Role of Cancer Associated Fibroblast Heterogeneity on Immunotherapeutic Potentials of Pancreatic Ductal Adenocarcinoma

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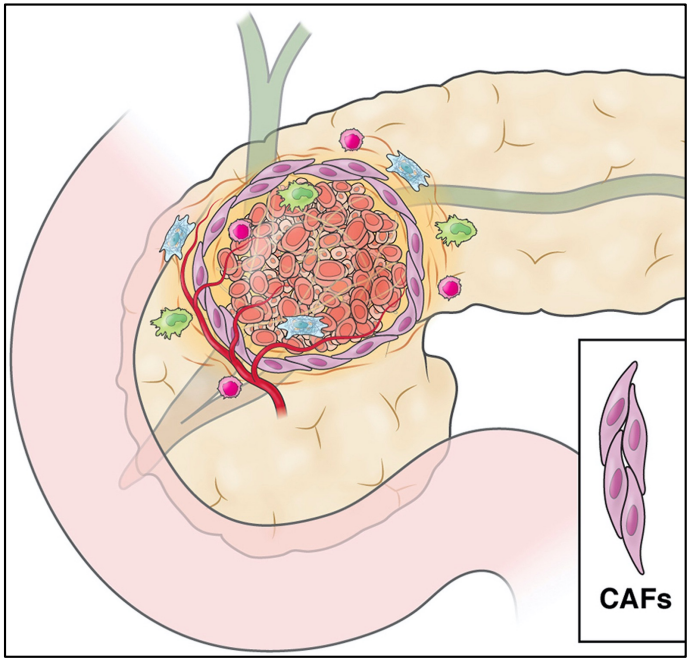
INTRODUCTION: Pancreatic Ductal Adenocarcinoma (PDAC)

- Poorest prognosis → **9% 5-year survival** [1]
- **4th** leading cause of cancer death worldwide [1]
- **20%** response to chemotherapy [2]
- **1-2%** approval for immunotherapies [3]
- **90% of tumor volume** is dense stroma and **cancer associated fibroblasts (CAF)** [3]
    - Barrier for **drug penetration**
    - **Potential target** for better treatment



CAFs surrounding PDAC tumor. Image obtained from [1]

