Single-Cell Cytokine Profiling of Tumor-Infiltrating T Cells to Measure Patient Responses to Anti-PD-1 Therapy

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BACKGROUND

- Functional alteration of tumor-infiltrating T lymphocytes (TILs) may serve as a predictor for clinical outcome in cancer patients receiving immunotherapy.
- The heterogeneity and small sample size of TILs in primary tumor tissues from patients with metastatic melanoma require single-cell highly-multiplexed analysis for precise yet comprehensive evaluation of TILs function kinetics.
- IsoPlexis IsoCode chip technology integrated with an automated bioinformatics platform simultaneously measures 17 cytokines secreted by single TILs, providing the full spectrum delineation of single TILs' cytokine profile unleashed by anti-PD-1 blocking.
- The IsoCode technology uniquely identifies the polyfunctional profile of single CD8+ TILs, indicating an induced quality immune response of T cells against tumor by checkpoint inhibitors.

METHODS

- Biopsied melanoma tissues were dissociated with Collagenase I (1 mg/ml) and DNase (20 µg/ml) at 37°C for 1-2 hours.
- CD8+ TILs from the digest were enriched by CD8 microbeads, stimulated with immobilized anti-CD3 antibody (10 µg/ml) at 37°C, 5% CO_2 for 24 hours, and loaded onto an IsoPlexis SCBC containing ~12000 microchambers pre-patterned with a complete, 17-plex, antibody array (see **Figure 1**).
- Cells on the SCBC were imaged to identify single-cell locations and incubated for 16 hours at 37°C, 5% CO_2; single-cell cytokine signals were then captured and digitized with a microarray scanner.
- The polyfunctional expression (2+ cytokines per cell, see **Figure 2**) of single CD8+ TILs was evaluated using IsoPlexis' software across four functional groups:
  - **Effector: Granzyme B, IFN-γ, MIP-1a, Perforin, TNF-a**
  - **Stimulatory: GM-CSF, IL-2, IL-5, IL-8, IL-9**
  - **Regulatory: IL-4, IL-10, IL-13, IL-22**
  - **Inflammatory: IL-6, IL-17A, MCP-1**

RESULTS

- The polyfunctional cytokine production of CD8+ TILs cells was found to be highly upregulated in patients who responded to anti-PD-1 antibody therapy, compared to both nonresponders and untreated patients.
- See **Figures 3-7** for detailed results.

Explanation: Platform for analyzing highly multiplexed, single-cell secretomics

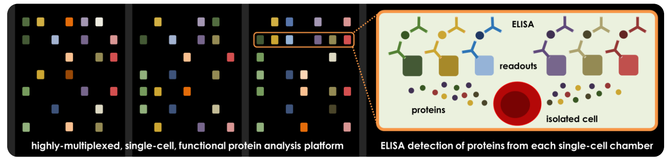

Figure 1: IsoPlexis' highly multiplexed, single-cell cytokine profiling. The IsoPlexis platform isolates thousands of single cells into individual chambers, each of which is pre-patterned with a complete copy of a 17-plex antibody array. Following a 16-hour incubation period, ELISA detection is used to determine which combinations of proteins are being secreted by each individual cell.

Explanation: Measuring a sample's single-cell Polyfunctional Strength Index (PSI)

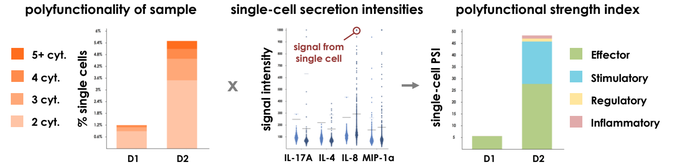

Figure 2: Measuring single-cell Polyfunctional Strength Index (PSI). A published IsoPlexis metric that quantifies the overall activity of a sample. Equivalent to the product of the percentage of polyfunctional cells (secreting two or more cytokines) in a sample and the average signal intensity of the secreted cytokines.

Upregulation in single-cell polyfunctionality of anti-PD-1-treated responders vs. nonresponders

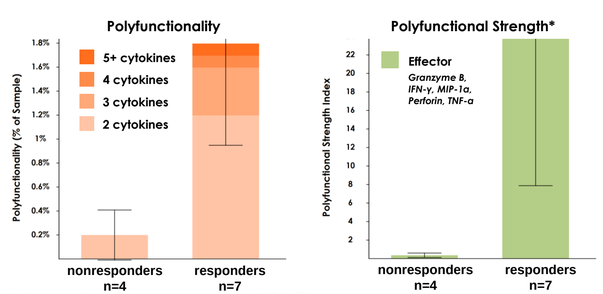

Figure 3: Polyfunctional overview of analyzed CD8+ TILs samples. Samples from responders have 9x more polyfunctional cells than non-responders, and 40x higher polyfunctional strength. Individual cells secreted a combination of effector cytokines (Granzyme B, IFN-γ, MIP-1a, Perforin and TNF-a) associated with anti-tumor immunity. *p= 0.0294

Single-cell functional heat map of anti-PD-1-treated responders vs. nonresponders

Figure 4: Heat map of most frequently secreted functional groups (left to right) by CD8+ TILs. Overall, CD8+ TILs from responding patients more frequently secreted polyfunctional effector groups secreting Granzyme B, IFN-g, MIP-1a, Perforin, and TNF-a in various combinations shown by the green dots. These groups were entirely absent from nonresponding TILs' profiles.

