Poster #123

Assessing Cytotoxic T Cell Responses in Live Tumor Fragments (LTFs™) with Multiphoton Microscopy

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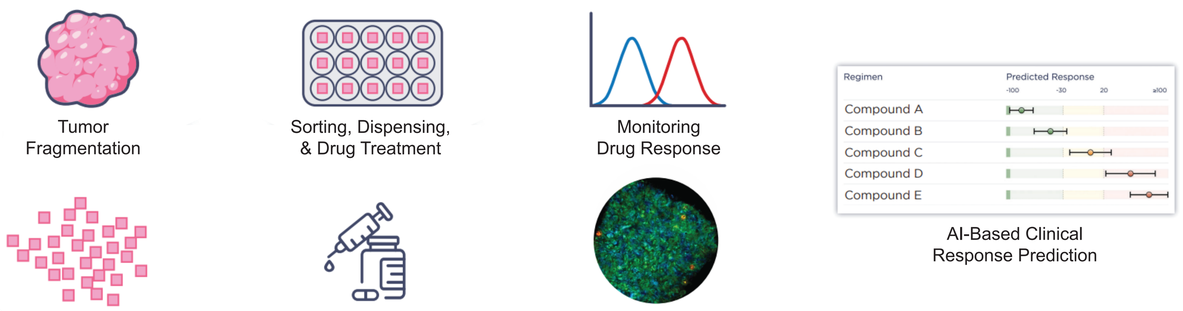
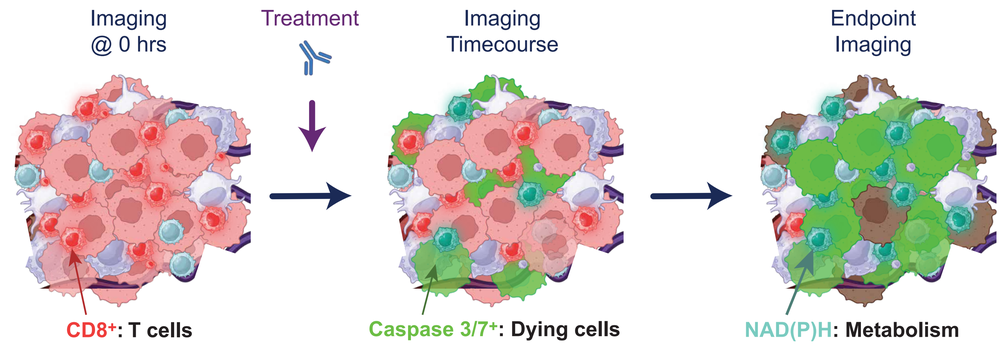
Introduction

- Immunotherapies provide remarkable clinical benefit, however, response rates are poor and robust biomarkers for predicting response remain elusive.
- Additionally, there is a lack of non-destructive technology that allows for the longitudinal assessment of the tumor microenvironment including T cell activation and resultant cytotoxicity in physiologically relevant tumor systems.
- To address this challenge, we have developed the Cybrid™ platform, which allows for the generation and interrogation of LTFs that preserve the tumor microenvironment, derived both from biopsies and resections, to characterize response to immunotherapies and predict clinical response.
- Advanced imaging techniques employed on the Cybrid platform, such as multiphoton fluorescence lifetime microscopy (MP-FLIM), are shown here to simultaneously enable longitudinal, non-destructive assessment of T cell activation and cytotoxicity in LTFs in response to immune checkpoint inhibitors (ICIs).

