Single-cell multiplex proteomics reveals synergistic impact of antigen and rimiducid-dependent stimulatory signals on promoting polyfunctional GoCAR-T cells targeting prostate stem cell antigen (PSCA)

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BACKGROUND

- Bellicum GoCAR-T cell products utilize a rimiducid (CID)-dependent MyD88/CD40 costimulatory molecule to enhance the proliferation and survival of CAR-T cells providing *in vivo* control over CAR-T cell dynamics in cancer patients.
- IsoPlexis single-cell IsoCode chip (SCBC) technology integrated with an automated bioinformatics platform simultaneously measures 32 cytokines secreted by single CAR-T cells, enabling the full characterization of functional attributes of GoCAR-T cell products.

METHODS

- GoCAR-T cells (**Figure 1**) were manufactured from human PBMCs of healthy donors transduced with a rimiducid-inducible costimulatory unit, MyD88/CD40 (iMC), and a first-generation CAR targeting PSCA.
- CD4+ and CD8+ GoCAR-T cells were enriched by microbeads and stimulated with the PSCA+ human pancreatic adenocarcinoma cell line (HPAC) in the presence or absence of CID (10 µm) at 37°C, 5% CO_2 for 24 hours.
- After co-culturing, the GoCAR-T cells were loaded onto an IsoPlexis SCBC containing ~12000 microchambers pre-patterned with a 32-plex, antibody array (**Figure 2**), imaged to locate single cells in microchambers and incubated at 37°C, 5% CO_2 for additional 16 hours.
- Single-cell proteins secretions were captured by antibody-barcoded slides; the polyfunctional profile (2+ proteins/cell, **Figure 3**) of single GoCAR-T cells was evaluated by IsoPlexis' software:
  - **Effector: Granzyme B, IFN-γ, MIP-1a, Perforin, TNF-a, TNF-β**
  - **Stimulatory: GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21**
  - **Chemoattractive: CCL11, IL-10, MIP-1β, RANTES**
  - **Regulatory: IL-4, IL-10, IL-13, IL-22, TGF-β1, sCD137, sCD40L**
  - **Inflammatory: IL-1β, IL-6, IL-17A, IL-17F, MCP-1, MCP-4**

RESULTS

- See **Figures 4-9** for detailed results.

CONCLUSIONS

- Single-cell multiplexed precision profiling reveals antitumor polyfunctional upregulation and heterogeneity of GoCAR-T cell products with the stimulation of PSCA+ tumor cells and/or iMC activation by rimiducid.
- The single-cell polyfunctional metrics may provide insights into quality check of pre-infusion GoCAR-T cell products and potential biomarker discovery to predict efficacy, persistence and safety of GoCAR-T cell therapy in solid tumor.

Bellicum GoCAR-T platform

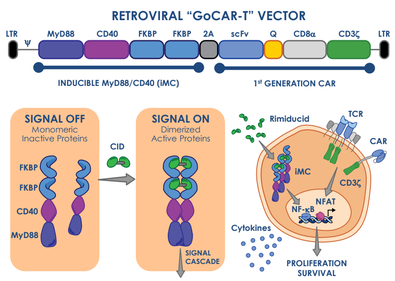

Figure 1: Bellicum GoCAR-T platform. In GoCAR-T cells, the MyD88/CD40 co-stimulatory activation signal is moved from the CAR to a rimiducid-controllable element. Cell proliferation and activation is rimiducid-dependent, and full activation requires a second, target-specific CD3ζ signal. This provides "on-demand" co-stimulation to T cells controlling CAR-T cell proliferation and survival.