PROBLEM: Cancer Associated Fibroblast Heterogeneity

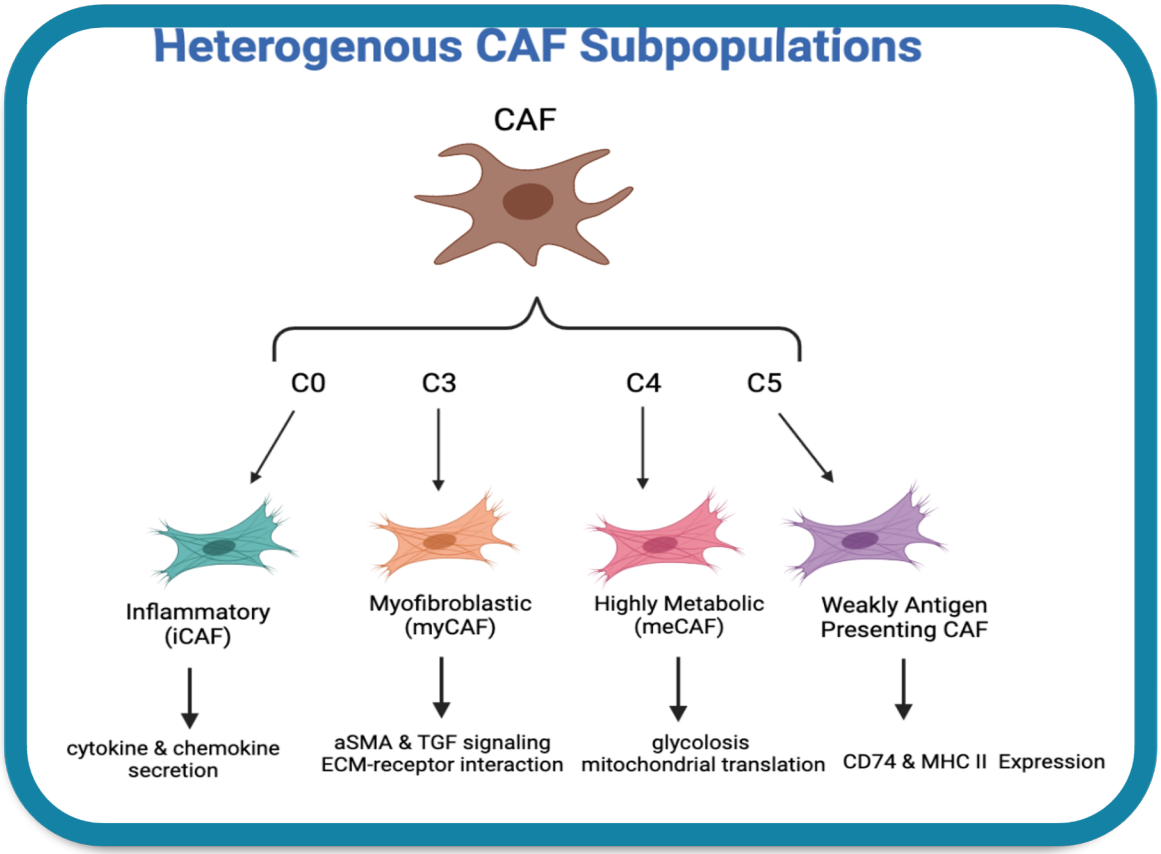


Diagram made by finalist using https://biorender.com/

- **Problem:** need for better understanding of CAF heterogeneity and stromal-immune crosstalk
- **Purpose:** help target pro-tumoral subtypes & create more effective immunotherapies

OBJECTIVES

I. **Characterize CAF subpopulation distribution among PDAC patients**

II. **Compare the overall survival of PDAC patients with a low vs. high expression of CAF subpopulations**

III. **Observe correlations between CAF subpopulation concentrations and immune cell concentration**

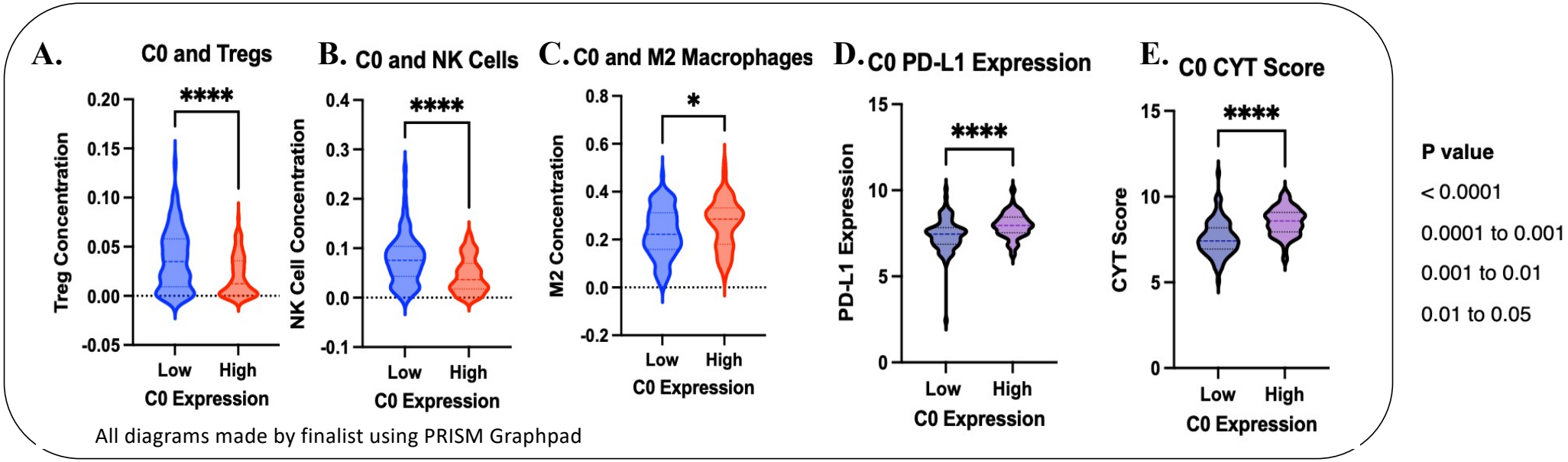
IV. **Predict immunotherapy response based on CAF subpopulation presentation**

