Abstract #6767 # Multivariate analysis of *ex vivo* kidney 3D immune-microtumors for functional precision medicine

pear bio
patient-centered cancer precision medicine

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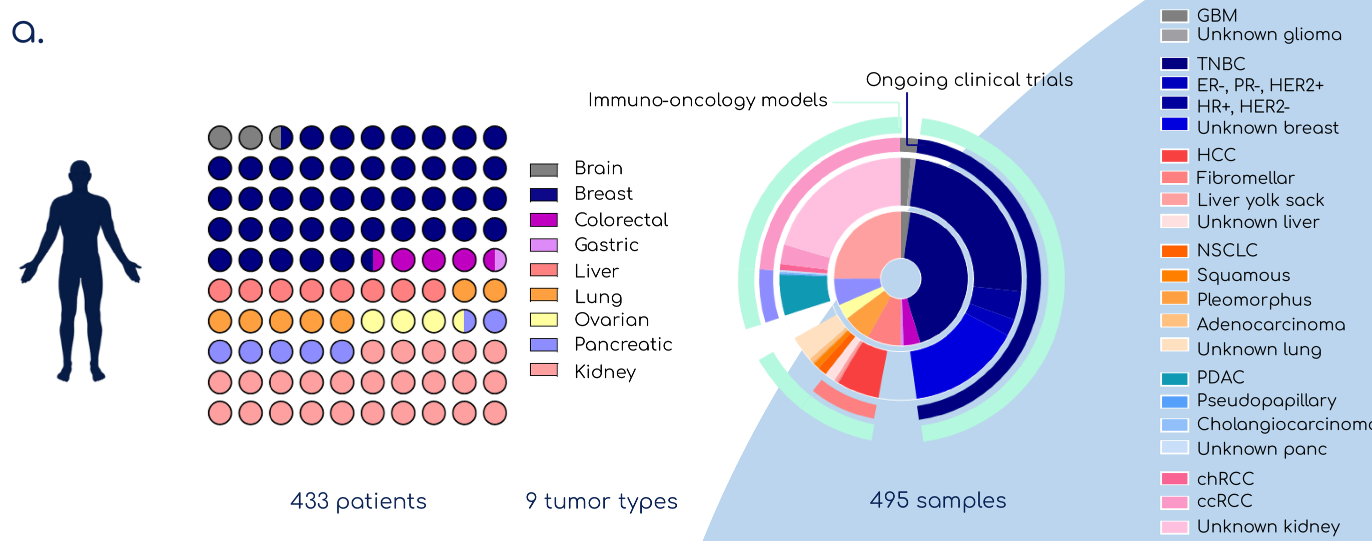
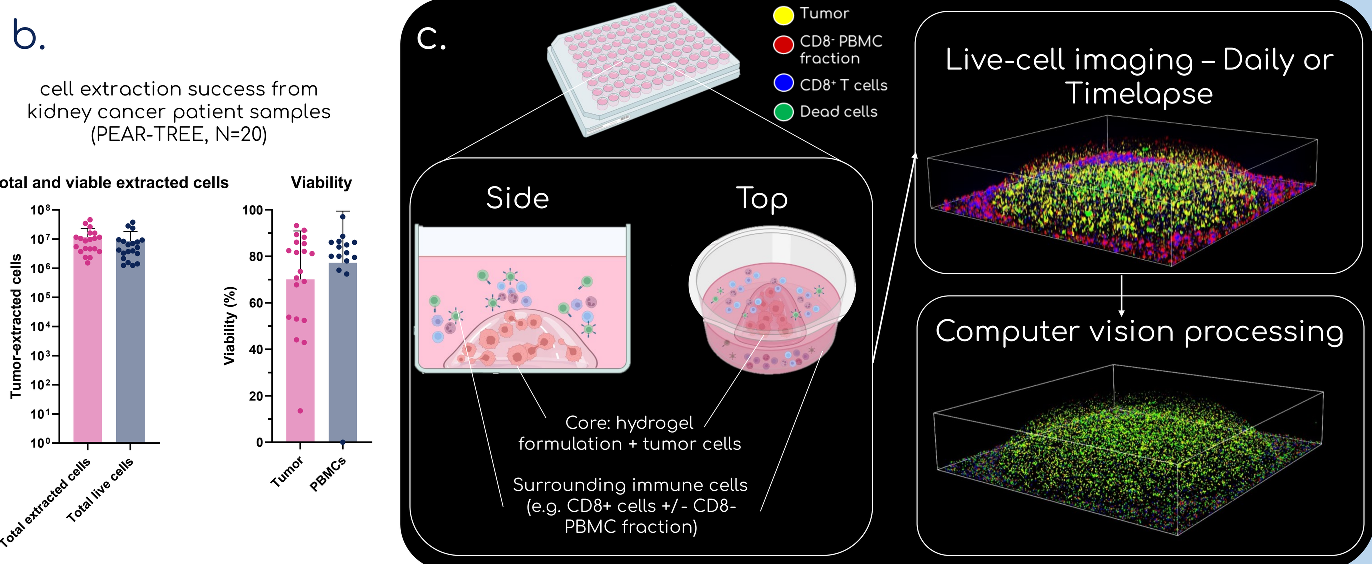
For more detail about our workflow take a look at our presentation or come talk to us at AACR booth #2612

