**COMPREHENSIVE ANALYSIS OF A NOVEL ANTI-PDL1 RESISTANT SARCOMA MOUSE MODEL**

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**Background** Soft Tissue Sarcoma (STS) is known to be resistant to cancer immunotherapy including the prototypical immune checkpoint inhibitor (ICI) anti-PD1 (1). It’s therefore crucial to develop innovative strategies aiming at improving current clinical benefit. To this end, a well-characterized sarcoma preclinical model that mimics resistance to immunotherapy is needed. Starting from the anti-PD(L)1-sensitive MCA205 sarcoma mouse model, we therefore aimed at developing and characterizing a novel model resistant to anti-PD1.

**Methods** Anti-PDL1 antibody was first administered to the ICI-sensitive MCA205 mouse sarcoma model (MCA205-S) and tumor growth was followed overtime. Tumor from a “non-responder” animal – tumor growth profile being similar as in the vehicle group – was then retrieved and processed for tissue dissociation and cell culture. Tumor cells were then amplified and inoculated into immunocompetent C57BL/6 mice that were exposed or not to anti-PD1 antibody, and tumor growth was monitored for a 8-week period. In addition, tumor samples from satellite animals were collected and processed for intratumoral immune landscape characterization by multiparametric flow cytometry as well as for gene expression analysis by RNA sequencing and spatial transcriptomics.

**Results** While anti-PD1 antibody demonstrated a strong anti-tumor effect in MCA205-S tumor-bearing mice, administration of anti-PD1 to the mice inoculated with the non-responder mouse-originating MCA205 cells did not yield to a significant anti-tumor effect thereby validating the resistant profile of this new MCA205-R cell line. Flow cytometry analysis of tumor-infiltrated immune cells revealed a higher abundance of macrophages (defined as CD11b+/F4:80+) in MCA205-R when compared to the MCA205-S model, which more likely harbor a M2 phenotype. Also, anti-PD1 treatment favored an important MCA205-R tumor infiltration by gMDSC which was more limited in the MCA205-S model. Data from gene expression analysis and spatial transcriptomics will be presented at the meeting.

**Conclusions** We developed and validated a novel preclinical mouse model of sarcoma (MCA205-R) characterized by its resistance to anti-PD1 and a high tumor infiltration by macrophages. This model – more reflecting the clinic in terms of immunotherapy sensitivity – can thus serve to evaluate novel immunotherapeutic regimens either alone or in combination with reference ICI, to ultimately translate into clinical benefit for STS patients treated with immunotherapy.