METHODOLOGY

All tests were performed computationally using bioinformatics techniques

- **Dataset**: PDAC bulk-RNA seq data was obtained from The Cancer Genome Atlas (TCGA, available online: https://cancergenome.nih.gov/)
- **Prism GraphPad**: produce violin plots and log rank survival curves based on CAF enrichment, immune cell concentration, and immunotherapeutic markers
- **Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT)**: determine immune cell concentration present across PDAC patients
- **Gene Set Variation Analysis (GSVA)**: determine variation in enrichment across CAF clusters
- **RStudio**: normalize bulk RNA-seq dataset, run GSVA and CIBERSORT
- **Excel**: classify and sort patients based on CAF enrichment, immune cell concentration, Cytolytic Score (CYT Score), and PD-L1 expression

Part I: C0 Subpopulation Analysis



All diagrams made by finalist using PRISM Graphpad

Fig. 1A. Patients with **low expression** of C0 CAFs showed **higher expressions of T regulatory cells (Tregs)**

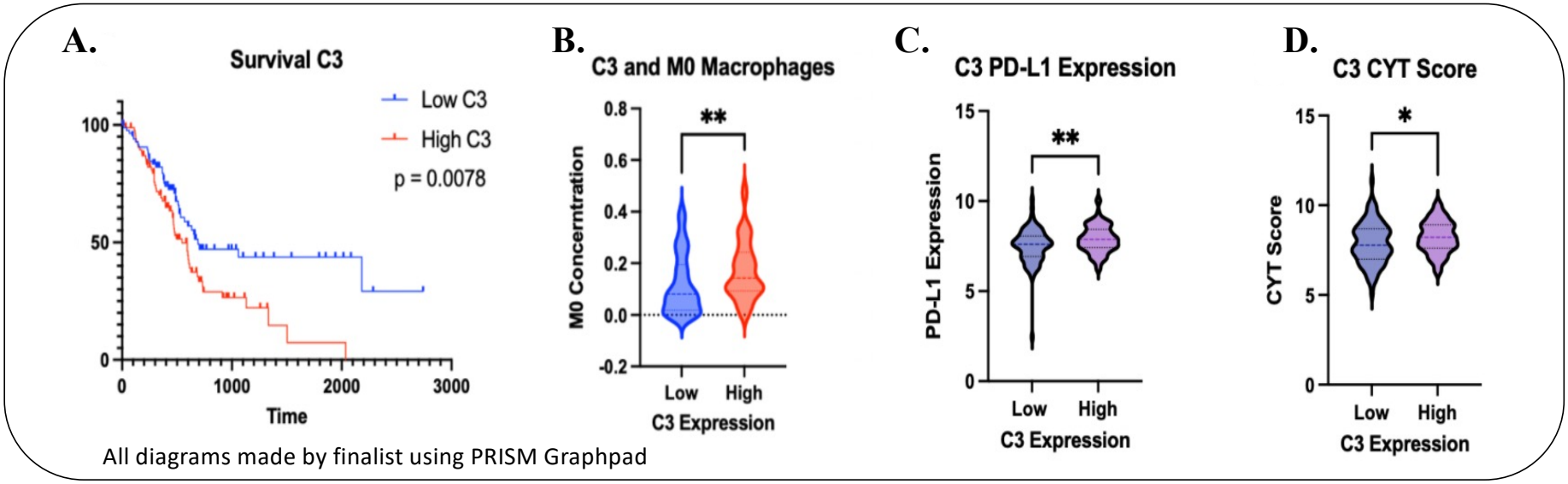
Fig. 1B. Patients with **low expression** of C0 CAFs showed **higher expressions of Natural Killer cells (NK cells)**

Fig. 1C. Patients with **low expression** of C0 CAFs showed **lower expression of M2 macrophages**

Fig. 1D. Patients with **higher expressions** of C0 CAFs showed **higher PD-L1 expression**

Fig. 1E. Patients with **higher expressions** of C0 CAFs showed **higher CYT score**

Part I: C3 Subpopulation Analysis



P value

< 0.0001

0.0001 to 0.001

0.001 to 0.01

0.01 to 0.05

Fig. 2A. Patients with **low expression** of C3 linked to **better overall survival**