Single-cell CD8+ TILs cytokine secretion distribution of anti-PD-1-treated responders vs. nonresponders

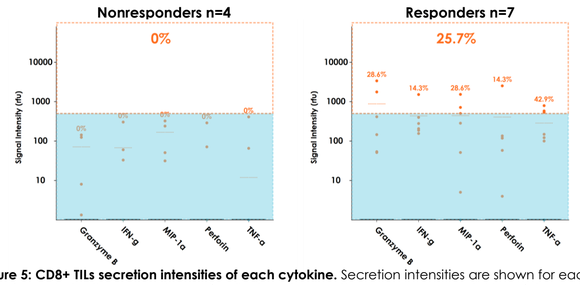

Figure 5: CD8+ TILs secretion intensities of each cytokine. Secretion intensities are shown for each single-cell. As a whole, the percentage of cells strongly secreting one or more cytokines (at an intensity of above 500) in responding patients' samples (25.7%) is higher than in nonresponding patients' samples (0%). Granzyme B has notably higher signal distribution in responding patients' samples vs. nonresponding patients' samples. MIP-1a, IFN-g, Perforin, and TNF-a were also elevated in responders.

Single-cell polyfunctional strength per cytokine of anti-PD-1-treated responders vs. nonresponders and untreated patients

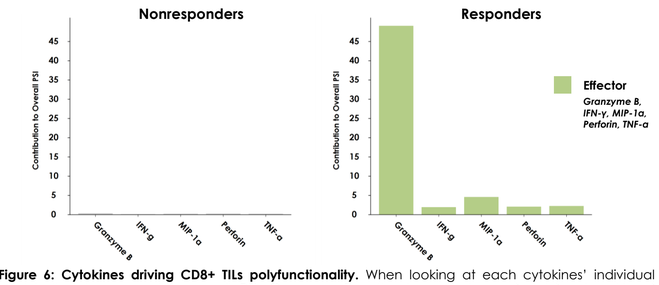

Figure 6: Cytokines driving CD8+ TILs polyfunctionality. When looking at each cytokines' individual contribution to a sample's overall PSI, Granzyme B is the biggest driver of polyfunctionality of responding patients' CD8+ TILs, followed by MIP-1a. However, there is a high degree of heterogeneity across responding patients' samples, as illustrated in Figure 7, suggesting that more than one secretion profile may be indicative of positive response to anti-PD-1 treatment.

CONCLUSIONS

- Single-cell analysis of CD8+ TILs samples revealed upregulation of polyfunctional cytokine production in patients who responded to anti-PD-1 antibody therapy (n=7) compared to nonresponders (n=4).
- The contributions to enhanced polyfunctional strength were dominated by effector and stimulatory cytokines – both positively associated with anti-tumor immunity.
- CD8+ TILs of patients responding to therapy exhibited highly polyfunctional subsets co-secreting Granzyme B, IFN-γ, MIP-1a, Perforin, and TNF-a, a potential biomarker to predict clinical outcome of melanoma patients treated by checkpoint immunotherapy.
- Single-cell multiplexed cytokine profiling is capable of dissecting the full spectrum of immune functions of heterogeneous TILs across patients with metastatic melanoma and more accurately measuring the function of TILs to predict the response of patients receiving anti-PD-1 blocking therapy.

ACKNOWLEDGMENTS

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Polyfunctional Activation Topology PCA of anti-PD-1-treated responders' CD8+ TILs

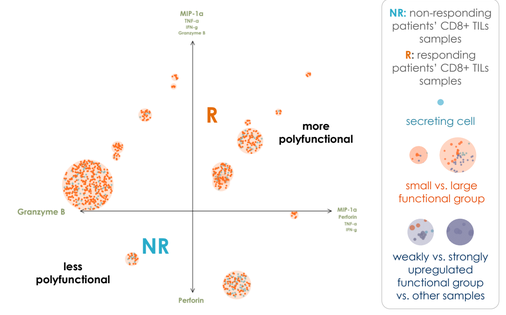

Figure 7: Polyfunctional Activation Topology (PAT) PCA of non-responding versus responding patients' CD8+ TILs profiles. Illustrates the activity of anti-PD-1-treated, responding patients' CD8+ TILs, relative to non-responding patients' TILs. All functional and polyfunctional groups are secreted at higher frequency in responders; most non-responders' single cells are secreting only Granzyme B or Perforin (small blue dots). Responders also secrete multiple effector-driven polyfunctional groups (towards the upper-right side of the graph.