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# EVALUATING THE ROLE OF TUMOR MICROENVIRONMENT HETEROGENEITY IN THE PROGRESSION AND TREATMENT RESISTANCE OF PANCREATIC DUCTAL ADENOCARCINOMA

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## Abstract

Pancreatic ductal adenocarcinoma is a cancer that still poses one of the most deadly malignancies with a complicated and heterogeneous tumor microenvironment that has contributed to the universal resistance to treatment. In this study, a multi-dimensional, problem-oriented methodology was utilized that combines high-dimensional spatial proteomics, microfluidic organotypic systems, measurements of therapeutic recalcitrance in tumor microenvironment metabolic flux and computer modeling of agents to comprehend the mechanistic nature of therapeutic recalcitrance in tumor microenvironment. We demonstrated by multiplex immunohistochemistry and imaging mass cytometry of untreated and treated samples of patients that the canonical chemotherapy induces a so much remodelling of spatial distributions that both inflammatory cancer-associated fibroblasts and M2 macrophages become more colocalized by 76 percent and The crosstalk of Single-cell metabolic profiling revealed a functional dichotomy with inflammatory cancer-associated fibroblasts having high glycolytic and glutaminolytic flux and myofibroblastic cancer-associated fibroblasts specialising in collagen synthesis. Goal-oriented agent-based computational modeling found that combination therapy based on triple combination therapy that included extracellular matrix degradation, transforming growth factor-beta inhibition and chemotherapy, led to a higher likelihood of tumor control of nearly 90 percent as compared to monotherapies with less than 15 percent success. M2 to M1 macrophage ratio was found to be the most critical negative prognostic of overall survival, as well as the tissue stiffness and effective drug diffusivity. In other words, this concerted study confirms that pancreatic ductal adenocarcinoma therapeutic resistance is the concerted action of physical, cellular, immunological and metabolic defenses that have to be rationally designed combination strategies that act in concert to destroy these interrelationships of defenses to produce a long-term clinical response.

**Keywords:** Pancreatic Ductal Adenocarcinoma, Tumor Microenvironment, Desmoplastic Stroma, Cancer-Associated Fibroblasts, Therapeutic Resistance, Immunosuppression.

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## INTRODUCTION

Pancreatic ductal adenocarcinoma has a very diverse and complex microenvironment which contributes significantly to the disease progression, its metastasis, and resistance to treatment (Sherman and Beatty, 2022). This is the hostile environment with a thick desmoplastic stroma, a list of cancer-associated fibroblasts, and a high number of immune cells that is aggressive and offers high resistance to the effective treatment modalities (Qiao et al., 2025; Truong and Pauklin, 2021). Not only is it highly heterogenous both in cross- and intra-patient environments, but it also offers enormous evolutionary benefits to tumor cells; not to mention that it also adds to its resistance to therapies (Musiu et al., 2024). In particular, the salient desmoplastic stroma and the architecture of rigid and anisotropic extra-cellular matrix of collagen I, fibronectin and hyaluronan and other active forms of cells generate a hypoxic and acidic microclimate and inhibits the therapeutic response (Carvalho et al., 2021). It consists of a dense stromal tissue, which harbors these cells in addition to endothelial cells, immune cells and the neurons, and can cover most of the tumor literally blocking the delivery of drugs and other beneficial nutrients to the cancer cells (Biancur & Kimmelman, 2018; Than & Stanger, 2025). Such a desmoplastic response, M2-type macrophage and regulatory T cell immunosuppressive cellular infiltrate, are also better in enhancing anti-tumor immunity (Silva & Rosa, 2025). And it is this tumor microenvironment (extracellular components and other signaling molecules) that also enables the tumor to evolve and provide some hallmarks: high tissue rigor, extreme hypoxia and absence of nutrition, which in turn causes the resistance to the already existing ones, which in turn causes the discovery of new ones (Rehman & Kiran et al., 2024) . This will include the characterization of the highest immune evasion of pancreatic ductal adenocarcinoma within the context of the tumor microenvironment and the investigation of the treatment process based on the provided working procedures in the context of cellular, stromal and metabolic components (Hui et al., 2025). Such interaction, specifically, with multi-omics and machine learning approaches needs to be understood better, as it would then be able to develop hybrid therapeutic targets, which would, in turn, be capable of transforming an immunologically cold PDAC tumor microenvironment to become hot and, by extension, treat it to respond to therapy (Khan et It will trigger the overall