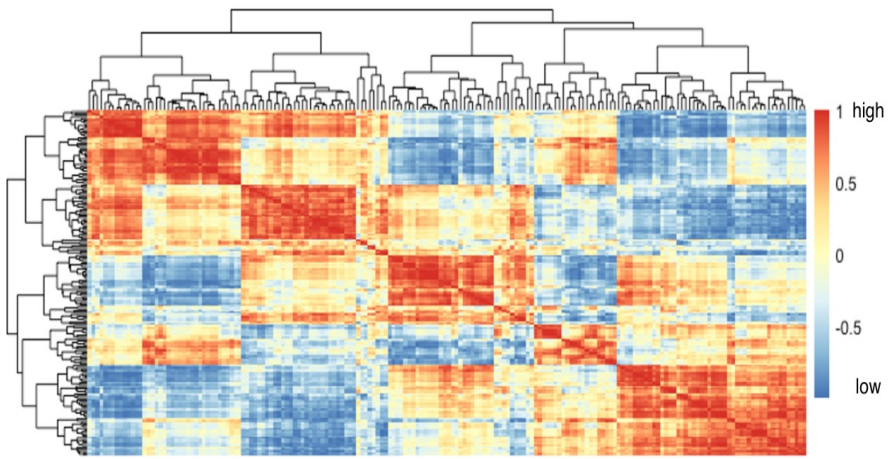
Fig. 2B. Patients with **low expression** of C3 CAFs showed **lower expression of M0 macrophages**

Fig. 2C. Patients with **higher expressions** of C3 CAFs showed **higher PD-L1 expression**

Fig. 2D. Patients with **higher expressions** of C3 CAFs showed **higher CYT score**

Part II: Population Level Clusters

A.



B.

Mean Values of CAF expression per Population Cluster				
	C0	**C3**	**C4**	**C5**
Population 1	0.2751	0.4015	-0.03635	-0.09259
Population 2	-0.1275	-0.3988	-0.1362	-0.1199
Population 3	-0.343	-0.2916	0.1231	0.1582
Population 4	0.2794	-0.2126	-0.3912	-0.2132
Population 5	-0.04527	0.4197	0.2259	0.06958

All diagrams made by finalist using Rstudio and PRISM Graphpad

Fig. 3 Five population-level clusters containing similar expressions of CAF subpopulations were identified