1 Introduction and 3D model

Precision medicine has the potential to revolutionize cancer treatment and drastically improve outcomes. While NGS has become the gold standard for personalized cancer therapy, its predictive power limited 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* immuno-oncology (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 biobank samples across **9 solid tumor types**. Ongoing observational clinical trials are aimed at establishing the tool's accuracy in triple-negative breast cancer (PEAR-TNBC, PEAR-MET), brain (PEAR-GLIO) and kidney cancer (PEAR-TREE2), as well as validating the technology in pancreatic, liver and lung cancers.





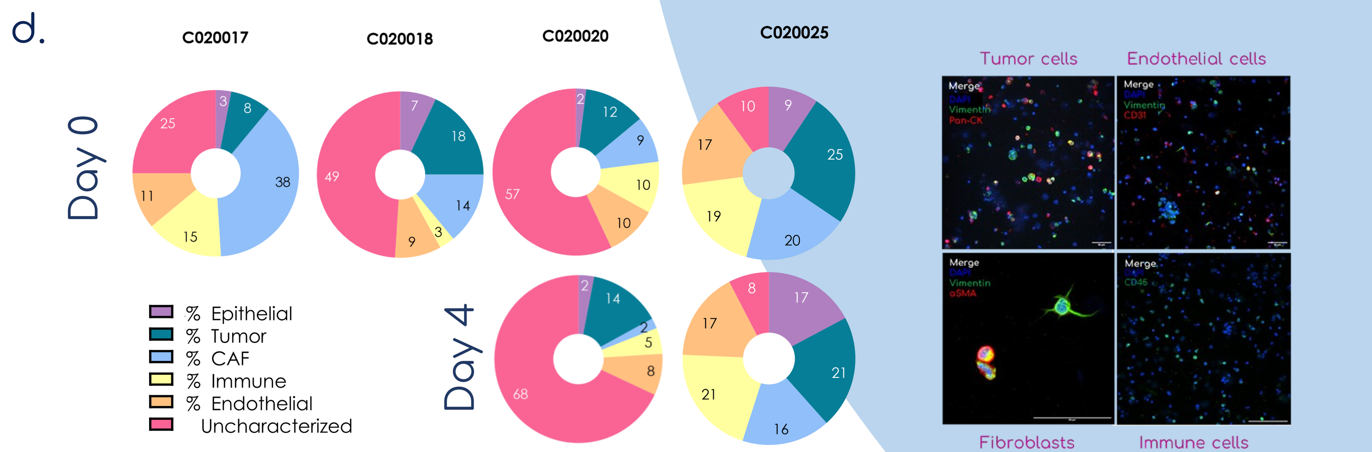


Figure 1. a) Pear Bio's patient cohort by cancer and subtype; b) cell extraction success from patient tumors and matched blood; c) 3D immuno-oncology model development, confocal imaging and computer vision processing of a kidney patient tumor sample with matched whole blood. After blood processing, effector 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). 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; d) immunofluorescence panels for the characterization and quantification of different cell subpopulations within the 3D cultures.