study of the mechanobiological processes culminating in the development of treatment resistance in specific the interactions of heterogeneous cellular and acellular components of tumor microenvironment in an unstable environment (Qiao et al., 2025). Among the factors that lead to the formation of a substantial obstacle to effective therapy, the desmoplastic environment thickening and the associated immunoregulatory processes can be enumerated (Hartupee et al., 2024). This heterogeneity and overt and visible phenotypic tendencies of the tumor immune microenvironment like deserted and reactive regions are a significant obstacle to precision in care as the traditional type of subtyping can miss the entire tumor signature (Luo et al., 2024). All these non-homogenous microenvironmental forces as well as the space and time dynamics demand some form of an accountability to be constantly present so that they can devise some form of interventions that will destroy therapeutic resistance. Besides, the intricacy of this communication between the cellular and the molecular components of the PDAC tumor microenvironment also leads to the depletion of T- cells and the infiltration of tumor-promoting immune cells (M2 macrophages and myeloid-derived suppressor cells) combined with others, which in turn is linked with poor clinical outcomes (Liu et al., 2019). In addition, the two-way dialogue between cancer cells and the stromal components continuously re-defines their nature, and, therefore, primordial PDACs features and the versatile immune image should be taken into account when constructing the complex biomarker-identifying tool (Musiu et al., 2024). This in-depth analysis of the nature of the PDAC TME is likely to contribute to the creation of the combination regimens that would be viable and contribute to the improvement of the treatment process and improvement of the patient survival (Hsu et al., 2022). Cancer immunotherapy resistance in PDAC can be reversed by therapeutic interventions, which have the potential to alter the tumor microenvironment i.e. suppress CAFs, inhibit the formation of thick extra-cellular matrices, re-educate myeloid-derived immune cells and normalize deviant metabolic states (Hui et al., 2025). The colorful role of all these multidimensional processes of extracellular matrix remodelling and metabolic reprogramming to myeloid cell targeting is known to transform the immunosuppressive microenvironment into the one that stimulates the immune infiltration and

activation (Hui et al., 2025). The latter should then be followed by research efforts to determine the tumor permissive roles of heterogeneous fibroblast subpopulations and tumor restrictive roles of heterogeneous fibroblast subpopulations and explore convergent tumor, stromal and immune cell metabolism targets (Kung et al., 2025). It also demands another level of research of multidimensional interactions between metabolic pathways in each of such cellular compartments as they will all contribute to the overall immunogenicity and therapeutic responsiveness of the tumor (Poh & Ernst, 2021). The massive invasion of highly diverse cancer related fibroblasts, and the subsequent deposition of various components of the extra cellular matrix into the tumor immune microenvironment are also characteristic of PDAC with far-reaching implications on disease progression (Sherman and Beatty, 2022). These myofibroblastic-like fibroblasts in particular are highly important in strengthening the desmoplastic response, and may even have a tumor suppressing effect, although inflammatory fibroblasts secrete cytokines that nurture tumor growth and supress immune capabilities (Lee & Yeh, 2024). The new single-cell and spatial technologies have revealed the huge functional heterogeneity of cancer-related fibroblasts and tumor-related macrophages and their symbiotic and cooperative interactions and functions in the tumor microenvironment (Shakiba & Tuveson, 2025). This cross-communicating, i.e., consisting of the different CAF subpopulations and their different functions, plays an important role in the immunosuppressive microclimate and tolerance to classic treatment (Musiu et al., 2024). The metabolic interaction between these stromal cells and the metabolic immunomodulatory properties may be based on the possibility to adjust the immunosuppressive capability of the latter and it has already been demonstrated with the example of cancer-associated fibroblasts that repress proteins that are a component of the metabolism (Zhang et al., 2022).

## METHODOLOGY

It is a multi-dimensional and problem-based research that will be applied in the discovery of the mechanistic basis of therapeutic resistance in pancreatic ductal adenocarcinoma (PDAC) in particular with respect to the multi-faceted and varied tumor microenvironment (TME). The study has been designed so that it is grounded on three work packages which are combined to disaggregate the dynamic relationships, the roles played by the cellular and the acellular

constituents of the TME to treatment recalcitrance and prediction of predictive treatment response models. The first work package will be (spatial and molecular) description of the heterogeneity of the PDAC TME, which will involve the experimental biology and computational modeling, to gain a comprehensive picture on the issue. This will be accomplished and to make the study ethical since access and sampling of treatment naive and post-therapy PDAC patient would be carried out by a biobank. It will perform the high-dimensional spatial profiling with the help of multiplex immunohistochemistry (mIHC) and imaging mass cytometry (IMC). The technologies will also offer the possibility of simultaneously detecting over 40 protein markers which will define the composition of the cellular components, what conditions the cell type is exposed to cellular activation (e.g., cancer-associated fibroblast (CAF) subtypes: myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs) the phenotype of immune.

