Single-Cell Polyfunctionality of CD4+ T Cells Shows Promise as a Predictor of Overall Survival of Pancreatic Cancer Patients Treated with GVAX Vaccine

Sean Mackay1, Brianna Flynn1, Jonathan Chen1 Patrick Paczkowski1, Elizabeth Jaffee2, Lei Zheng2, Jing Zhou1

1. IsoPlexis Corporation, 33 NE Industrial Road, Branford, CT 06405
2. The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

**BACKGROUND**

The therapeutic GVAX vaccine boosts the body’s immune system T cells to fight pancreatic cancer. However, it remains challenging to identify clinical correlates that can sensitively detect T-cell functional kinetics in patients with pancreatic cancer who had GVAX vaccination.

IsoPlexis single-cell IsoCode chip (SCBC) technology integrated with an automated bioinformatics platform simultaneously measures 32 cytokines secreted by single T cells, providing the unmet need of precision profiling functional heterogeneity of T cells in response to therapy.

**METHODS**

- CD4+ T cells were isolated with anti-CD4 microbeads from pre- and post-vaccination patient PBMCs.
- Enriched CD4+ T cells were stimulated with anti-CD3/CD28 at 37°C, 5% CO2 for 24 hours and loaded onto an IsoPlexis SCBC containing ~12000 microchambers pre-patterned with a complete, 32-plex, antibody array.
- Cells on the SCBC were imaged to identify single-cell locations and incubated for additional 16 hours at 37°C, 5% CO2 single-cell cytokine signals were then captured and digitized with a macroarray scanner.
- The polyfunctional expression (2+ cytokines per cell, see Figure 2) of ~1000 single CD4+ T cells were evaluated across five functional groups (IL-5/TNF-β excluded):
  - Effectors: Granzyme B, IFN-γ, MIP-1α, Perforin, TNF-α, TNF-β
  - Stimulatory: GM-CSF, IL-1β, IL-5, IL-6, IL-7, IL-9, IL-12, IL-15, IL-21
  - Regulatory: IL-4, IL-10, IL-13, IL-17, TGF-β1, IL-16, IL-17A, IL-17F, IL-23, IL-12, IL-21
  - Inflammatory: IL-6, IL-8, IL-17F, MCP-1, MCP-3, MCP-4
  - Chemotactic: CCL-11, IP-10, MIP-1β, ENKES

**RESULTS**

- Single-cell multiplexed analysis demonstrates a marked upregulation of polyfunctional CD4+ T cells across 5 patients after GVAX vaccination compared to pre-vaccination CD4+ T cells.
- The enhanced polyfunctional strength index (PSI) of CD4+ T cells by GVAX was predominated by antitumor-associated effector proteins including Granzyme B, IFN-γ, MIP-1α, Perforin, and TNF-α, mixed with small amounts of MIP-1β and sCD137 secretions.
- Most importantly, post-versus pre-vaccination fold-change of PSI was significantly associated with patient overall survival (OS, P=0.001), indicating a potential of PSI in predicting GVAX vaccine efficacy and prognostic outcomes of patients with pancreatic cancer.

**CONCLUSIONS**

- Single-cell multiplexed proteomic profiling provides a comprehensive assessment of 1 cell function and identifies PSI change between pre- and post-vaccination CD4+ T cells as a novel correlate to OS in pancreatic cancer patients with GVAX treatment.
- Novel visualization methods reveal a diverse landscape of polyfunctional response by GVAX vaccination, providing a precise platform for capturing efficacy and durability data for therapy development.
- These novel biomarker capture and analysis methods have the potential to guide more sensitive and potentially predictive quality assessments, which may enable vaccines with more efficacious and persistent profiles.