Development and technical validation of tetramer staining for use as a biomarker for assessing gluten-specific T cells in clinical studies

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Introduction

- Gluten-challenges in subjects with celiac disease results in a transient upregulation of gluten-specific CD4+ T cells in the blood. Despite the increase, these cells are quite rare requiring a selective and sensitive assay for detection.

Method and results

- We have developed a 12-colour tetramer flow assay to enable detection and immunophenotyping of the gluten α-I/α-II CD4+ T cells in the blood.

Figure 1. Experimental procedure

Step 1. Separation and storage of PBMCs
- For clinical application, trial sites can collect whole blood
- PBMCs isolate using SepMate™ tubes – Ficoll
- PBMCs frozen to maintain viability and allow batch testing
- Longitudinal samples from each subject to be tested together

Step 2. Gliadin monomers are tetramerized with streptavidin phycoerythrin (PECy7) fluorophore Tetramers are pooled for staining
- Tetramer α I (GLQPGPFPEPQ)
- Tetramer α II (POPELPYPQPE)

Step 3. Isolation of CD4+ T cells from thawed PBMCs
- PBMCs are thawed and CD4+ cells isolated via magnetic bead based separation
- Post purity >95% prior to staining

Step 4. Stain isolated CD4+ T cells
- Controls for staining include:
  - Unstained samples to measure auto-fluorescence
  - Single-colour controls, entire antibody panel minus 1 stain for each marker
  - PBMCs frozen to maintain viability and allow batch testing

Step 5. T cell stain full antibody panel
- This includes C3D3, CD4, CD38, B7, tetramer, CD95RA, CD69, Alpha, a4, CCR7, CD27, lineage depletion (CD11c, CD14, CD45, CD62L) and live/dead viability dye

Step 6. Detection with BD LSRFortesa™ 5-S成为一名18 colour system
- Flow cytometry with a ThermoFACSFlow™ C8 analyser

Table 1. Assay validation parameters with pre-set acceptance criteria

Table 2. Assay criteria were met for the validation parameters

Conclusions

- This gliadin α-I/α-II DQ2 tetramer flow cytometric assay was developed and validated to be sensitive and selective. Technical validation of the assay was successfully met and the assay performs within the precision parameters.

- This assay will enable characterization of the gliadin α-I/α-II CD4+ T cells in the blood for celiac patients, providing a more comprehensive evaluation of response to new therapies and may reduce invasive biopsy-based measurements.

References


Disclosures

GMS, RH and JG are employees of Takeda, LA. AB and DP are employees of Precision for Medicine. VM has no disclosures to report.

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