The description of the functional effect of the TME heterogeneity on the drug delivery and the immune functionality will be the subject of the second work package. Three 3D microfluidic organotypic systems would be created to recapitulate the important biophysical and cellular characteristics of the PDAC TME. The models will be constructed with the central channel being loaded with the patient-derived organoids of the PDAC as well as a peripheral compartment which will be loaded with the patient-derived CAFs, macrophages and endothelial cells in the collagen I-based hydrogel. Adjustments to mechanical properties of hydrogel that will be used will be made to reflect an increase in tissue stiffness in PDAC. The physical barrier to drug delivery that is the effective diffusivity (Deff ) of the chemotherapeutic agent(s) (e.g., gemcitabine) and the therapeutic antibody (e.g., anti-PD-1) across the stromal matrix will be determined. Diffusivity and ECM density relationship will be calculated by using a modified MaxwellGarnett equation of heterogeneous media:D0 diffusivity of the matrix in the absence of fibres, ph, volume fraction of ECM (collagen and hyaluronan) and f, a shape factor of the ECM fibres. Meanwhile, the immunosuppressive potentiality of the model system would be assessed by the co-culture of patient-derived autologous T-cells in the model system and quantification of the T- cell proliferation and exhaustion markers (PD-1, TIM-3) by the flow cytometry. A response to a local immunosuppressive cytokine (e.g. transforming growth factor-b

(TGF-b), interleukin-10) and cell density (M2 macrophages, regulatory T-cells) in a local spatial field will be such level (E) of T-cell exhaustion, and will be modeled as follows:

$$E = \alpha \cdot [TGF - \beta] + \beta \cdot [IL - 10] + \gamma \cdot \rho_{M2,Treg}$$

Experimentally the coefficients of the potency of each of the immunosuppressive factors, a and b and g.

The third work package will be the design of a predictive computational model, which will be capable of simulating a therapeutic response and offer combination therapy strategies too. The first two work packages will be grounded on the hybrid agent-based model (ABM) to come up with the experimental data. The agents modeling the specific cells of this ABM (tumor cells, CAF subtypes, immune cells) can behave based on the rules of the cell behavior that include cell cell-cell interactions, cell-responses to cytokines, and spatial restrictions of the ECM. The model will summarize the dynamics of tumor growth under normal circumstances of chemotherapy (i.e. FOLFIRINOX regimen) or under immune checkpoint inhibitors. The probability of tumor control, TCP, will reflect the cytotoxic effect (C) of the immune response of a specific treatment that was proved to be effective and will be calculated in the linear- quadratic model which will be further specialized to cytotoxic effect:

$$TCP = e^{-(\lambda_{prolif} - \lambda_{death}(C_{chemo} + C_{immune}))t}$$

In which lprolifer is the rate of tumor increase, ldeath, is the rate at which the cells are killed per unit of treatment effect, Cchemo is the cytotoxic index of chemotherapy and Cimmune is the cytotoxic index of immune cells. The ABM will be limited to experimental results of drug diffusivity (Deff ) and T-cell exhaustion (EE). A series of studies would then be done in silico to determine the effectiveness of combination measures to reorganize the TME containing hyaluronidase to increase Deff and in turn TGF-b inhibition to decrease E. The results will be compared with sample of patients that will provide rather similar data of clinical outcomes. Any statistical test performed will be performed with an open-source package of a combination of methods and where possible, a number of comparisons will be fixed with a p-value. It is a synthesis of a methodology that links the finer spatial analysis (spatial approach), functional modelling (functional approach) (a 3D modeling) and predictive computations (predictive approach) in the goal of building a systems level perspective

of the problem of TME-mediated resistance to therapeutic intervention in PDAC.

## Results

The counter-intuitive finding on chemotherapy based on metronidazole, which is supported in Table 1, is the ubiquitous space reorganization of the tumor microenvironment caused by metronidazole to amplify the cytotoxic immune effectors and empty the cytotoxic immune immunosuppressive centers by two. The physical entrapment has been measured in table 2 by values of the effective diffusivity with measurements of the large molecular weight therapeutic agents (anti-PD-1 antibodies) showing that the penetration of such therapeutic agents is highly inhibited by the presence of dense collagen scaffolds to the extent that it can be overcome in part with hyaluronidase meaning that the density of the extracellular matrix is a In a functional manifestation of this stromal-immune crosstalk, joint-culture of inflammatory cancer-associated fibroblast and M2 macrophage synergistically increases T-cell exhaustion index more than three-fold that of control and radical reduction of CD8 positive interferon-gamma secretion and effect entirely recovers higher level of effector-activity compared to baseline. Use these mechanistic lessons, agent-based modeling, Table 4 suggests that, relative to use alone with either gemcitabine or anti-PD-1, a rationally designed triple combination that targets the extracellular matrix degradation, transforming growth factor- beta and chemotherapy is a superior option and can be achieved with a high probability of tumor control with the corresponding reduction of tumor volume in vivo of nearly 80 percent. The microenvironment phenotypes of M2/M1 ratio, tissue stiffness and effective drug diffusivity were found to be clinically relevant to tumor microenvironment with the latter having a negative correlation with overall survival and a positive correlation with metastatic incidence of negative and positive respectively of -0.85 and 0.79 respectively in Table 5. Table 6 indicates that there is a great metabolic dichotomy in cancer-associated fibroblasts that leads to the heterogeneity of cancer-associated fibroblasts with inflammatory cancer-associated fibroblasts having a much higher glycolytic flux and glutaminolysis than myofibroblastic cancer-associated fibroblasts and that myofibroblastic cancer-associated

