Abstract #187

Murine live tumor fragments (mLTF™) can be used to assess immunotherapy response

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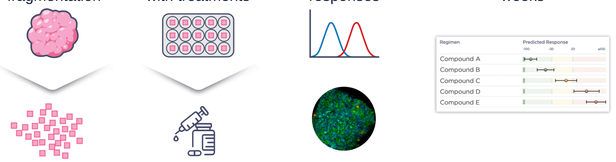
Introduction

- Despite increasing cancer rates and increasing investment into IO, no Dx solution exists
- 4 in 5 patients fail to respond to IO regimens today
- Only 4 FDA-approved biomarkers for marketed IO therapies
- Current ex vivo models have reduced translatability to human in vivo outcomes due to the loss of native tumor architecture and microenvironment
- Elephas' Cybrid platform significantly de-risks drug development end-to-end with rich, orthogonal data sets delivered in less than two weeks

Elephas Cybrid™ Platform overview



Fragmentation creates homogeneous groups

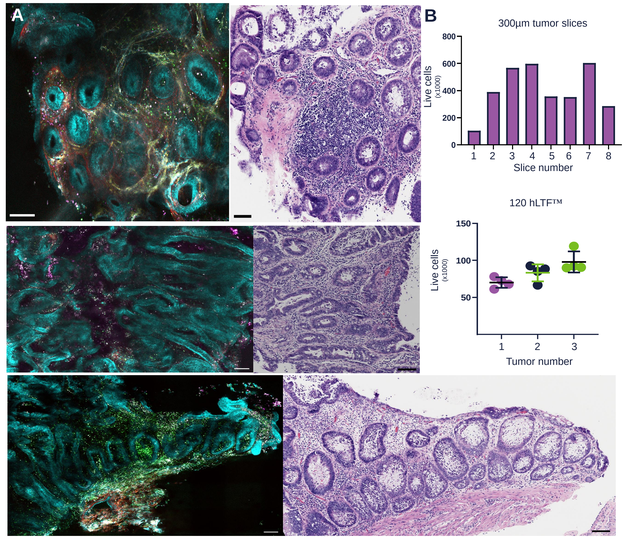


Figure 1. A) Multiphoton images of a live human colorectal cancer showing NADH, propidium iodide, cleaved caspase 3/7 and connective tissue demonstrating phenotypic heterogeneity. Scale bars = 100µm B) Top – Flow cytometry data from 300µm slices of the same tumor with 600µm between each slice showing heterogeneous cellularity across the excision. Bottom – Groups of 120 human live tumor fragments (hLTF™) measuring 300x300x300µm cubes created with the Elephas Cybrid Platform showing normalization of cell numbers across groups cut from the same tumor.

TILs expanded from mLTF™ are cytotoxic

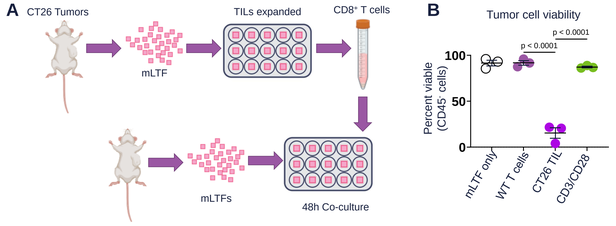


Figure 2. A) Experiment diagram depicting tumor infiltrating lymphocyte (TIL) expansion from CT26 mLTF and subsequent treatment of fresh CT26 mLTF with CD8⁺ T cells isolated from expanded TILs. B) mLTF were cultured in complete RPMI 1640 alone, with wild type CD8⁺ T cells isolated from solenocytes that were expanded under the same conditions as the CT26 TILs, CD8⁺ T cells isolated from expanded TILs or with CD3 and CD28 activating antibodies. Shown is the percentage of tumor cells that are viable after 48 hours as determined by flow cytometry assessing the CD45⁻ viability dye⁻ cells as a percent of total CD45⁻ cells.

