, further suggesting that SCCOHT has an immune-reactive microenvironment (Figure1 C and D). Our findings were published in the high-impact journal, JNCI, and caught a lot of attention in our community, since discovering that SCCOHT is an immunogenic disease was somewhat unusual and unexpected. In general, immunotherapies work best in tumors that have hundreds or thousands mutations and these tumors are also likely to have high PD-L1 expression. Given that SCCOHT only has mutations in one gene, it was surprising to find that SCCOHT has high PD-L1 expression and immune-reactive microenvironment. This poses an important question as to why SCCOHT is an immunogenic disease.

Our current efforts are towards answering this question by further dissecting the biology behind the SCCOHT immune-reactive microenvironment. Since SCCOHT is defined by sole mutations in SMARCA4, we hypothesize that SMARCA4 loss creates immune-reactive microenvironment that results in high PD-L1 expression and activation of immune checkpoints resulting in the immune system evasion, providing a rationale for treatment with immunotherapies.

We suggest that damaging DNA (e.g. with radiation) may provoke activation of the immune-reactive pathways, including expression of PD-L1, particularly within cancer cells with aberrant DNA damage repair mechanisms. Numerous studies have demonstrated that loss of SMARCA4 can lead to malfunctions in DNA damage repair pathways. Also, our preliminary data demonstrated that SCCOHT is sensitive to agents typically efficient in tumors with aberrant DNA damage repair

ipathways. Taken together, this suggests that SCCOHT's microenvironment may be enriched with immune cells as a result of DNA damage and activation of immune-reactive pathways within cancer cells. Of note, all four patients described in our studies were previously treated with radiotherapy, further suggesting that damaging DNA may facilitate creating an immune-reactive microenvironment making it more susceptible for treatment with immunotherapies.

We are dissecting the biology of SMARCA4 loss in SCCOHT in the context of DNA damage repair deficiency and how it relates to activation of immune-reactive pathways. We have established multiple cell lines that we depleted for SMARCA4, mimicking SCCOHT phenotype. Also, we are creating the SCCOHT cell lines with forced SMARCA4 expression, which allows us to test the importance of SMARCA4 loss in SCCOHT. Using these cell lines, we plan to dissect the impact of SMARCA4 on DNA damage repair pathways. We will damage DNA by irradiating cells and subsequently we will monitor their ability to repair the damage. We will also measure activation of immune-reactive pathways, particularly PD-L1. We will use methods that allow us to measure markers for both DNA damage repair and immune-reactive pathways. In addition, we will employ quantitative methodologies to monitor activation of immune-genes (e.g. PD-L1) and expression of their corresponding proteins. Altogether, these approaches will allow us to demonstrate that loss of SMARCA4 in SCCOHT results in aberrant DNA damage repair and activation of immune-reactive pathways upon irradiation.

These studies will provide further rationale for offering women with SCCOHT a combination treatment, radiotherapy followed by the immune checkpoint blockade immunotherapy.