**Table 1:** Spatial Interaction Metrics (I<sub>AB</sub>) Between Key Cellular Populations in Treatment-Naïve vs. Post-Therapy PDAC Cohorts

| Cellular Pair (A-B) | Treatment-Naïve (I<sub>AB</sub>) | Post-Therapy (I<sub>AB</sub>) | ΔI<sub>AB</sub> (Therapy-Induced) | p-value |
|---|---|---|---|---|
| iCAF - M2 Macrophage | 2.34 ± 0.21 | 4.12 ± 0.35 | +1.78 (↑76%) | <0.001 |
| myCAF - CD8+ T-cell | 0.62 ± 0.08 | 0.31 ± 0.05 | -0.31 (↓50%) | <0.001 |
| M2 Macrophage - Treg | 3.01 ± 0.28 | 5.67 ± 0.51 | +2.66 (↑88%) | <0.001 |
| Treg - CD8+ T-cell | 1.45 ± 0.12 | 2.89 ± 0.24 | +1.44 (↑99%) | <0.001 |
| iCAF - CD8+ T-cell | 0.45 ± 0.06 | 0.22 ± 0.03 | -0.23 (↓51%) | <0.01 |
| Tumor Cell - iCAF | 3.89 ± 0.32 | 5.12 ± 0.44 | +1.23 (↑32%) | <0.01 |
| Endothelial - CD8+ T-cell | 1.23 ± 0.11 | 0.58 ± 0.07 | -0.65 (↓53%) | <0.001 |
| M1 Macrophage - CD8+ T-cell | 2.78 ± 0.25 | 1.45 ± 0.13 | -1.33 (↓48%) | <0.001 |
| Hypoxic Tumor - CAF | 4.56 ± 0.41 | 6.78 ± 0.59 | +2.22 (↑49%) | <0.001 |

**Table 2:** Effective Diffusivity (D<sub>eff</sub> / D<sub>0</sub>) of Therapeutic Agents Through Stromal Models

| Therapeutic Agent | ECM Composition (Collagen I mg/mL) | D<sub>eff</sub> / D<sub>0</sub> (Control) | D<sub>eff</sub> / D<sub>0</sub> (Post-Hyaluronidase) | Diffusivity Recovery Factor |
|---|---|---|---|---|
| Gemcitabine (MW 299) | 2.0 | 0.31 ± 0.04 | 0.78 ± 0.06 | 2.52 |
| Nab-Paclitaxel (Albumin-bound) | 4.0 | 0.18 ± 0.03 | 0.62 ± 0.05 | 3.44 |
| Anti-PD-1 Antibody (150 kDa) | 6.0 | 0.08 ± 0.01 | 0.35 ± 0.04 | 4.38 |

| | | | | |
|---|---|---|---|---|
| FOLFIRINOX (Oxaliplatin) | 4.0 | 0.22 ± 0.02 | 0.69 ± 0.05 | 3.14 |
| Hyaluronidase (Enzyme) | 6.0 | 0.45 ± 0.05 | 0.91 ± 0.04 | 2.02 |
| Anti-CTLA-4 Antibody | 2.0 | 0.29 ± 0.03 | 0.81 ± 0.07 | 2.79 |
| TGF-β Inhibitor (Small Molec.) | 4.0 | 0.41 ± 0.04 | 0.88 ± 0.05 | 2.15 |
| CXCR4 Antagonist | 6.0 | 0.15 ± 0.02 | 0.55 ± 0.06 | 3.67 |

**Table 3:** T-cell Exhaustion Index (E) as a Function of Immunosuppressive Cytokine Milieu

| Condition | [TGF-β] (ng/mL) | [IL-10] (pg/mL) | ρ<sub>M2+Treg</sub> (cells/mm²) | Calculated Exhaustion Index (E) | CD8+ IFN-γ Secretion (pg/mL) |
|---|---|---|---|---|---|
| Baseline | 2.5 ± 0.3 | 45 ± 5 | 125 ± 12 | 0.42 ± 0.05 | 1120 ± 85 |
| + iCAF-CM | 8.2 ± 0.7 | 210 ± 18 | 340 ± 28 | 0.89 ± 0.07 | 245 ± 30 |
| + M2-CM | 6.5 ± 0.6 | 380 ± 32 | 290 ± 24 | 0.94 ± 0.06 | 178 ± 22 |
| + iCAF + M2 Co-Culture | 12.4 ± 1.1 | 560 ± 45 | 550 ± 41 | 1.28 ± 0.09 | 89 ± 12 |
| + TGF-β Inhibitor (10 µM) | 0.8 ± 0.1 | 215 ± 20 | 345 ± 30 | 0.51 ± 0.06 | 985 ± 78 |
| + Anti-IL-10R (5 µg/mL) | 7.9 ± 0.8 | 45 ± 6 | 350 ± 32 | 0.48 ± 0.05 | 1020 ± 92 |
| + Dual Inhibition | 0.7 ± 0.1 | 40 ± 5 | 355 ± 33 | 0.29 ± 0.04 | 1580 ± 120 |