2 Measuring *ex vivo* response biomarkers

Human renal tumor resections and matched blood (PEAR-TREE, N=20, ISRCTN10001405) are processed for histology and isolation of single tumor cells and PBMCs (Fig.1b). Tumor-dissociated (tumor, stromal, immune, etc.) cells and PBMC subsets are characterized by flow cytometry, IF (Fig.1d) and bulk RNAseq. Target cells (tumor) and effector cells (αCD3-treated PBMCs and subsets thereof, CD8+) are stained with different fluorescent dyes including viability probes (dead dye cocktail) and encapsulated in hydrogels that recapitulate human TiME physiology (Figure 1c). The 3D immune-microtumors cultures are treated with approved regimens including immune checkpoint (ipi-, nivo-, pembro-) and receptor-tyrosine kinase (TKI) (cabozantinib, lenvatinib, axitinib) inhibitors in SoC combination. Cells are tracked up to 7 days using 3D time-course confocal microscopy. Computer vision analysis detects and quantifies spatio-temporal cell behaviors in 3D such as immune infiltration, immune/tumor cell migration, T cell-mediated tumor killing and tumor viability in response to treatments (Fig.2).

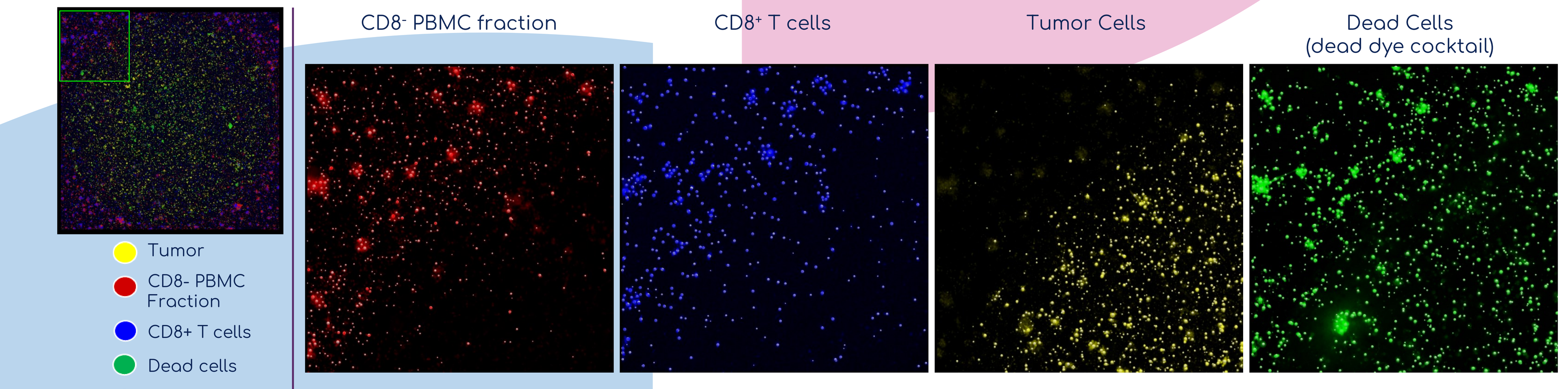


Figure 2. Proprietary automated computer vision pipeline detects single cells in 3D co-culture model across time points. Tumor cell death over time is quantified via detection and co-localization of orange dye (tumor) and green dye (dead cell dye cocktail).

Treatment with FDA-approved combo therapies resulted in patient-specific responses, quantifiable through a variety of functional immune and tumor metrics (Fig3-4)(N=9). Our preliminary data shows that activation with αCD3 results in higher immune cell infiltration in the microtumor (Fig.3a, Fig.4b/c). Microtumor viability showed patient-dependant, activation/treatment-dependant responses and was lower in positive controls (Fig.3b, Fig4a). Microtumor volume was inversely correlated with immune cell infiltration (Fig.3c). 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 (Fig.3d). FCm was used to characterize tumor cell subpopulations (cancer, endothelial, immune) and the expression of druggable targets in each patient. Treatment with IO drugs led to differential upregulation of pro-inflammatory and anti-inflammatory cytokines (Fig.4a, N=3). Treatment with TKIs led to reduced phosphorylation of VEGFR1/2, PDGFRβ and HGFR amongst others (Fig.4b, N=2).

