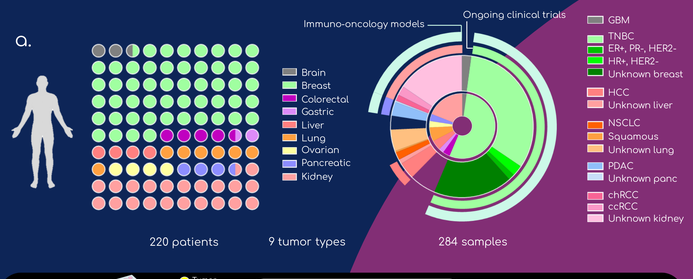
Development of a Kidney Cancer *ex vivo* Tumor Model and Image-Based Artificial Intelligence Tool for Precision Immunotherapy and Combination Therapy Testing

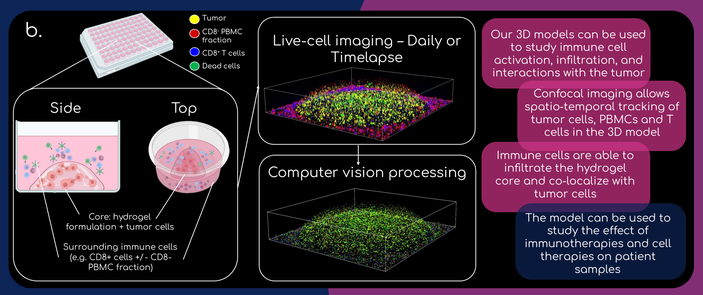
For more detail about our workflow take a look at our presentation or come talk to us at AACR booth #1730

1 Introduction

Precision medicine has the potential to revolutionize cancer treatment and drastically improve outcomes. While genomic screening has become the gold standard for personalized cancer therapy, its predictive power is confined to select cancers and drug modalities, and is yet to show promise as a standalone drug selection tool for improving patient outcomes.

Pear Bio has developed an *ex vivo* IO model and multivariate analysis to predict clinical drug efficacy by combining patient tumor samples, functional assays, artificial intelligence and omics. This model recapitulates each patient's unique tumor-immune microenvironment (TiME) and allows time-course bulk and single-cell resolution analyses of functional metrics in 3D. Initial development was performed on retrospective biobank samples across **9 solid tumor types**. Ongoing observational clinical trials are aimed at establishing the tool's sensitivity and specificity in triple negative breast cancer (TNBC) in the neoadjuvant and metastatic settings, as well as validating the technology in renal, pancreatic, liver, brain and lung cancers.





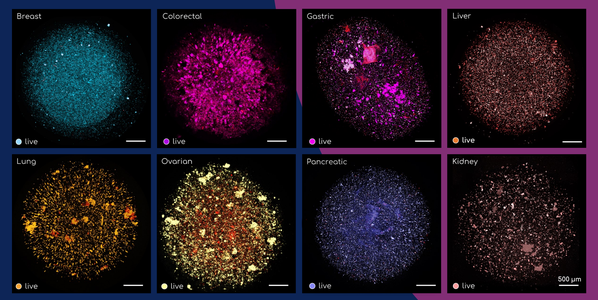


Figure 1. a) Pear Bio's patient cohort by cancer and subtype; b) 3D immuno-oncology model development, confocal imaging and computer vision processing of a kidney patient tumor sample with matched whole blood. After blood processing, effector cells (CD8+ T cells) are isolated from the peripheral blood mononuclear cells (PBMCs) population, stained (violet- 405), and re-combined with the CD8- fraction of PBMCs (red- 630). Parallelly tumor cells are isolated from the tumor sample, stained (orange- 546) and encapsulated in Pear Bio's physiological hydrogel. Immune cells are added, free-floating on top, and tracked over 4 days using time-lapsed confocal microscopy to determine functional metrics such as tumor cell migration, immune cell infiltration, and cell killing; c) physiologically-relevant 3D models across eight solid tumors - apoptotic cells NucView (red 528); scale bar 500 µm applies to all.

2 Measuring *ex vivo* response biomarkers

Human renal tumor resections and matched blood (N=20) are processed for spatial transcriptomics and isolation of single tumor cells and PBMCs. Tumor-dissociated (tumor, stromal, immune, etc.) cells and PBMC subsets are characterized by flow cytometry (FCm). Target cells (tumor) and effector cells (PBMCs and subsets thereof, CD8+) are stained with different fluorescent dyes including viability probes (caspase 3/7, SYTOX) and encapsulated in hydrogels that recapitulate human TiME physiology (Figure 1b). The immuno-oncology (IO) cultures are treated with approved regimens including immune checkpoint (ipilimumab, pembrolizumab) and receptor-tyrosine kinase (TKI) (cabozantinib, lenvatinib, axitinib) inhibitors. Cells are tracked up to 7 days using 3D time-course confocal microscopy. Computer vision analysis detects and quantifies cell behaviors such as immune infiltration, immune/tumor cell migration, T cell-mediated tumor killing and tumor viability in response to treatments.