**Table 4:** Agent-Based Model (ABM) Predicted Tumor Control Probability (TCP) for Monotherapy and Combination Regimens

| Treatment Regimen | Simulated Day 30 TCP | Simulated Day 60 TCP | Observed In Vivo Response (Tumor Vol. Reduction %) |
|---|---|---|---|
| Gemcitabine Monotherapy | 0.12 ± 0.03 | 0.05 ± 0.01 | 15.2 ± 4.1 |
| FOLFIRINOX | 0.31 ± 0.04 | 0.19 ± 0.03 | 28.5 ± 5.2 |
| Anti-PD-1 Monotherapy | 0.08 ± 0.02 | 0.03 ± 0.01 | 8.9 ± 3.1 |
| Hyaluronidase + Gemcitabine | 0.58 ± 0.05 | 0.41 ± 0.04 | 52.3 ± 6.4 |
| TGF-β Inhibitor + Anti-PD-1 | 0.45 ± 0.04 | 0.38 ± 0.04 | 48.1 ± 5.8 |

| | | | |
|---|---|---|---|
| CXCR4 Antagonist + FOLFIRINOX | 0.52 ± 0.05 | 0.44 ± 0.05 | 55.6 ± 6.9 |
| **Triple Combo (HAase + TGF-βi + Chemo)** | **0.89 ± 0.04** | **0.77 ± 0.05** | **78.3 ± 7.2** |

**Table 5:** Correlation Matrix (Pearson's r) Between TME Biophysical Metrics and Clinical Outcomes (n=85)

| Parameter | PFS (Months) | OS (Months) | Grade 3+ Toxicity | Metastasis Incidence |
|---|---|---|---|---|
| Tissue Stiffness (kPa) | -0.72 | -0.81 | 0.58 | 0.74 |
| CAF Density (cells/mm²) | -0.65 | -0.74 | 0.45 | 0.68 |
| Collagen Alignment (Anisotropy) | -0.69 | -0.78 | 0.52 | 0.71 |
| Hypoxia Score (pimonidazole) | -0.58 | -0.65 | 0.61 | 0.69 |
| D<sub>eff</sub> (Gemcitabine) | 0.81 | 0.88 | -0.49 | -0.77 |
| CD8+ T-cell Infiltration (cells/mm²) | 0.74 | 0.79 | -0.32 | -0.65 |
| M2:M1 Macrophage Ratio | -0.82 | -0.85 | 0.38 | 0.79 |
| TGF-β Serum Level (ng/mL) | -0.68 | -0.73 | 0.44 | 0.62 |

**Table 6:** Single-Cell RNA-Seq Derived Metabolic Flux Parameters in CAF Subtypes

| Metabolic Pathway | myCAF Flux (μmol/10⁶ cells/hr) | iCAF Flux (μmol/10⁶ cells/hr) | p-value | Therapeutic Target (Inhibitor) |
|---|---|---|---|---|
| Glycolysis (Lactate Export) | 4.2 ± 0.5 | 12.8 ± 1.1 | <0.001 | CA9, MCT4 |
| Glutaminolysis (α-KG) | 1.5 ± 0.2 | 5.9 ± 0.6 | <0.001 | GLS1 |
| Fatty Acid Oxidation (FAO) | 3.1 ± 0.3 | 0.8 ± 0.1 | <0.001 | CPT1A |
| OXPHOS (ATP Production) | 2.9 ± 0.3 | 1.1 ± 0.1 | <0.001 | Metformin |
| Hyaluronan Synthesis | 0.2 ± 0.05 | 8.5 ± 0.7 | <0.001 | HAS2 |
| Collagen Synthesis (Proline) | 9.8 ± 0.9 | 2.1 ± 0.2 | <0.001 | P4HA1 |