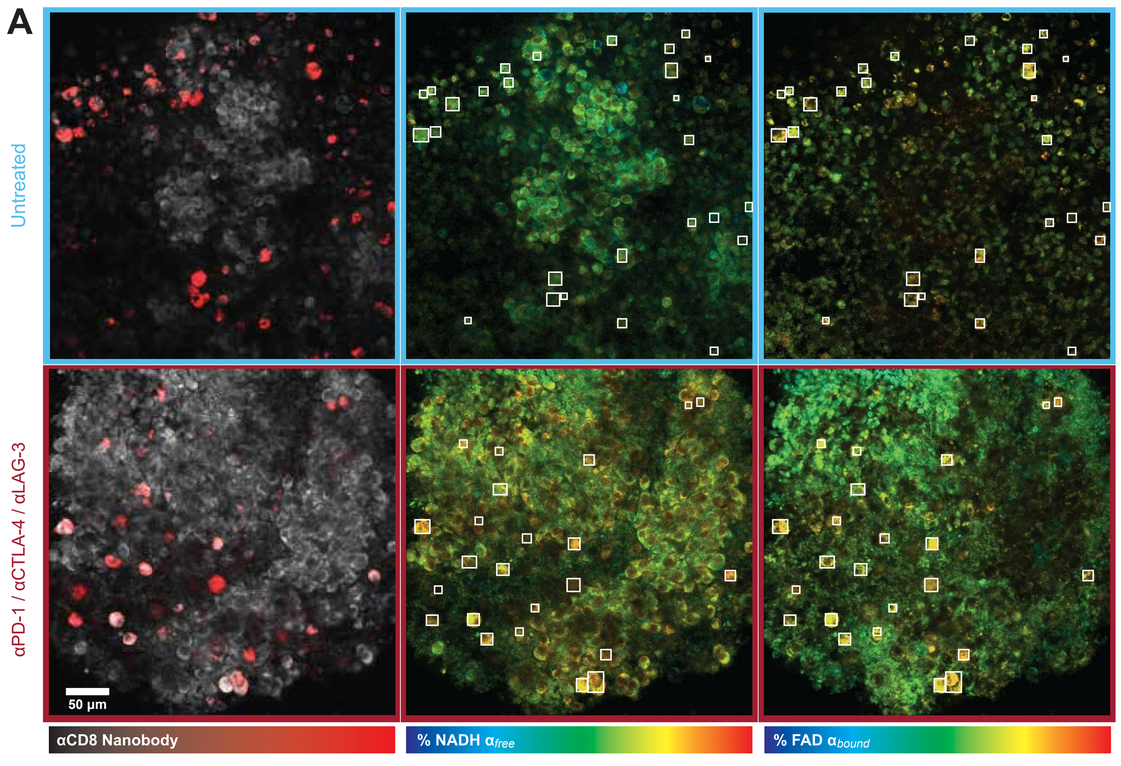


Methods



Murine syngeneic CT26 tumors were processed using the Cybrid platform to create murine LTFs which were sorted into well plates, cultured, and treated with a combination of ICIs targeting PD-1, CTLA-4 and LAG-3. For metabolic assessment of individual T cells following treatment, we imaged the intrinsically fluorescent metabolic co-factors nicotinamide dinucleotide (NAD(P)H) and flavin adenine dinucleotide (FAD) using MP-FLIM. A αCD8 nanobody conjugated to AF594 was used to label native cytotoxic T cells in a manner spectrally independent from NAD(P)H and FAD emission. FLIM data were analyzed via phasor ratio analysis using two reference lifetimes (T_1 and T_2 for short and long lifetimes, respectively) to generate images of lifetime parameters, including the fractional contributions (α_1 is the shorter lifetime component). Cytotoxic T cell-mediated killing of tumor cells was assessed with an exogenous marker for apoptosis (Caspase 3/7 Green). Due to baseline cutting damage, (i.e., high density of Caspase 3/7 positivity at the fragment periphery), a 3D region of interest was identified to only include regions greater than 45 microns from any edge.

T Cell Response to ICI Treatment in Murine LTFs



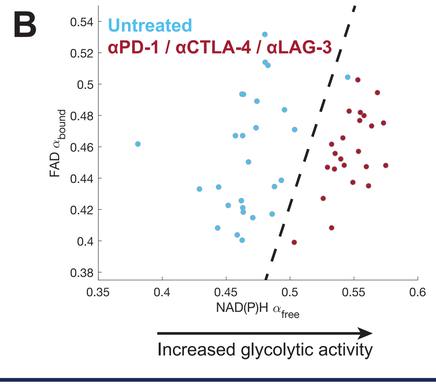


Figure 1. Metabolic assessment of ICI-mediated T cell activation.