Enhanced population cytokines of GoCAR-T cells co-cultured with HPAC ± CID

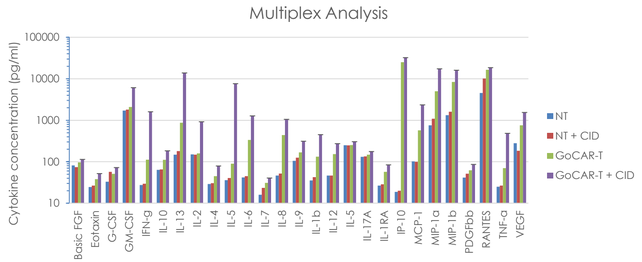

Figure 4: Population cytokines measured by Luminex. PBMCs transduced with or without the PSCA-specific GoCAR-T construct were co-cultured with HPAC ± CID at 37°C, 5% CO_2 for 24 hours at a 1:1 ratio of T cells: target cells. Cytokines from supernatants were measured by Luminex. GM-CSF, IFN-g, IL-13, MIP-1a, MIP-1b were greatly upregulated by stimulation of HPAC + CID.

IsoPlexis detection platform for analyzing highly multiplexed, single-cell secretomics

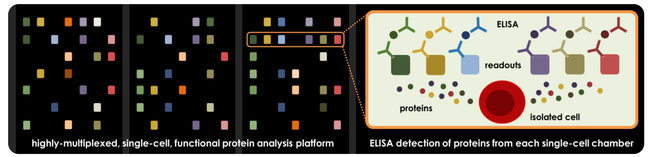

Figure 2: IsoPlexis' highly multiplexed, single-cell cytokine profiling. The IsoPlexis platform isolates thousands of single cells into individual chambers, each of which is pre-patterned with a complete copy of a 17-plex antibody array. Following a 16-hour incubation period, ELISA detection is used to determine which combinations of proteins are being secreted by each individual cell.

Enhanced cytokine secretion distribution of single GoCAR-T cells stimulated by HPAC ± CID

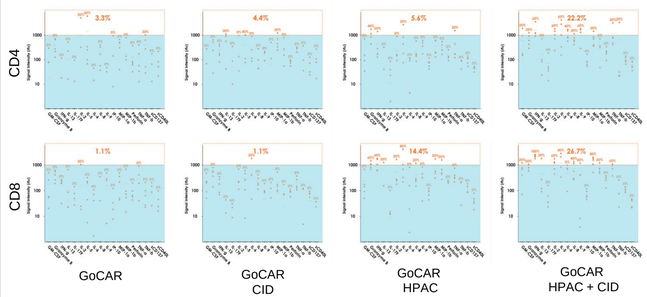

Figure 5: Cytokine secretion intensities of single GoCAR-T cells. Enhanced cytokine secretion intensities were seen in single GoCAR-T cells stimulated with HPAC ± CID compared to GoCAR-T cells ± CID. There was approximately a 4x increase in CD4 GoCAR-T cells and a 2x increase in CD8 GoCAR-T cells by HPAC + CID relative to HPAC alone.

Measuring a CAR-T sample's single-cell Polyfunctional Strength Index (PSI)

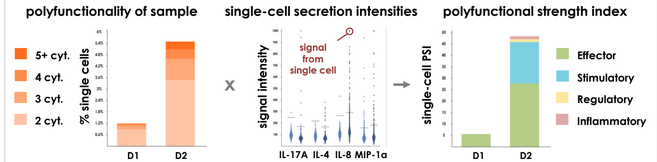

Figure 3: Measuring single-cell Polyfunctional Strength Index (PSI). A published IsoPlexis metric that quantifies the overall activity of a sample. Equivalent to the product of the percentage of polyfunctional cells (secreting two or more cytokines) in a sample and the average signal intensity of the secreted cytokines.

Enhanced PSI of single GoCAR-T cells by stimulation of HPAC ± CID

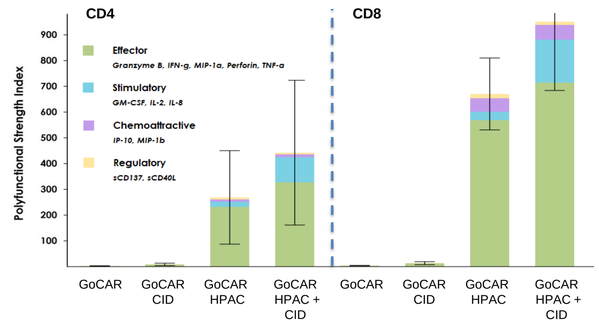

Figure 6: PSI in single GoCAR-T cells. Enhanced antitumor PSI was shown in both CD4 and CD8 GoCAR-T cells by HPAC ± CID stimulation relative to the controls of GoCAR-T cells ± CID. A roughly 2-fold increase of PSI was induced by HPAC + CID compared to HPAC alone.

