

TCR $\gamma\delta$ + TIL FROM PATIENTS WITH PANCREATIC CANCER RECOGNIZE AUTOLOGOUS TUMOR CELLS RESTRICTED BY CD1D

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Background Pancreatic cancer is the 7th leading cause of cancer-related deaths worldwide. Treatment options are still limited; immunotherapeutic approaches using tumor infiltrating lymphocytes (TIL) represent a viable option for PDAC patients. TILs from PDAC lesions will include $\alpha\beta$ + and $\gamma\delta$ + T-cells, that recognize tumor cells restricted by non-classical MHC I molecules.^{1,2} $\gamma\delta$ + TILs are generally associated with a more favorable prognosis³ and present promising candidates for adoptive cellular therapy as cellular products, or by cloning the molecular blueprint of cancer-specific TCRs that could be transferred into recipient immune cells. The study focused on the expansion of TIL containing TCR $\gamma\delta$ +, molecular analysis of TCR $\gamma\delta$ + cells, and testing anti-tumor directed TIL specificity defined by IFN-gamma production upon NRLP3 pathway stimulation.

Methods Freshly harvested tumor tissue was used to propagate TIL using cytokines in combination with an NRLP3 pathway activator for 2-3 weeks. $\gamma\delta$ + T-cell expansion and T-cell activation/exhaustion were tested by flow cytometry and autologous tumor recognition assays were performed using blocking mAbs and IFN-gamma as the biological readout; molecular recognition analysis was performed using synthetic peptides derived from tumor exome sequencing. TCR deep sequencing was performed in sorted TCR $\gamma\delta$ + TIL. Tumor infiltration by TCR $\gamma\delta$ + was gauged by immunohistochemistry and spatial transcriptomics.

Results NRLP3 stimulation results in increased TCR $\gamma\delta$ + cells which – after sorting (>90% purity) – recognized autologous and allogeneic pancreatic cancer cells in a CD1d-restricted fashion defined by IFN-gamma production. TCR sequencing revealed a focused TCR repertoire with unusual Vgamma3 and Vgamma8 usage. NRLP3 stimulation increased the frequency of $\alpha\beta$ + T-cells against autologous tumor cells and mutant target epitopes defined by IFN-gamma production, abrogated with an NRLP3 inhibitor. Spatial transcriptomics revealed co-localization of CD1d and perforin+granzymeA +granzymeB $\gamma\delta$ + T-cells; TCR-sequencing of PDAC tissue and corresponding TIL exhibited an oligoclonal TCR repertoire. NRLP3 stimulation increased mRNA and protein expression of CXCL9 and CXCL10, which facilitates tissue invasion and favored CD1d restricted recognition of damaged mitochondria by $\gamma\delta$ + T-cells.

Conclusions NRLP3 pathway stimulation increased tumor-reactive and mutant epitope specific TILs, increased expression of chemokines facilitating tissue invasion and expansion of $\gamma\delta$ + TIL engaged in surveillance of damaged mitochondria. This allows a clinically relevant expansion of TIL for patients with PDAC that recognized tumor associated antigens restricted by classical and non-classical MHC molecules. CD1d restricted and tumor-reactive $\gamma\delta$ TCRs can be used as blueprints for anti-cancer directed receptors that could be transferred into recipient cells for active cellular immunotherapy for patients with PDAC.

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Ethics Approval This study was approved by the Champalimaud Foundation's Ethics Committee. Research complied with the corresponding ethical principles and the applicable international, EU and national directive laws (EU Directive 2004/23/EC).

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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