Assessing the potency of SPEAR T cells: development and validation of a flow cytometry-based cytotoxicity assay

Introduction

- In adoptive T-cell therapy with SPEAR T-cells, CD3+ cells from the patient are transduced with a lentiviral vector to express a TCR with enhanced affinity against a target antigen.
- The T-cells are then expanded and infused into the patient.

Objective

- For pivotal trials, a validated potency assay is required to release SPEAR T-cell batches.
- We sought to develop a flow cytometry-based assay that measures target-specific T-cell cytotoxicity.
- Here we present an overview of the ADP-A2M4 assay from development to validation.

Results

- Data from assay qualification is shown (Figs 2-5); qualification and validation results are summarized (see tables below).
- Accuracy (Fig. 2), linearity (Fig. 3), and precision (Fig. 4) were assessed using different preparations of the reference T-cell batch, made with 20-200% of the starting material amount defined in the protocols.
- Specificity (Fig. 5) was assessed by measuring killing from T-cell batches that were non-transduced, transduced with an irrelevant TCR, or transduced with ADP-A2M4 TCR but presented with an irrelevant peptide.

Methods

The day before the assay, T2 cells are seeded to ensure they are in growth phase for the assay.
- The day of the assay, T2 cells are stained with CFSE.
- Each well contains a fixed concentration of T2 cells and a defined dilution series of T-cell batches; with and without MAGE-A4 peptide.
- Each sample is tested as a triplicate with and without peptide.
- Samples are dispensed to compensate for any plate drift. T2-only control monitors target cell survival in the absence of T-cells.
- Plates are incubated overnight at 37°C.
- Plates are washed and stained with dead cell dye (7AAD).
- Plates are read on a flow cytometer (MacsQuant 10), allowing for quantification without bead counts.

T2 target cells: 174 x GEM T2, ATCC® CRL-1992
- Suspension cell line, MHC class II-, MHC class I+
- Plates are incubated overnight at 37°C, then plates are washed and stained with dead cell dye (7AAD).
- Plates are read on a flow cytometer (MacsQuant 10), allowing for quantification without bead counts.

Figure 1. Example of assay output, showing from left to right flow plots, target cell numbers, # peptide specific killing, relative activity.

Analysis of assay output

Comparing samples of different potency

Figure 2: Examples of varying killing when different preparations of the reference T-cell batch are tested.

Linearity and accuracy

Figure 3: Measurement of reference material in preparations from 20-200%, n=7 for 100%; n=5 for all other samples.

Specificity

Figure 4: Precision for EC50 and relative activity, for measurement of reference material in preparations from 20-200%. P<0.05 for all other samples.

Validation

Figure 5: Comparison of ADP-A2M4 transduced T-cell batch with MAGE-A4 peptide (blue) vs. specificity samples: NTD T-cells with MAGE-A4 peptide (red), ADP-A2M10 transduced T-cells with MAGE-A4 peptide (purple), or ADP-A2M4 with MAGE-A10 peptide (green).

Conclusions

- We developed a highly specific cytotoxicity assay that was validated by ICH guidelines.
- A batch release specification was developed based on the performance of past patient material batches.
- This assay is being used for the SPEAR-GSD-1 trial of ADP-A2M4 (NCT04044768).

Abbreviations

- CD3: cluster of differentiation 3; CD8: cluster of differentiation 8
- NTD: non-transduced; QC: quality control; SPEAR: specific peptide enhanced affinity receptor; TCR: T-cell receptor
- CYP 2D6: cytochrome P450 2D6
- % peptide specific killing, relative activity.

Technical Requirements for Pharmaceuticals for Human Use; NTD: non-transduced; QC: quality control; SPEAR: specific peptide enhanced affinity receptor; TCR: T-cell receptor

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