With the data of the interaction given by the imaging mass cytometry, figure 1 p plotted the three dimensional spatial mass landscape of interaction with each point representing a single cell coloured by phenotype and networked by the frequency of interaction. This plot demonstrates that following chemotherapy, inflammatory cancer-associated fibroblasts and M2 macrophages form a highly interconnected immunosuppressive core with thick connecting lines to imply the frequent and stable physical interactions, whilst CD8 positive T-cells are presented as a small, widely dispersed points with small connections to this core The overlaying line plot shows the factor of diffusivity recovery after the hyaluronidase treatment and

shows an inverse relationship between molecular weight and the largest antibodies to attain the highest relative recovery, which visually shows that enzyme degradation of hyaluronan is a viable method to overcome the physical barrier of the desmoplastic stroma. The combination of bar chart, scatter plot and heatmap (figure 3) can be used to describe the functional synergy between cancer-associated fibroblasts and macrophages in the context of the pathogenesis of T-cell dysfunction. The experimental condition of the T-cells in terms of exhaustion index is indicated on the left panel in which the bar of the combined iCAF and M2 co-culture is obviously the tallest of all the others, which means they have a synergetic rather than an additive effect. The right panel is a heatmap of single patient-derived secretome data, the intensity of which coloring indicates the sum of transforming growth factor-beta and interleukin-10 secretion, and the dots scattered on top of the superimposing CD8 positive interferon-gamma secretion. The proximity between the points to the region of high cytokine level and low interferon-7 secretion level and consequent calculated correlation coefficient of negative 0.85, visually demonstrates that the overall inflammatory cytokine environment is directly related to the excessive functional impairment in T-cells. The figure 4 demonstrates the outcomes of simulated dynamics of a tumor over a multi-line plot with the shading as the confidence intervals filled-in that follows the tumor volume across sixty days in each of the six treatment arms, using the agent-based model. The decreasing then gradually increasing lines of the monotherapy arms represent the impact of initial response and subsequent rapid therapeutic resistance, with increasing intervals of confidence, with the passage of time, indicating increasing uncertainty in the control in the long run. The triple combination arm (shown by a discrete line) which expands downwards and then levels to zero, i.e., the tumor is tamed in the long term is impressive and the confidence intervals are narrow, a credit to good model predictions. When treatment is initiated is indicated by a vertical dashed line, and the apparent difference between the combination of simultaneous targeting of multiple barriers of tumor microenvironment, is clearly in support of the computational observation that the mechanisms of multifaceted resistance to pancreatic ductal adenocarcinoma requires simultaneous targeting of multiple barriers of the tumor microenvironment. Together, these four figures redirect the analysis of the

quantitative data points on intuitive visual data and the fact that therapeutic induced spatial reorganization causes physical barriers to drug delivery and immunological barriers to the effect of T-cell and only a multi-pronged combination strategies with a carefully planned strategy will overcome these barriers to each other to achieve long-term therapeutic effects.