Cytokine contributions to enhanced PSI of single GoCAR-T cells by HPAC + CID

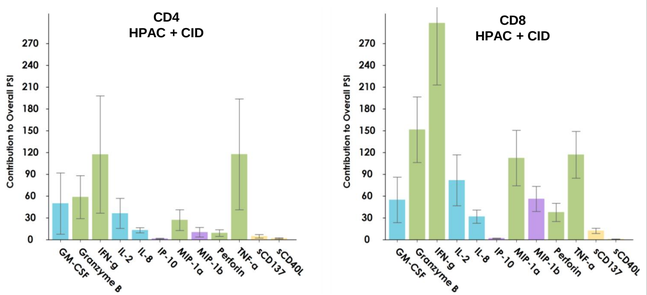

Figure 7: Cytokines driving GoCAR-T cell polyfunctionality induced by HPAC + CID. IFN-γ and TNF-α play a primary role in driving enhanced polyfunctionality of CD4 CAR-T cells, followed by Granzyme B, GM-CSF, IL-2 and MIP-1α, and to a small degree by IL-8, MIP-1α, Perforin and sCD137. In contrast, IFN-γ followed by Granzyme B, MIP-1α, TNF-α and IL-2 are the major contributors to the enhanced polyfunctional CD8 CAR-T cells while GM-CSF, IL-8, Perforin and sCD137 to a lesser extent.

Distinct combinations of cytokine secretions of CD4 GoCAR-T cells by HPAC + CID

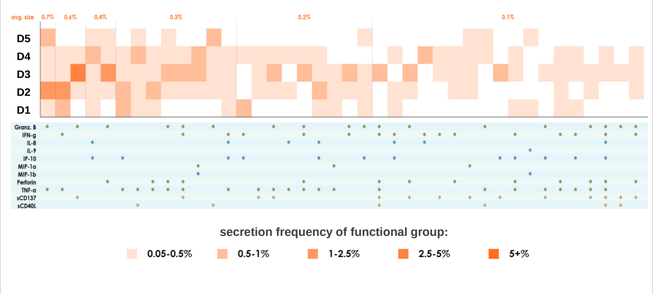

Figure 8: Polyfunctional heat map of HPAC + CID stimulated CD4+ GoCAR-T cells. The heatmap shows the distinct functional and polyfunctional profile groups secreted by each donor, with the color indicating how commonly each donor is secreting the corresponding functional group. Donor 3 followed by donor 2 and 4 showed the higher frequencies of most expressed functional groups. Donor 1 had fewer polyfunctional cell subsets than donor 2, 3, 4 while donor 5 showed the least polyfunctional response to HPAC + CID.

PAT PCA heterogeneity of CD4 GoCAR-T cells by HPAC + CID

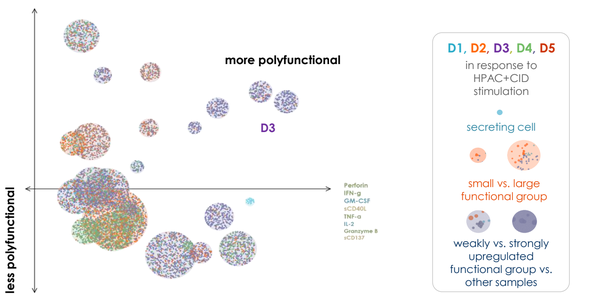

Figure 9: Polyfunctional Activation Topology (PAT) PCA of single CD4 GoCAR-T cells by stimulation of HPAC + CID. PAT PCA revealed the polyfunctional heterogeneity of different donors in response to HPAC+CID stimulation. D3 showed the most upregulated polyfunctional cell subsets, followed by D2 and D4. D1 and D5 exhibited fewer polyfunctional cell subsets induced by HPAC + CID.