(A) CT26 murine LTFs were treated with the indicated combination of ICIs or left untreated for 48 hours. αCD8 nanobody intensity was used to identify and segment individual T cells for quantification of their NAD(P)H α_{free} and FAD α_{bound} fraction via phasor ratio analysis.

(B) NAD(P)H FLIM signature showed an increased glycolytic state in ICI-treated CD8 T cells compared to untreated.

Increased T Cell Killing in ICI Treated Murine LTFs

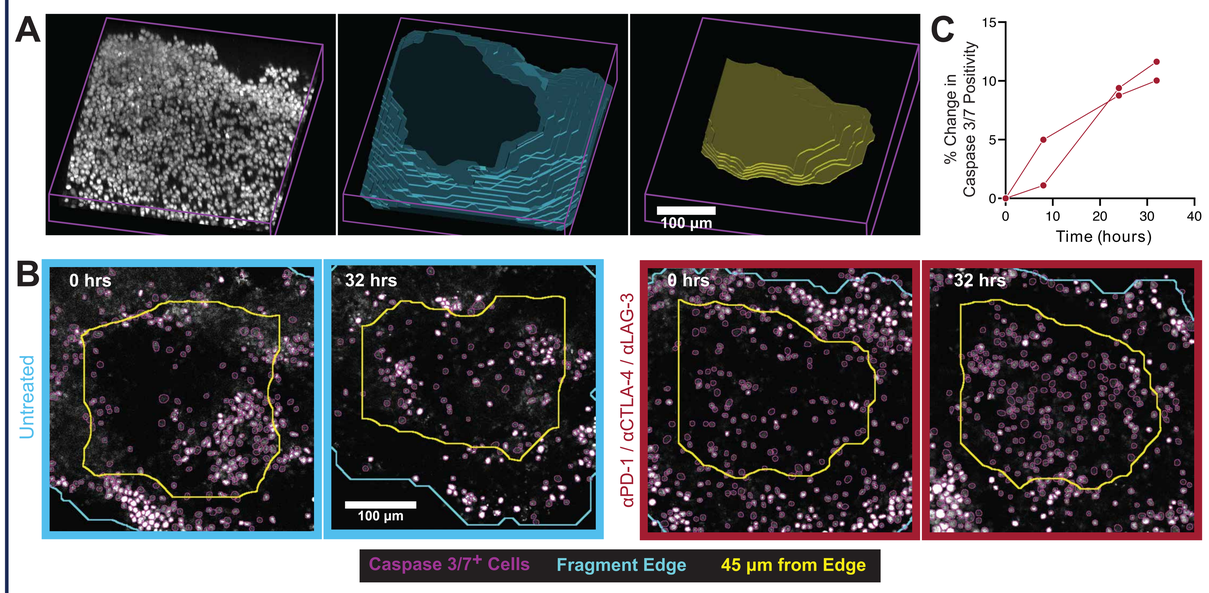


Figure 2. Longitudinal assessment of ICI-mediated T cell killing.

(A,B) CT26 murine LTFs were labeled with an exogenous apoptosis marker (Caspase 3/7 Green). Volumetric labeling was captured at baseline (t_0) followed by treatment with or without the indicated combination of ICIs and imaged at 8, 24, and 32 hours post-treatment. A single optical plane from an untreated (left) or ICI treated (right) fragment are shown from 0 and 32 hours.

(C) Line graph shows increase of 3D ROI positive for Caspase 3/7 for 2 individual ICI-treated fragments, normalized against a single control fragment.

Summary

- We show here preliminary data on MP-FLIM to simultaneously assess ICI-mediated T cell activation and cytotoxic killing of tumor cells.
- We identified T cells with a αCD8 nanobody and measured their activation with NADPH FLIM to assess metabolic state.
- Tumor cell death was measured with a Caspase 3/7 probe.
- Unlike flow cytometry and transcriptional profiling, our MP-FLIM assay is non-destructive and enables longitudinal assessment of the tumor microenvironment.
- We will confirm these studies in additional models and in human tumors.
- This imaging approach is a component of the Cybrid platform, which will enable the assessment of immunotherapy response to predict clinical outcomes in individual patients.

See More Here!