Flow cytometric profiling of the human tumor microenvironment of biopsies and surgical resections to guide clinical development of immunotherapeutics

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A challenge for the development of immuno-modulating therapeutics for the treatment of cancer has been understanding the basis of response and resistance (innate and acquired). This is due to the complexity of factors impacting the anti-tumor immune response and the limitations of assessing the tumor immune infiltrate by standard diagnostic methods. A flow cytometry pipeline to characterize the human tumor microenvironment in freshly obtained clinical samples has been established to delineate the cellular makeup of the human tumor immune infiltrate. In addition the expression levels of known modulators of the anti-tumor response have been determined. Using surgical resections from a variety of tumor types as the initial set of samples, we established a multi-panel flow cytometry analysis and demonstrated that data acquired within hours of surgical resection agreed with standard IHC analyses for a set of well characterized biomarkers including PDL1, PDL2, CD3, CD8, and CD4. Additionally, the flow cytometry analysis was able to quantify rare or highly relevant cell populations that are unable to be identified by standard IHC, such as CD8+T-cells expressing multiple exhaustion markers. As an example, flow cytometry profiling of a panel of approximately 30 pleural mesotheliomas, identified a subset of patients with characteristics of a highly suppressive microenviroment with elevated levels of infiltrating exhausted T-cells, including a number of patients with significant populations of CD8+ T-cells expressing both PD-1 and Tim-3. We would anticipate that patients with this type of a profile would be good candidates for immunocheckpoint blockade and that the combination of anti-PD1 and anti-Tim3 would be a rational combination to explore in such a patient population. We are further adapting the flow cytometry profiling to evaluate fresh biopsy samples including core needle biopsies, fine needle aspirates, and endoscopic biopsies. Promising early data suggests that this methodology is feasible in the context of clinical trials where sequential biopsies are obtained at enrollment and upon disease progression. Data from these studies will help understand response and resistance to immuno-agents and potentially provide rationale for future combination therapies.