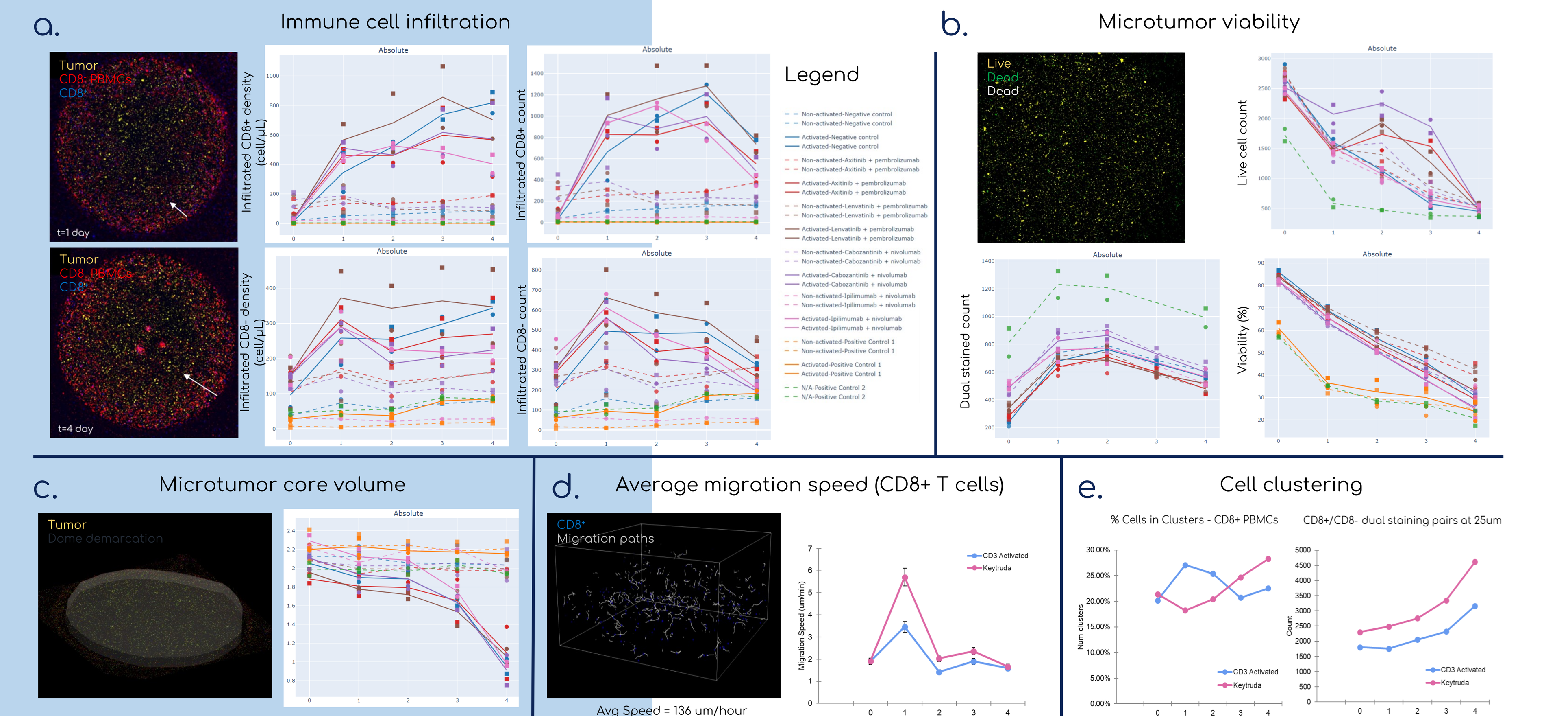


Figure 3. Computer vision metrics of time-course confocal imaging in immune-oncology 3D cell culture of primary kidney tumor with and without CD3 activation and treatments. a) PMBC (CD8+ top; CD8- bottom) infiltration into microtumor over time; b) Tumor cell viability metrics (tumor cell count, dual stained count, viability); c) Microtumor culture dome volume; d) CD8+ T cell migration speed within the microtumor; e) Clustering of CD8+ (left) and CD8+/CD8- cells (right). N=9, 1 representative patient shown, CD8+ cells, violet- 405; CD8- fraction of PBMCs, red – 630; tumor cells, orange – 546; apoptotic + necrotic cells, green – 488; images acquired on Nikon XR and processed using proprietary computer vision pipeline.

