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

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INTRODUCTION

- Immuno-Oncology (IO) therapies provide remarkable clinical benefits, but overall response rates are only ~20%, and no diagnostic tools predict IO response with high accuracy.
- As more IO drugs and combinations are approved, selecting the best IO-based regimen for each patient will become more complex.
- The Elephas live tumor fragment (LTF) platform retains the patient's tumor microenvironment, including immune cells, enabling development of AI-based models to predict IO response and thereby individualize therapy.

LTFs ARE VIABLE AND PROLIFERATING

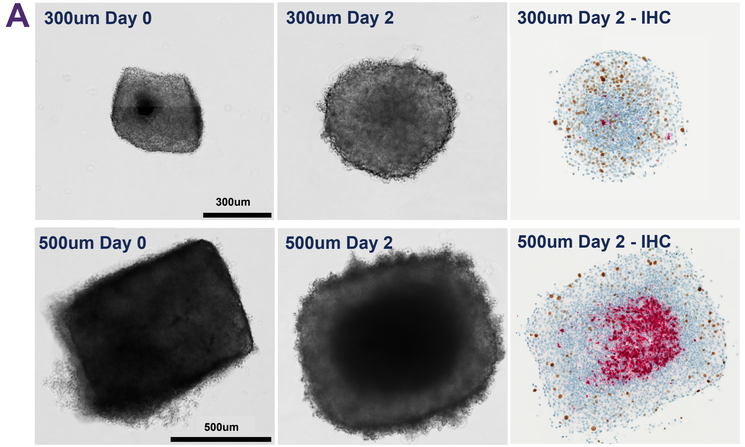


Figure 1. A: Subcutaneous CT26 tumors were cut into LTFs and monitored over time with brightfield microscopy to observe growth. After 48h, proliferation and apoptosis were assessed by Ki-67 (Brown) and cleaved caspase 3 (Red). LTFs cut to 300x300x300 um were viable and proliferating, while LFTs cut to 500x500x500 um harbored areas of central apoptosis. B: A human kidney tumor obtained from the University of Wisconsin, Madison (IRB-approved) was cut into LTFs, cultured for 48h, and cell viability and T cell content were quantified by flow cytometry.

T CELL MOTILITY IS ALTERED BY αPD1/αCTLA4

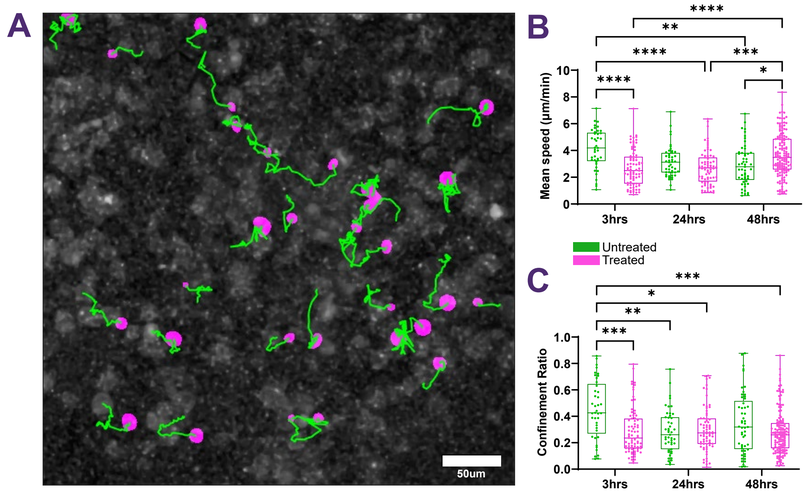
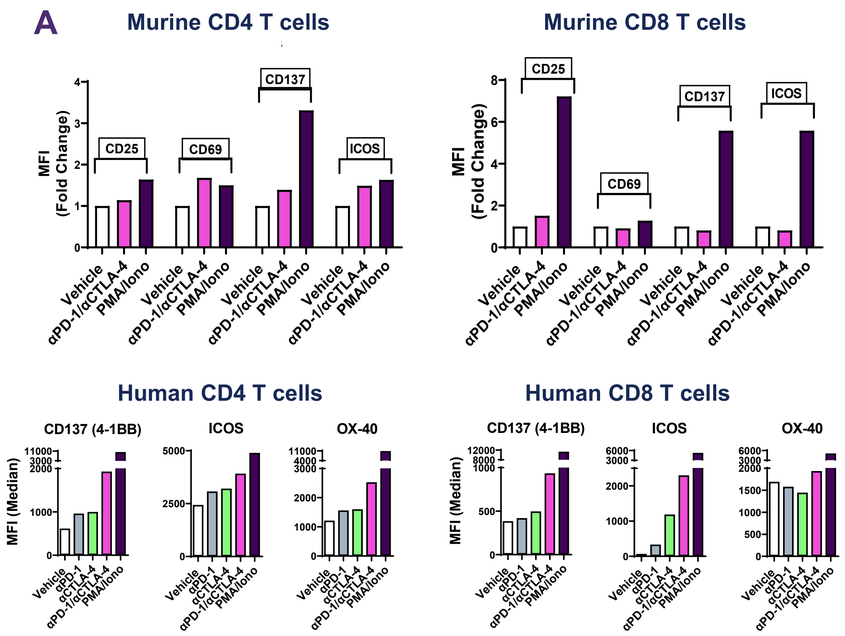
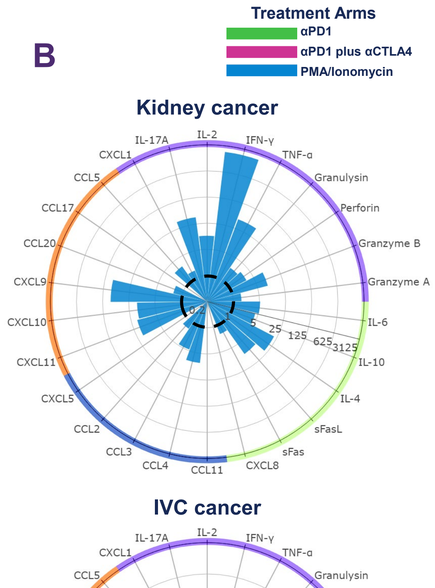