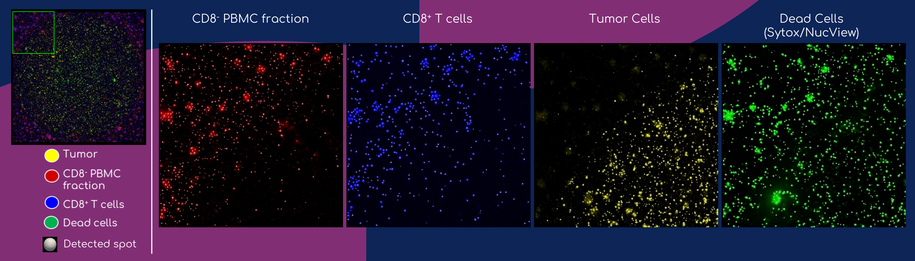


Figure 2. Proprietary computer vision pipeline detects single cells in 3D co-culture model across time points. Tumor cell death over time is quantified via detection and co-localization of orange dye (tumor) and green dye (dead cell dye cocktail).

Our preliminary data shows that treatment with pembrolizumab resulted in a 26% increase in the infiltration of CD8- PBMC fraction into the microtumor core and a 14% increase in infiltration of the CD8+ subset. Tumor cell death was 15% higher in pembrolizumab-treated samples compared to tumor cultures with CD3-activated PBMCs and no treatment, and 30% higher compared to tumor cultures alone (no PBMCs). Migration speed of immune cells was found to be higher as cells invaded and slowed during engagement/killing, peaking at day 1 (3µm/min) and slowing to 2 and 1.5 µm/min for CD8+ and CD8- cells by day 3. FCm was used to characterize tumor cell subpopulations (cancer, endothelial, immune) and the expression of druggable targets in each patient. Treatment with TKIs led to reduced phosphorylation of VEGFR1/2/3, FGFR, PDGFRβ, HGFR, c-Ret, and upregulation of ErbB2/3 (N=3).

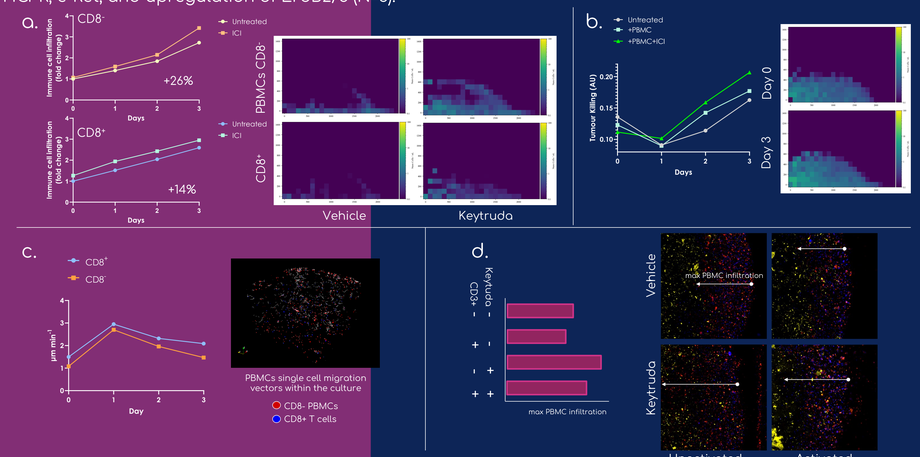
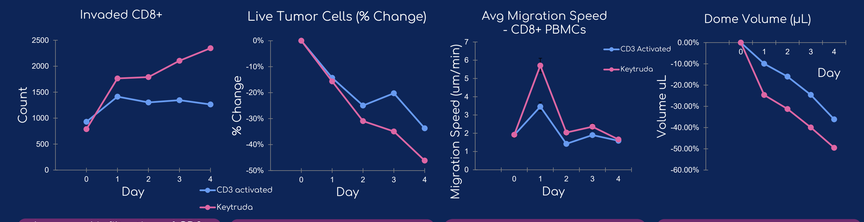