3 Population-level statistics

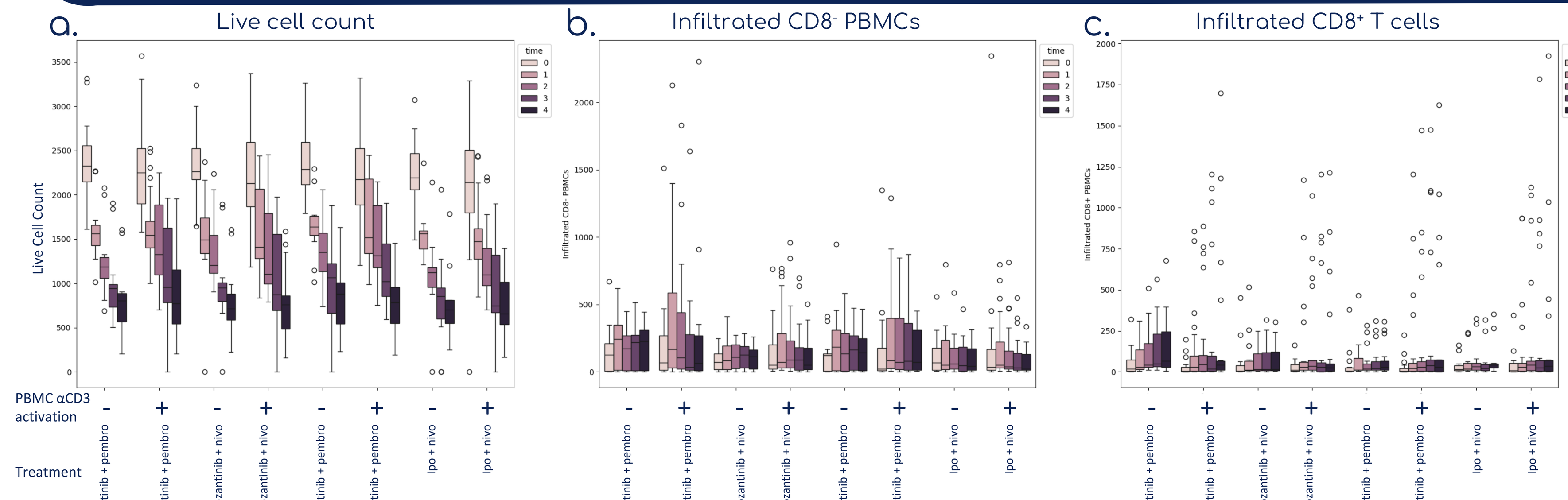


Figure 4. Effect of αCD3 PBMC activation and combination treatment on 3D patient cell culture a) live cell count, b) number of infiltrated CD8- PBMCs, c) number of infiltrated CD8+ PBMCs, N=9.

