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

Sean Mackay¹, Brianna Flynn¹, Kevin Morse¹, Colin Ng¹, Patrick Paczkowski¹, Aruna Mahendravadha², Nicholas Shinnier², David Spencer², Aaron Foster² and Jing Zhou¹

¹. IsoPlexis Corporation, 35 NE Industrial Road, Branford, CT 06405
². Bellicum Pharmaceuticals, Houston, TX 77030

BACKGROUND

• Bellicum GoCAR-T cell products utilize a rigidulin (CD122)-dependent MyD88/CD40 costimulatory mAb 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 PBMC of healthy donors transduced with a rigidulin-inducible costimulatory mAb MyD88/CD40 (mAb), and a first-generation CAR targeting PSA.
• CD4+ and CD8+ GoCAR-T cells were enriched by microbead stimulation and incubated with x human prostate-specific adenosinereceptor cell line (HPAC) in the presence or absence of CID (10 μM) at 37°C, 5 % CO₂ for 24 hours.
• After co-culturing, the GoCAR-T cells were loaded onto an IsoPlexis SCBC containing ~1000 microchambers pre-patterned with a 32plex antibody array (Figure 2). Image to locate single cells in microchambers and incubated at 37°C, 5 % CO₂ for additional 16 hours.
• Single-cell protein secretions were captured by antibody-capture based flow, the polyfunctional profiles (2+ proteins/cell). Figure 3 of single GoCAR-T cells was evaluated by IsoPlexis' software:
  - Effectors: Granzyme B, IFN-γ, TNF-α, perforin, TNF-α, TNF-β – Stimulatory: GM-CSF, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-21 – Chemokine:</p>

RESULTS

• Single-cell multiplexed precision profiling reveals antihuman polyfunctional upregulation and heterogeneity of GoCAR-T cell products with the stimulation of PSA+ tumor cells and/or IL-23 activation by rigidulin.
• 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.

CONCLUSIONS

Bellicum GoCAR-T platform

- Enhanced population cytokines of GoCAR-T cells co-cultured with HPAC ± CID is improved.
- Cytokine contributions to enhanced PSI of single GoCAR-T cells by HPAC ± CID are shown.
- Distinct combinations of cytokine secretion of CD4 GoCAR-T cells by HPAC + CID are observed.
- Measuring a CAR-T sample's single-cell Polyfunctional Strength Index (PSI) can provide functional context.

GoCAR-T cells targeting PSCA: Induced CD40+GoCAR-T cells by HPAC ± CID is elevated.

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

Enhanced cytokine secretion of single GoCAR-T cells by HPAC ± CID is achieved.

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