Figure 2. LTFs were cut from an LLC1 tumor that was grown in mice heterozygous for CD2-dsRed, CD11c-eYFP, and CX3CR1-eGFP transgenes. A: T cells were segmented as DsRed+ YFP- GFP- from 3D multiphoton images using a Laplacian of Gaussian filter to emphasize round objects. T cells were tracked over time (green paths). The mean speed (B) and confinement (C) of T cells from αPD-1/αCTLA-4-treated and untreated LTFs were calculated at 3, 24, and 48 hours after treatment. Confinement was calculated as: (ending track position – starting track position)/total track length; index > 0.7 indicates a directional motility, and lower values indicate confined motility: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

LTFs RESPOND TO CHECKPOINT BLOCKADE





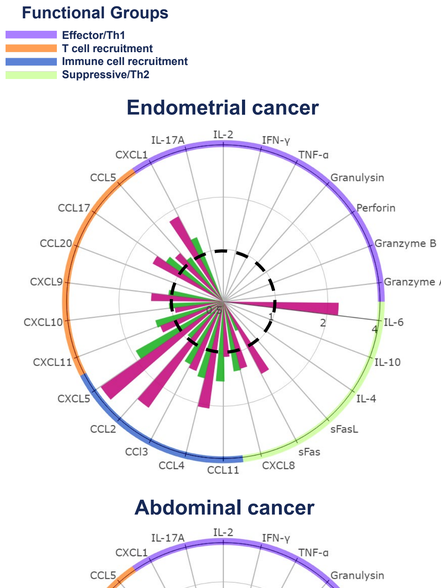


Figure 3. Murine LTFs were cut from subcutaneous LLC1 tumors, and human LTFs were cut from tumor excisions obtained from the University of Wisconsin, Madison. All LTFs were cultured for 48 hours in the presence of indicated treatments. Flow cytometry and cytokine bead assays were used to quantify T cell surface activation marker expression and secreted factor production. A: T cell responses to immune checkpoint blockade were evident by flow cytometry. B: Human LTFs were generated from kidney, inferior vena cava (IVC), abdominal, and endometrial cancers and cultured with vehicle, αPD-1, dual αPD-1/αCTLA-4, or PMA/ionomycin. Shown are the fold change over vehicle, demonstrating that the Elephas LTF platform can distinguish between responses to different IO agents in multiple human cancer types.

SUMMARY

- Live Tumor Fragments (LTFs) maintain viability and immune infiltration
- T cells are motile in LTFs and alter their movements with checkpoint blockade
- Murine and human LTFs respond to immune activators by increasing surface marker expression and cytokine/chemokine production
- Combination immunotherapy was distinguishable from single agent therapy in human LTFs
- Upcoming clinical trials will be used to train and validate deep neural networks to predict response to IO using the Elephas platform