Figure 3. Computer vision metrics of time-course confocal imaging in immuno-oncology 3D cell culture of primary kidney tumor with and without CD3 activation or pembrolizumab (Keytruda) treatment. a) PMBC (CD8- top; CD8+ bottom) infiltration into microtumor in untreated and Keytruda-treated (ICI) conditions; b) Tumor cell death over time in tumor-only condition, tumor and PBMCs or tumor, PBMCs and Keytruda conditions; c) PBMC (CD8- red; CD8+ blue) immune cell speed/activity within the microtumor; d) PBMC infiltration in response to CD3 activation and Keytruda treatment. N=3, CD8+ cells, violet- 405; CD8- fraction of PBMCs, red – 630; tumor cells, orange – 546; apoptotic + necrotic cells, green – 488; images acquired on Leica Stellaris V and processed using proprietary computer vision pipeline.

3 Immuno-oncology *ex vivo* 3D metrics



Increased infiltration of CD8+ cells into microtumor in Keytruda-treated sample

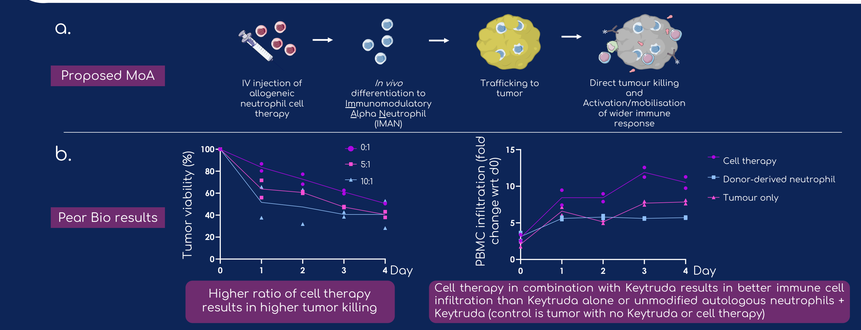
Increased tumor cell death in Keytruda-treated sample

Quantitative CV readout shows Keytruda achieves a higher CD8+ cell migration peak speed compared to CD3 activation.

Keytruda results in more microtumor shrinking compared to CD3 activation

Figure 4. Pear Bio's proprietary computer vision pipeline detects cells and their position over time. Metrics such as invaded immune cells, changes in tumor cell numbers, cell migration speed and microtumor dome volume are quantified. Migration tracks of CD8+ cells over a 3D time-lapse are used to quantify motion. Changes in microtumor size over time are tracked through microtumor-dome shape detection.

4 Cell therapy testing in Pear IO model



Higher ratio of cell therapy results in higher tumor killing

Cell therapy in combination with Keytruda results in better immune cell infiltration than Keytruda alone or unmodified autologous neutrophils + Keytruda (control is tumor with no Keytruda or cell therapy)

Figure 4. Testing efficacy and mechanism of action (MoA) of preclinical allogeneic cell therapy using Pear Bio's IO 3D kidney model. a) proposed cell therapy MoA and b) results obtained in the 3D model.

5 Conclusions

Our platform allows time-course analysis of functional cell response metrics. The model tests treatment combinations across multiple modes of action and quantifies cell response including viability, death, migration, immune infiltration and immune-surveillance. The technology has also been validated across 9 solid tumor types, where dissociated tumor cells show high viability in their physiologically-relevant 3D TiMEs. Future work aims to further develop the platform to predict patient responses in triple-negative breast cancer (NCT05435352), kidney, liver, pancreatic and brain tumors. Early R&D is progressing to include the testing of additional therapeutic modalities including immunotherapies, cell therapies, antibody-drug conjugates, small molecules, chemotherapies and combination therapies.

Oncology/immuno-oncology diagnostics						
Commercial milestones			Pharma preclinical services	Launch lab developed test	FDA De Novo approval	FDA PMA and pharma CDx contracts
R&D stage	Early R&D	Optimise analytical workflow	Retrospective testing	Prospective testing	Prospective outcome prediction trial	Interventional trial
Breast			PEAR-TNBC	PEAR-ME1		NHS Barts Health NHS Trust
Kidney			PEAR-TREE	PEAR-TREE2	MED-TT1	
Liver		PEAR-PAL	UK			NHS Royal Free London NHS Foundation Trust
Brain		PEAR-BRAIN				NHS Guy's and St Thomas' NHS Foundation Trust
Lung						
Colorectal						NHS King's College Hospital NHS Foundation Trust
Ovarian						
Gastric						
Pancreatic		PEAR-PAL	UK			

Figure 6. Pear Bio commercial, R&D and clinical trials timeline.

6 Get in touch

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