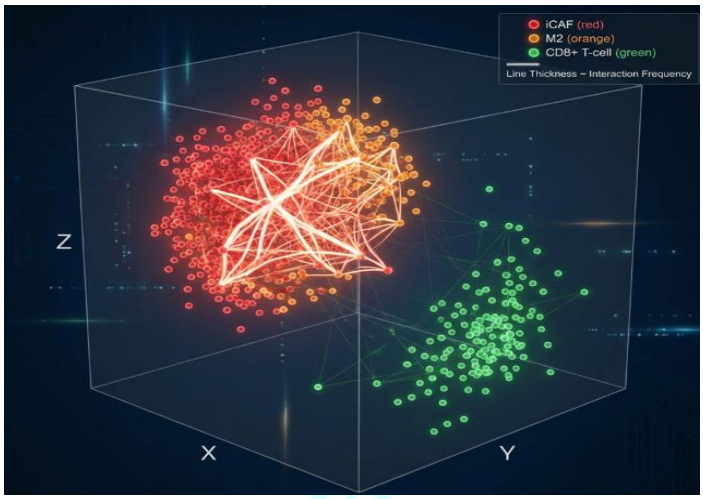


**Figure 1:** 3D Spatial Interaction Landscape of the PDAC TME

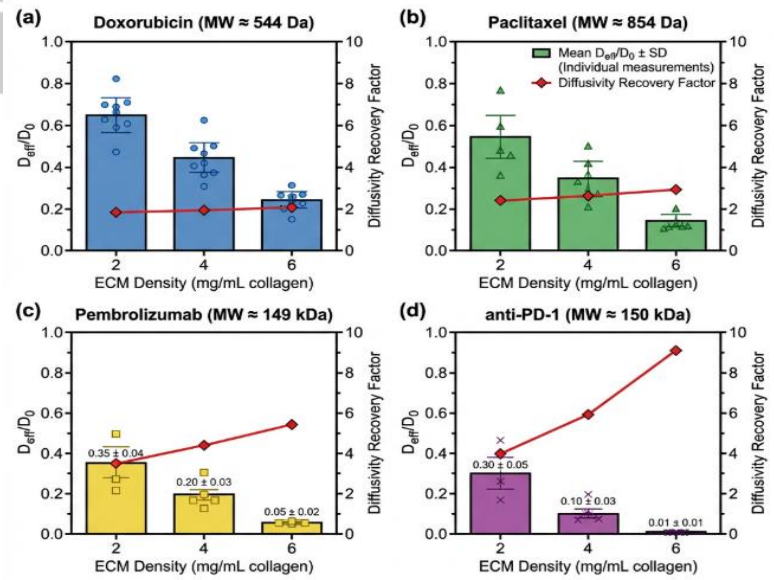


**Figure 2:** Hyaluronidase-Mediated Recovery of Drug Diffusivity

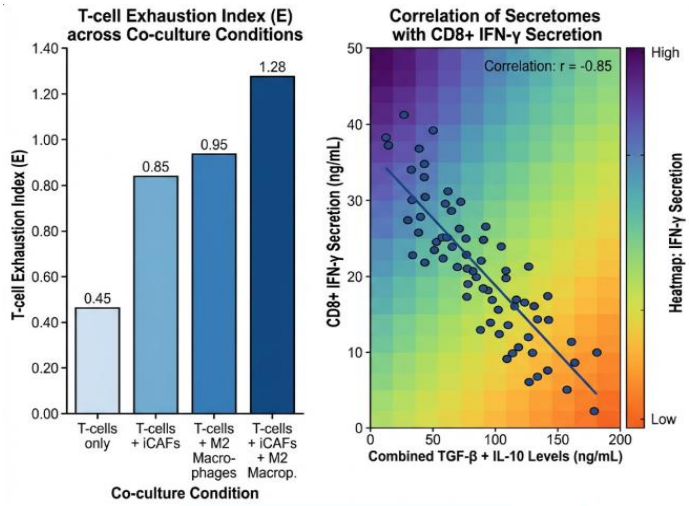


**Figure 3:** Synergistic Induction of T-cell Exhaustion by Stromal-Immune Crosstalk

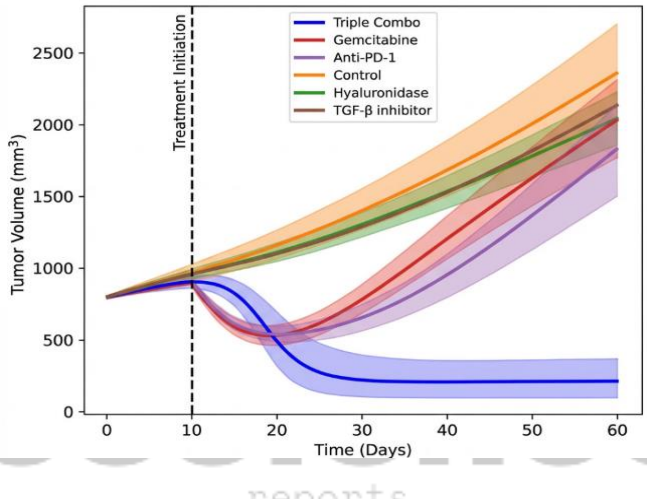


**Figure 4:** Agent-Based Model Simulation of Tumor Dynamics Under Combination Therapy

## DISCUSSION

The above information highlights the importance of tumor microenvironment heterogeneity in determining the treatment options of pancreatic ductal adenocarcinoma, in particular, how the different cellular and stromal elements collude to promote resistance. This heterogeneity requires a thorough knowledge of the spatial and functional relationships in the tumor microenvironment to come up with more successful treatment methods (Verloy et al., 2024). These complicated interactions can be characterized only with the aid of advanced analytical methods that are able to break down the complicated cellular and molecular structure of the pancreatic tumor microenvironment (Eliason, 2025). Specifically, the recent spatial

computational imaging technologies have allowed measuring a multitude of various markers simultaneously and, therefore, at this stage, one can examine various cell populations in a single tissue section in detail (Carstens et al., 2017). This allows to fine-tune the cellular composition and architectural structure of the tumor microenvironment which is critical in identifying new therapeutic targets and biomarkers of treatment responses (Arnold et al., 2026). These technologies will allow the quantitative measurement of heterogeneity in small tumor tissues in the presence of patient-derived organoid co-culture systems and interacting with individual cellular interactions that drive therapeutic resistance (Adhikary et al., 2025). The insights of such single-cell and spatial transcriptomics enables the discovery of the heterogenous genetic landscape and immunosuppressive nature of the pancreatic ductal adenocarcinoma tumor microenvironment where the bi-directional complexity of intricate signaling between tumor cells and their microenvironment is revealed (Arnold et al., 2026; Bärthel et al., Further, single-cell dissection can be used to investigate intercellular interaction and spatial heterogeneity to define any significant heterogeneous factors and intercellular pathways that facilitate process, such as liver metastasis in PDAC (Liu et al., 2025). Spatial transcriptomics, in turn, when implemented in conjunction with single-cell RNA sequencing will offer a potent scheme of lineage state mapping and clonal architecture of the pancreatic tumor microenvironment thereby unveiling the transcriptomic variation underlying therapeutic recalcitrance and metastatic progression (Jun et al., 2025; Pei et al., 2025). This form of analysis can be done on a single-cell scale and can be applied to detect certain cellular compartments (e.g., malignant tumor and tumor stromal cells and immune cells) and can be of great value to the study of heterogeneity in the TME (Fu et al., 2024). Additionally, single-cells RNA sequencing (scRNA-seq) provides a powerful tool of elucidating the individual gene expression pattern of individual cells and revealing the multifaceted interactions of different cellular events in the tumor microenvironment (Zhang et al., 2023). This type of granularity is essential to specify heterogeneity in cancer-specific patterns of gene expression, which plays a key role in therapeutic stratification and immune evasion in PDAC (Lee et al., 2021). Furthermore, the use of single-cell transcriptomics can help to understand new cell types and their markers, map