Part II: Population Level Clusters

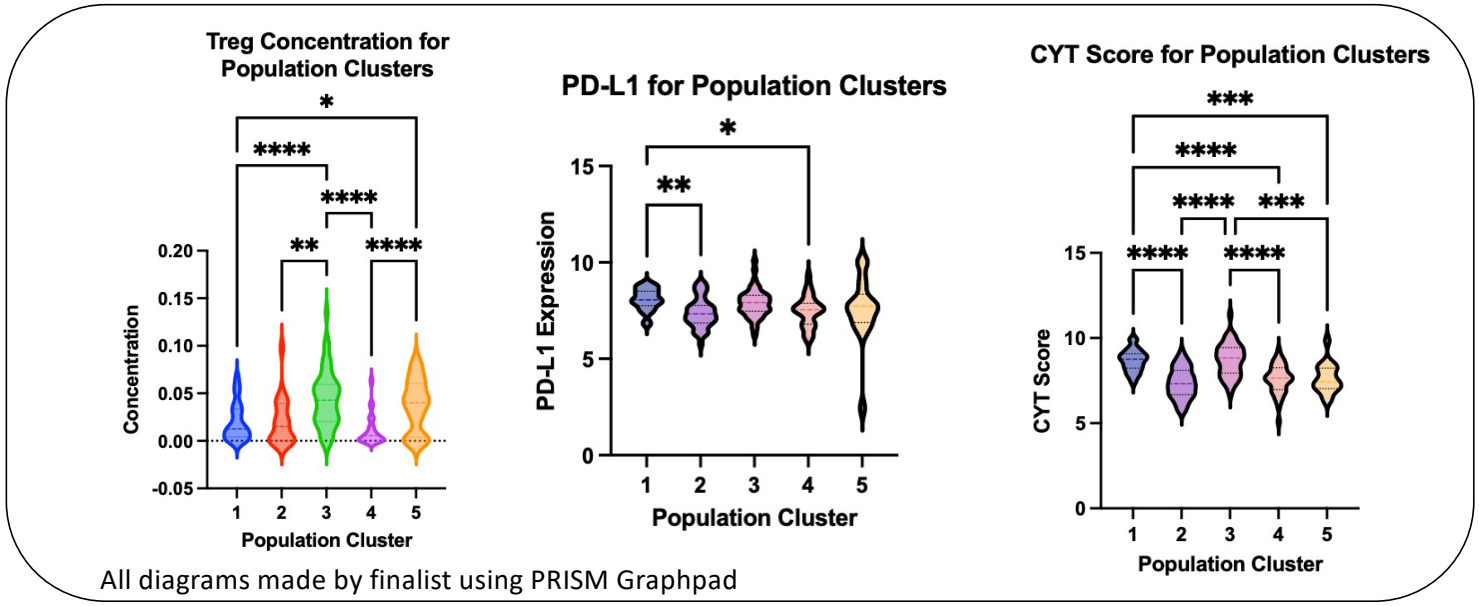


Figure 4. Population-Level Cluster Analysis
A) Immune Cell Concentration Analysis: one-way ANOVA violin plot of Treg cell concentration across population-level clusters **B) PD-L1 Immunotherapy Marker Analysis:** one-way ANOVA violin plot of PD-L1 expression across population-level clusters **C) CYT Score Analysis:** one-way ANOVA violin plot of CYT score across population-level clusters

- Immune Cell Concentration ➔ **Population 3 & 5 (higher C4 & C5 CAFs expression)** had **higher Treg cell concentration**, correspond to an **immunosuppressive microenvironment**
- Immunotherapy Markers
  - **Population 1 (higher C0 & C3 CAF expression)** had **higher PD-L1 expression** and **higher CYT**, corresponds to **better anti-PD-L1 therapy response**

CONCLUSION

I. **Identified populations of CAFs that correspond to differential survival and immunotherapeutic response**

II. **Differing levels of fibroblast populations in the TME corresponded to distinct immune environments that can provide insights into stromal-immune crosstalk**

III. **Identified 5 population level clusters of patients based on differential presence of fibroblast subpopulations**

IV. **Population level clusters may have differential response to immunotherapy and can inform treatment decision making**

IMPORTANCE & APPLICATIONS

- Integral to understanding the **connection between CAFs and the immune microenvironment** of PDAC
- More **personalized** and **effective immunotherapies**, leading to better overall survival
- Potential **increase in immunotherapy approval** due to targeted treatment

References

[1] Y. Zhang, J. Yang, M. S. Bronze, C. W. Houchen, and M. Li, “Interplay of Tumor Microenvironment Factors and Cancer Stem Cell Enrichment in Pancreatic Ductal Adenocarcinoma,” *Gastroenterology*, vol. 161, no. 6, pp. 1800–1802, Dec. 2021, doi: 10.1053/j.gastro.2021.09.024.

[2] R. L. Siegel, K. D. Miller, and A. Jemal, “Cancer statistics, 2020,” *CA: A Cancer Journal for Clinicians*, vol. 70, no. 1, pp. 7–30, 2020, doi: 10.3322/caac.21590.

[3] S. Gillen, T. Schuster, C. Meyer zum Büschenfelde, H. Friess, and J. Kleeff, “Preoperative/Neoadjuvant Therapy in Pancreatic Cancer: A Systematic Review and Meta-analysis of Response and Resection Percentages,” *PLoS Med*, vol. 7, no. 4, p. e1000267, Apr. 2010, doi: 10.1371/journal.pmed.1000267.

[4] M. Orth *et al.*, “Pancreatic ductal adenocarcinoma: biological hallmarks, current status, and future perspectives of combined modality treatment approaches,” *Radiation Oncology*, vol. 14, no. 1, p. 141, Aug. 2019, doi: 10.1186/s13014-019-1345-6.

[5] Y. Wang *et al.*, “Single-cell analysis of pancreatic ductal adenocarcinoma identifies a novel fibroblast subtype associated with poor prognosis but better immunotherapy response,” *Cell Discov*, vol. 7, no. 1, pp. 1–17, May 2021, doi: 10.1038/s41421-021-00271-4.

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