Transgenic T cells respond to Ag⁺ mLTF™

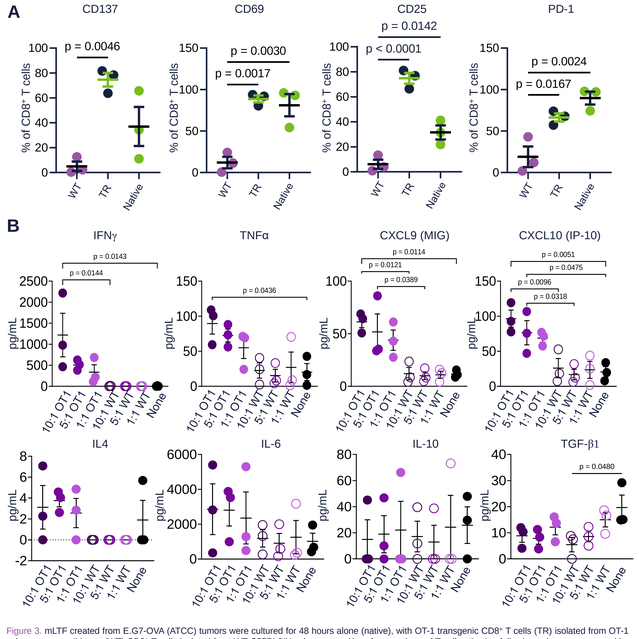


Figure 3. mLTF created from E.G7-OVA (ATCC) tumors were cultured for 48 hours alone (native), with OT-1 transgenic CD8⁺ T cells (TR) isolated from OT-1 splenocytes or wild type (WT) CD8⁺ T cells isolated from WT C57BL6/N splenocytes. A) surface markers of T cell activation following culture as assessed by flow cytometry on CD45⁺CD20⁻CD14⁻CD3⁺CD8⁺ cells. B) mLTF were cultured for 48 hours with no added T cells or a 10:1, 5:1 or 1:1 effector:target ratio of OT-1 or WT T cells after which culture media was assess for cytokine and chemokine secretion using the LEGENDplex bead capture assay. Th1 biased cytokines and chemokines are shown in the top row and demonstrate significant increases over their WT counterparts while Th2 biased cytokines are show in the bottom row where no significant differences were observed.

