康龙化成 PHARMARON

CELL THERAPY

Potency Assays CAR-T Cells

Pharmaron is able to support the preclinical demands for a CAR-T candidate to be IND-ready or IMPD-ready for First-in-Human (FIH) clinical trials. Pharmaron's global team of cell and gene therapy drug developers are experienced in the case-by-case testing strategy based on the diversity and specific inherent biological properties of a CAR-T cell therapy. Potency assays are critically important in the development of CAR-T cell therapies and guide pre-clinical and clinical development. Here we show a potency assay approach measuring the ability of the CAR T cell to eliminate tumor cells and release IFN-ɣ upon activation by tumor cells.

Potency Assays

Background

Gene modified cell therapies require potency assays at various steps to monitor (1) vector to expression and (2) its ability to exert its intended function. Validated potency assays are required at the time of product licensing. However, potency assays play a critical role in preclinical and process development, dosing, comparability, and stability and should be developed and qualified early on.

Matrixed approach

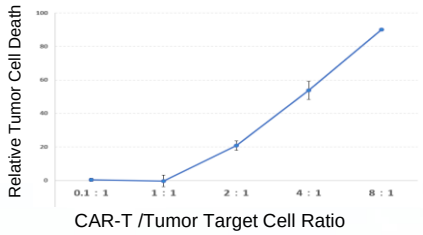
Regulatory guidance suggest to develop multiple, orthogonal, assays to assess potency. As more data becomes available during preclinical and clinical development the number of assays can be reduced. The potency of current approved CAR-T cell products is primarily measured by IFN-ɣ release upon activation with tumor target cells in combination with other factors, such as viability, unique for each product.

Pharmaron Experience

Our multidisciplinary team of experts develop potency assays supporting all stages of your product development.

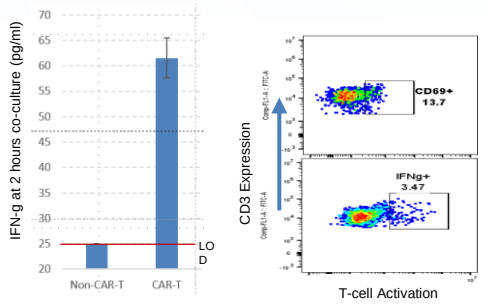
Potency Assay CAR-T Cells

Flow Cytometric Toxicity Assessment



Anti CD19 CAR-T cells induce cell death of CD19 positive tumor cells via lysis in a dose depend fashion shown here after 90 minutes in co-culture. The graph shows the specific lysis of the tumor targets at different E:T ratios as the data is corrected for tumor cell lysis occurring in co-cultures with unmodified T cells.

IFN-ɣ production and CAR-T Cell Activation



Tumor cell activated CAR-T cells show secreted INF-ɣ (left panel) as well as intracellular IFN-ɣ (lower right) as well as upregulation of CD69; a cell surface T cell activation marker (upper right)

Experiment

Set-up

Commercially obtained anti-CD19 CAR-T cells and CD19 expressing tumor cells were co-cultured to assess the killing potential of the CAR-T cells. Co-cultures of various Effector (CAR-T cells) and Target (Tumor cells) ratios were analyzed over a time course of 90 minutes to 24 hours). Unmodified T cells were used as a comparator.

Analysis

Primarily, the co-cultures were stained with anti CD3, anti-CD19, and viability dyes (Annexin-V or 7-AAD). Additional antibodies for T cell activation and intracellular IFN-ɣ were also used. Finally, supernatant was analyzed for IFN-ɣ via ELISA.

Results:

Significant cell death of tumor cells was demonstrated at E:T ratios of 4:1 and higher and as early as 90 minutes of co-culture. The cytotoxic activity coincided with higher levels of CAR-T cell activation as measured by IFN-ɣ (co-culture supernatant and intracellular).

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