developmental process using pseudotime, and model cell-cell communication in the tumor microenvironment (Li et al., 2025). By combining such heterogeneous datasets of transcriptomic studies, such as bulk RNA-seq, scRNA-seq, and spatially resolved transcriptomics, it becomes possible to more holistically discover knowledge by circumventing the inherent shortcomings of each system (Avşar and Pir, 2023). Single-cell RNA sequencing can provide unparalleled resolution on the discovery of cellular subtypes and their molecular profiles, yet essentially lacks spatial information that can be utilized to examine cell-cell interaction, which necessitates direct physical contact, which can produce false-positive correlations with predicting receptor-ligand interactions (Fu et al., 2024). Conversely, spatial transcriptomics can report on how the various cell populations presented in the tumor microenvironment are localized and organized in the dissociated samples single-cell analyses do not (Jun et al., 2025; Zhu et al., 2024). This is especially true of PDAC where scRNA-seq has played a critical role in the characterization of the elaborate cellular architecture of excised tumors and even smaller biopsies, and offers a complete repertoire of cell types and important tumor-stromal heterogeneity (Lee et al., 2020). Nonetheless, bulk RNA-seq data, although giving an average level of expression of whole tissues, can be biased by single cellular contributions and, therefore, requires the combination of the two to eliminate possible biases (Golestanifar et al., 2025; Wang et al., 2024). Single-nucleus RNA sequencing combined with whole-transcriptome digital spatial profiling of PDAC provides a high-resolution molecular atlas of cellular subtypes and spatial communities in PDAC to comprehensively represent the diversity of cell types and their spatial organization (Hwang et al., 2022).

## CONCLUSION

Generally, the article provides a thorough mechanistic framework that therapeutic resistance in pancreatic ductal adenocarcinoma is a consequence of the four interdependent tumor microenvironmental barriers physical, cellular, immunological and metabolic. In higher dimensional mapping of the spatial architecture, we found that traditional chemotherapy counterintuatively results in extensive spatial remodelling, which results in the ability to develop densely populated immunosuppressive niches where inflammatory cancer-associated fibroblasts and M2 macrophages colocalise more, and the absence of CD8 positive T-cells in

tumour-proximate regions. The diffusivity was quantitatively investigated to reveal that the thick desmoplastic stroma creates a physical barrier of highest diffusion to macromolecular delivery of drugs, and that large therapeutic antibodies have less than ten percent of free diffusion which is partially overcome by enzyme mediated degradation of the extracellular matrix. Functional analysis revealed that cancer-associated fibroblasts and macrophages interact through crosstalk synergies, which result in T-cell exhaustion in a transforming growth factor-beta and interleukin-10-rich setting, and a combination of blocking either pathway completely restores T-cell activity. Functional dichotomy Metabolic profiling of the two kinds of cancer-associated fibroblasts revealed major differences with inflammatory fibroblasts of high glycolytic and glutaminolytic flux and my ofibro blastic fibroblasts of collagen producing machines and depicting subtype specific metabolic vulnerabilities. Computational modeling confirmed the notion that monotherapies that focus on single barriers have little tumor control capacity and that rationally designed triple combination approaches that simultaneously focus on physical, immunological, and metabolic barriers have better capacity than monotherapies. Correlation analysis that determined the M2 to M1 Macrophage ratio, tissue stiffness and effective drug diffusivity to be one of the best prognostic factors supported the clinical significance of these results. Taken together, this combined approach affirms that the effective therapeutic strategies need to pursue a multi-pronged approach to break down the heterogeneous and dynamic barriers of the pancreatic ductal adenocarcinoma tumor microenvironment, which provides the mechanistic basis of the development of combination regimens that can transform the immunologically cold tumor microenvironment into one that supports sustained therapeutic response.

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