ICB responses in mLTF™ indicate the magnitude of growth suppression *in vivo*

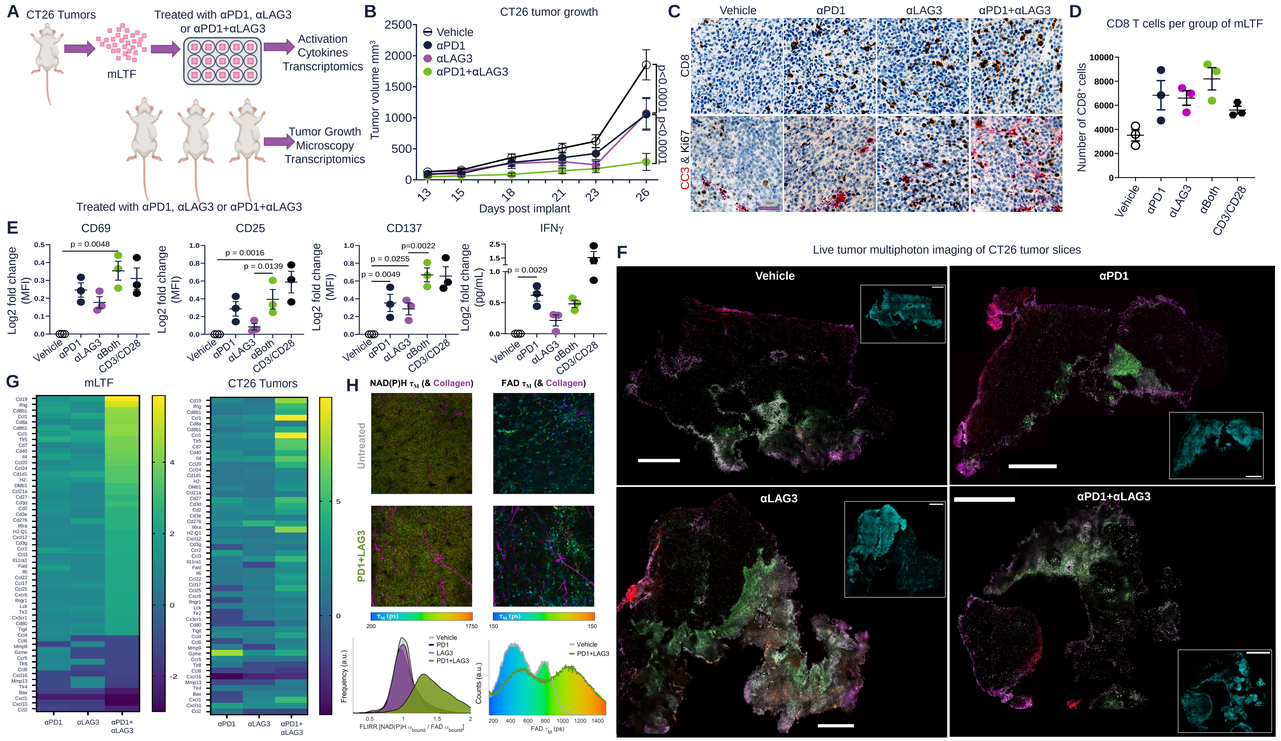


Figure 4. CT26 mLTF and in vivo tumors were treated with vehicle, αPD1, αLAG3 or αPD1 and αLAG3 immune checkpoint blocking (ICB) antibodies. A) Experiment diagram depicting mLTF creation and 48-hour culture with concurrent in vivo therapy designed to test correlation of the Cybrid platform with in vivo therapeutic responses. B) In vivo growth of CT26 tumors treated with vehicle, αPD1, αLAG3 or αPD1 and αLAG3 showing delayed tumor growth with each individual treatment that was further delayed in the αPD1 and αLAG3 group. C) Immunohistochemistry conducted on in vivo tumors harvested 26 days after implant showing differences in CD8⁺ T cell infiltration and cleaved caspase 3 staining. D) CD8⁺ T cell numbers from mLTF cultured for 48 hours with each treatment or CD3 and CD28 stimulating antibodies showing increased T cell retention in treatment groups when compared to vehicle, analogous to the in vivo results show in C. E) Surface marker expression and cytokine secretion levels from mLTF following 48-hour culture demonstrating increases with monotherapies when compared to vehicle and further increase with the combination, indicating that these parameters could be used to infer the magnitude of anti-tumor responses in vivo. F)) Multiphoton microscopy conducted on live 300µm slices of CT26 tumors treated in vivo NADH, propidium iodide, cleaved caspase 3/7 and connective tissue, scale bars = 1mm. G) Gene expression analysis of treated mLTF and *in vivo* tumors showing the fold change over vehicle for the most upregulated and down regulated genes in the mLFT αPD1 and αLAG3 showing similarities in gene expression changes during treatment of mLTFs and *in vivo* tumors with ICB. H) NADH and FAD by fluorescence lifetime imaging microscopy (FLIM) as a measure of cellular metabolic activity demonstrating a reduction in both when comparing the combination treatment to vehicle control.

METHODS

Resections of a human colorectal cancer were sliced into 300µm slices in sets of three with one slice digested for flow cytometry, one imaged using multiphoton microscopy and the third formalin fixed and embedded in paraffin (FFPE) for H&E and immunohistochemistry (Figure 1A and B left). Using the Cybrid™ platform resections of three different human tumors were cut into 300x300x300µm human live tumor fragments (hLTF), randomized, and pooled into groups of 120. Each pool was digested in Miltenyi tumor disassociation enzymes and assessed by flow cytometry (Figure 1B right). Tumor infiltrating lymphocytes (TILs) were expanded from 300x300x300µm mouse live tumor fragments (mLTF) created from subq CT26 tumors on the Cybrid platform and CD8 T cells were isolated using a negative selection magnetic bead separation. CD8 TILs were then cultured with additional mLTF for 48 hours and tumor cell viability was assessed by flow cytometry (Figure 2). CD8 T cells were isolated from OT-1 transgenic mice and incubated with E.G7-OVA mLTF for 48 hours after which media was harvested and assessed for secreted cytokines and chemokines while CD8 T cells were assessed by flow cytometry (Figure 3). Subq CT26 tumors were grown until their volume reached 200-300mm3 after which mLTF were created and treated with vehicle, αPD1, αLAG3 or αPD1+αLAG3 immune checkpoint blockade (ICB) for 48 hours. Culture media was collected for cytokine analysis by IFNγ ELISA, cells were assessed by flow cytometry (Figure 4E), and additional cells were reserved for RNA isolation. BALB/c mice implanted with CT26 tumors were treated with PBS vehicle control or 100µg αPD1, αLAG3 or αPD1+αLAG3 (N=30 per group) starting five days post implantation and continuing twice every week until tumors in the control group reached 2000mm3. All mice were sacrificed and tumors with median volumes were used for RNA isolation, FFPE immunohistochemistry and multiphoton imaging as described in the figure legends. Intrinsically fluorescent metabolic co-factors nicotinamide dinucleotides (NAD(P)H) and flavin adenine dinucleotide (FAD) were imaged using multiphoton fluorescence intensity and fluorescence lifetime imaging microscopy (MP-FLIM). We analyzed the data by fitting fluorescence decay curves using a bi-exponential model to generate images of lifetime parameters, including the mean (τM) and individual component lifetimes and amplitudes (e.g., αfree is the shorter lifetime component of NAD(P)H). Corresponding collagen intensity images were overlaid for additional structural visualization (Figure 4 F and H). All statistics were conducted with Graphpad Prism 9.4.1 using one- or two-way ANOVA with Dunnett's or Tukey's multiple comparisons test to compare means to the control or to one another. P values of interest are indicated on the figures. All mice were treated in accordance with IACUC approved research protocols.

Summary

- Cybrid platform produces fragments that retain viability including innate immune cells
- Cybrid LTF strategy addresses heterogeneity of tumors and creates homogeneous treatment groups
- Adoptive T cell responses can be detected using the Elephas Cybrid platform indicating CAR T cells can also be assessed
- Expanded TIL anti-tumor responses can be observed using the Cybrid platform
- mLTF responses to ICB therapy correlate to *in vivo* tumor responses
- Gene expression changes observed in the Cybrid platform are similar to those seen *in vivo*