1030 PERIPHERAL IMMUNOLOGICAL DYNAMICS OF RESPONSE IN PANCREATIC CANCER TREATED WITH IMMUNE CHECKPOINT INHIBITORS

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Background Pancreatic cancer (PC) is the fourth leading cause of cancer death in the United States,[1] as it is highly resistant to conventional treatments,[2–4] and nearly all patients develop metastases and progress. Immunotherapy with immune checkpoint inhibitors (ICI) has shown strong, durable responses in patients with various types of cancer,[5] but these successes have not yet completely translated to PC. Here we analyzed samples from a phase II clinical trial where patients with PC were treated with a combination of radiotherapy and ICI to understand the peripheral immunological dynamics of responding patients.

Methods Peripheral blood mononuclear cells (PBMCs) were isolated from patients with pancreatic cancer enrolled in the CheckPAC protocol (NCT02866383[6]) and subjected to multiomic single-cell analyses. In detail, CITESeq was performed on baseline and on-treatment (+8 weeks) blood samples from responders (n=5) and non-responders (n=6) in the trial to obtain both gene expression and protein expression data. The data was further analyzed with the R package Seurat.[7]

Results By exploring gene and surface protein expression on multiple immune cell subsets, we identified increased numbers of effector cells (CD8 T cells, Natural Killer cells, and γδ T cells) in the PBMCs of responders, consistent with increased immune activation leading to tumor regression. The expression of Granulysin in effector cells (GNLY) strongly correlated with clinical responses, confirming previous reports where increased levels of GNLY have been associated with clinical responses.[8] Specifically, γδ T cells were the most enriched cell type for GNLY expression, with a statistically significant higher expression in responders at baseline, suggesting the role of GNLY+ γδ T cells as a predictive biomarker population. Furthermore, surface protein analyses confirmed that the specific subset of Vδ2γ9 T cells was associated with response and showed high expression of other effector molecules like Granzyme B or Tumor Necrosis Factor, confirming its highly cytotoxic phenotype. Of note, analysis of TCGA datasets showed that PC has a high expression of the genes BTN3A1 and BTN2A1, which are necessary for phosphoantigen presentation and tumor recognition by Vδ2γ9 cells.[9] Expression of GNLY, as well as other cytotoxic markers, will further be validated by flow cytometry on the responding patients.

Conclusions This data sheds light on the peripheral immunological dynamics of patients with PC who respond to ICI therapy and suggest a highly cytotoxic subset of GNLY+ Vδ2γ9 T cells as the potential driver of response.

REFERENCES

1. Siegel RL, *et al.* Cancer statistics, 2020. *CA Cancer J Clin. CA Cancer J Clin*; 2020
2. Von Hoff DD, *et al.* Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med*; 2013
3. Sonbol MB, *et al.* Second-line treatment in patients with pancreatic ductal adenocarcinoma: A meta-analysis. *Cancer*; 2017
4. Rahma OE, *et al.* Second-line treatment in advanced pancreatic cancer: a comprehensive analysis of published clinical trials. *Ann Oncol*; 2013
5. Postow MA, *et al.* Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol*; 2015
6. Chen IM, *et al.* Randomized Phase II Study of Nivolumab With or Without Ipilimumab Combined With Stereotactic Body Radiotherapy for Refractory Metastatic Pancreatic Cancer (CheckPAC). *J Clin Oncol*; 2022
7. Butler A, *et al.* Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol*; 2018
8. Pagès F, *et al*. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med*; 2005
9. Rigau M, *et al.* Butyrophilin 2A1 is essential for phosphoantigen reactivity by gd T cells. *Science*; 2020

Ethics Approval This study was conducted using PBMCs from patients enrolled in clinical trials performed at the National Center for Cancer Immune Therapy (CCIT-DK), Department of Oncology, Copenhagen University Hospital, Herlev, Denmark. All procedures were performed in compliance with clinical protocols approved by the Ethics Committee of the Capital Region of Denmark (Reference H-20070020) and national regulations for biomedical research, including appropriate data protection of human participants (Reference P-2021–303). Written informed consent was provided by all patients before obtaining any samples.

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