A live tumor fragment (LTF) platform with real-time imaging for immune response assays

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Introduction: Immuno-Oncology (IO) therapies provide remarkable clinical benefits. However, too few patients respond, and there are no diagnostic tools that predict IO response with high accuracy. Moreover, as more IO drugs and combinations are approved, selecting the best IO-based regimen for each patient will become more complex. To facilitate this selection, we are developing an ex vivo live tumor fragment (LTF) screening platform that retains representation of a patient's tumor microenvironment (TME) including immune cells, enabling development of deep neural networks to predict IO response and thereby individualize therapy. Here we present preliminary data from our proof-of-concept platform that performs tissue fragmentation/sorting, liquid handling, drug treatment, high-resolution dynamic imaging, and multiplex immuno-assays.

Methods: Human tumor excisions were obtained from the Univ. of Wisconsin (IRB approved), and CT26 tumors were grown in mice subcutaneously. Live tumors were cut into 300 x 300 µm fragments of 100 - 300 µm thickness, sorted into multi-well plates, and cultured for 48 or 72 h in the presence or absence of anti-PD1 (nivolumab or mouse equivalent), anti-PD1 plus anti-CTLA4 (ipilimumab or mouse equivalent), or concanavalin A (ConA) as a positive control. Cell viability was measured by ATP luminescence assay or flow cytometry. Motility of CD8+ cells was tracked in human LTF using camelid VHH anti-hCD8a-AF594 and 3D multiphoton microscopy for 30 min. Supernatant cytokines were measured using bead immunoassay and T cell markers by flow cytometry.

Results: T cells were retained within LTFs, and the proportion of lymphocytes in LTFs was independent of fragment thickness (1.6%/2.1% for 300 µm, 1.4%/2.3% for 200 µm and 1.4%/2.8% for 100 µm thickness, forCD4+ and CD8+ T cells, respectively). Total cell viability and T cell viability exceeded 80% at 48h, and 3D LTF structure remained intact for at least 48 h. We treated LTFs with anti-PD1, anti-PD1 plus anti-CTLA4, or ConA and confirmed the presence of IFN-γ and 10 (mouse) or 16 (human) other cytokines associated with immune activation in both the ConA and anti-PD1 treated samples, but not the control. In human LTFs, cytokine panel upregulation was observed for anti-PD1 vs. control($p=1.1e-5$) and anti-PD1 plus anti-CTLA4 vs. anti-PD1 ($p=3.7e-9$). Using multiphoton microscopy and CD8-binding nanobodies, we observed vigorous CD8+ T cell motility in human LTFs, with a speed of 10 µm/min, which is comparable to that reported in vivo.

Conclusion: Our LTF platform has an immuno-competent TME in which we can detect cellular and secreted immune response markers, compare alternative treatments, and track the surveillance activity of infiltrating T cells. Future work will further advance the platform, enabling clinical trials for training and validating deep neural networks to predict response to checkpoint inhibitors and other IO drugs.

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