4 Kidney therapies MoA validation

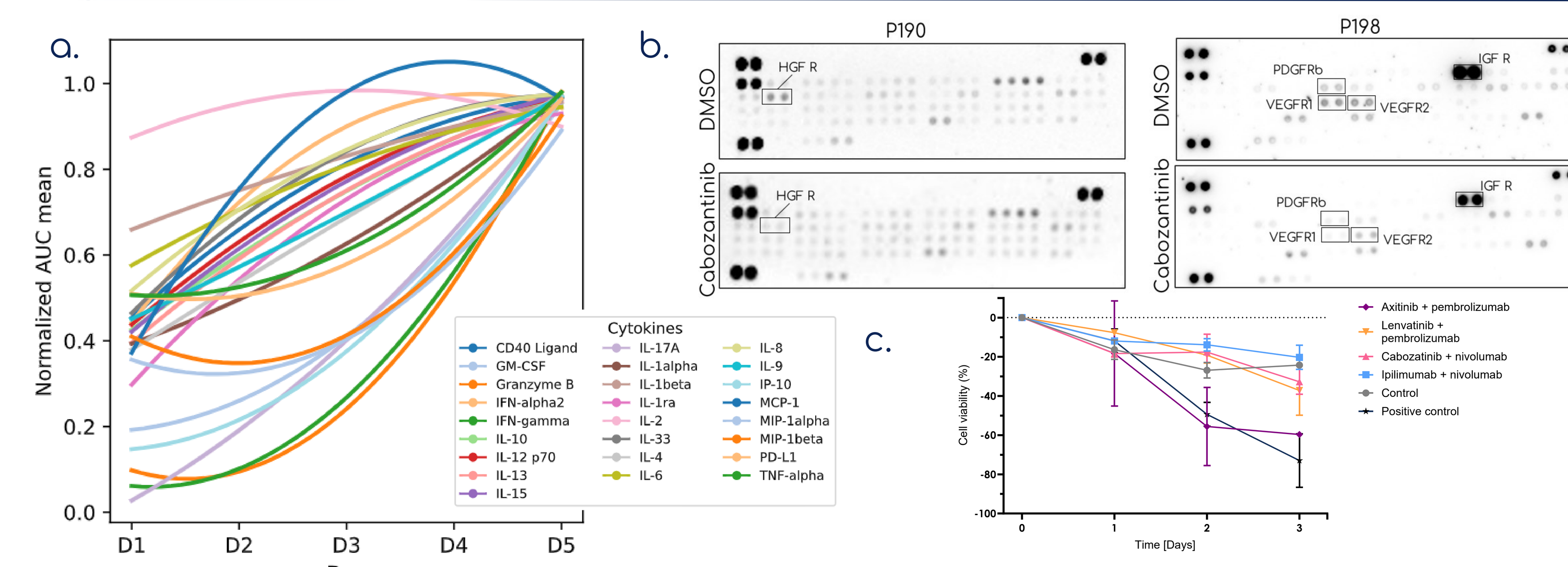


Figure 4. Testing efficacy and mechanism of action (MoA) of a) immunotherapies, b) tyrosine kinase inhibitors, and c) combination therapies using a) Luminex, b) phospho-RTK array and c) Pear Bio's functional assay.

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. We showed MoA-agnostic, patient-dependent responses to FDA-approved clinical treatment regimens via molecular and functional spatio-temporal analyses. Clinical outcome correlation is currently underway in our advanced RCC study PEAR-TREE2 (N=200) as well as in breast NCT06182306, NCT05435352 and brain NCT06038760 tumors.

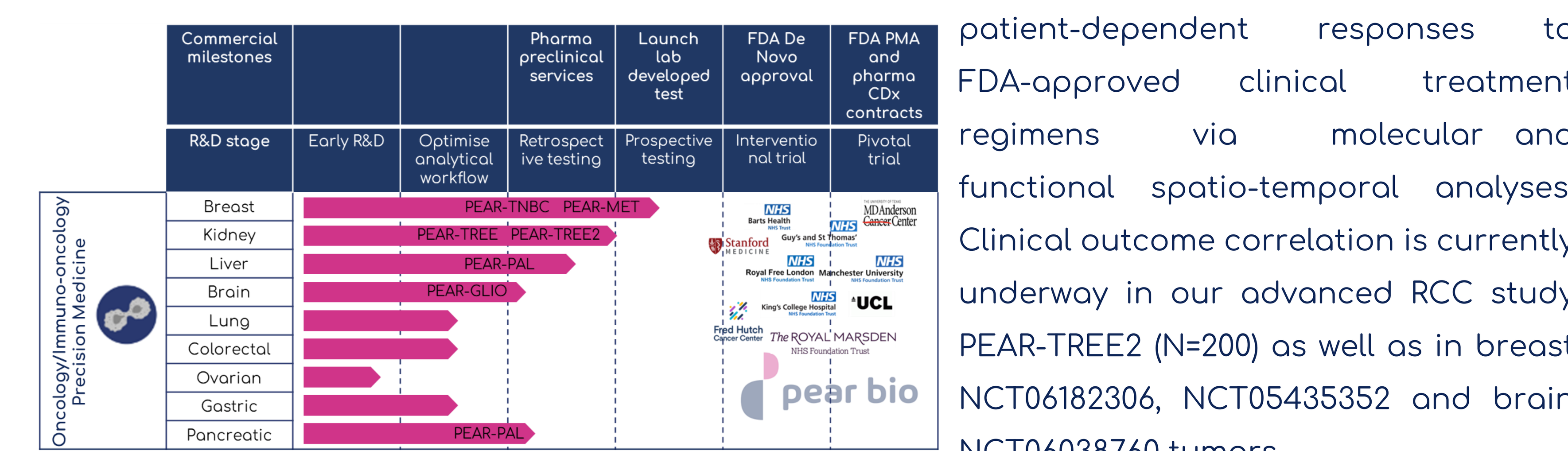


Figure 6. Pear Bio commercial, R&D and clinical trials timeline.

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6 Get in touch

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