Background Immune checkpoint inhibitors (ICI) provide significant clinical benefits for advanced melanoma patients. However, most patients do not respond or develop resistance after initial tumor regression. In this ongoing study, we explore the role of tumor immune microenvironment (TIME) architecture with an emphasis on tumor infiltrating lymphocytes (TIL)' quantitative and spatial characteristics in tumor inflammatory neighborhoods in the pre-ICI tumor samples.

Methods We identified total of 63 patient tumors to evaluate their tumor TIL population, both quantifiably and spatially, in inflammatory neighborhoods. To investigate the heterogeneity of the TIME in melanoma tumors, we used deep proteomics to generate spatially resolved cellular profiles in pre-ICI samples of advanced melanomas and determine the association between TIL characteristics and innate inflammation markers. We have performed our protein marker analyses by using two platforms; CyTOF for immune phenotyping and multiplex IHC for innate inflammatory markers of oxidative stress. Then, we aligned subsequent slides to perform hotspot analyses and explore TIL characteristics in proposed inflammatory neighborhoods by using the Opal Polaris and VisioPharm & R softwares to analyze images, including cell segmentation, phenotyping, and cell quantitation.

Results We have profiled total of 63 patient samples from various subtypes of melanoma, including rare melanomas. Patients were selected who had melanoma tumor tissue banked prior to receiving anti-PD1 +/- anti-CTLA4 immunotherapy either in the adjuvant (N=26) or definitive (N=60) setting at MD Anderson Cancer Center. Patients had no significant comorbidity or other cancers and tissue collected within 1-year prior to ICI initiation, received no other systemic treatment between the time of tissue collection and ICI treatment initiation. We first examined both high-TIL and low-TIL regions on these pre-ICI treatment tumor samples and found significant differences in their cellular landscape. We observed significantly higher innate inflammatory enzymes, iNOS and mPGES1, expressing tumor cells in low-TIL areas (p<0.0004) and they were surrounded by CD4 cells, while high-TIL areas were surrounded by CD20, CD68 and CD56 cells. Further phenotyping analyses revealed that M1 macrophages were located in high-TIL areas with fewer tumor cells around while M2 macrophages were located wide-ranging area in the entire tumor tissue (p<0.008). NK cells were spatially closer to TIL in high-TIL areas but quantitatively higher in number in low-TIL areas. Association studies with clinical information of these findings are currently underway.

Conclusions Understanding the tumor architecture by proteomics approaches in intact tissue provides significant knowledge on TIME and supports clinical decision making by comprehensive modeling.

Ethics Approval The use of clinical samples was approved by the Institutional Review Board of MD Anderson Cancer Center (no. LAB01-717 and LAB00-063) and informed consent forms were signed by each patient before participation.