In-depth Characterization of Cell Therapy Products Using Mass Spectrometry-based Proteomics

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Outline

- Cell therapy and iPSC platform overview
- MS-based proteomics to tackle challenges in cell surface marker characterization
- Analytical Strategies and Objectives
- Protein-level differences revealed in various cell therapy products
- Conclusion
Chimeric antigen receptor (CAR) T cell therapy

- Ex vivo engineered T cells
- Next-generation anti-cancer therapy
- Several recent FDA approvals
- Little proteomics level understanding

Current CAR-T Approaches and Associated Risks

- **Autologous** (Patient Derived)
  - T-cell disfunction
  - Harvest/manufacture failure
  - Disease progression during manufacturing
  - Cost & supply chain

- **Allogeneic** (Healthy Donor)
  - Rejection
The versatile iPSC platform

- induced Pluripotent Stem Cell (iPSC)-derived CAR T Cells

### iCAR-T Platform

- Derived T cells (iT)

### iCAR-NK Platform

- Derived NK cells (iNK)

#### Why iCAR-T/iCAR-NK?

- Versatile platform
- Improved patient access
- Higher consistency, better quality
- Affordability

#### Critical need for in-depth characterization:

- Cell-based assay characterization
- RNA sequencing
- **Proteomics: cell surface markers**
Bottom-up proteomics & challenges in cell surface marker characterization

• Bottom-up proteomics is a powerful approach to determining the protein make-up of a complex sample.

• Why is cell surface marker characterization challenging?
  – Marker proteins are membrane proteins
  – Membrane proteins are usually present in low abundance with poor solubility and lack of trypsin cleavage sites

• **KEY: reduction of sample complexity!**
Subcellular proteome fractionation to reduce sample complexity

1. Plasma membrane disruption

Trypsin digestion of subcellular fractions of interest

- Nuclei fraction
- Plasma membrane fraction
- Cytosolic fraction
- Organelle + Plasma membrane
- Organelle fraction

2. Slow/short-time spin down
3. Fast/long-time spin down
4. Add strong lysis buffer & spin down
5. Precipitate in PBS

Analytical goals
- Discover unique cell surface marker proteins
- Characterize & quantify CAR construct on transduced CAR-T/NK cells
Feasibility study: successful detection of CAR in primary CAR-T cells

• Results demonstrated great potential of proteomics approach to characterize therapeutic cell products.

Plasma membrane fraction digest of primary CAR-T cells

Isoforms Separated

Total ID (all fractions) = 6713
Plasma Membrane Fraction ID = 5017

• 74% CAR sequence coverage achieved
• Thousands of other non-membrane proteins identified/quantified
• Cell surface markers enriched in plasma membrane fraction
Characterization of various cell therapy products using established proteomics workflow

- 14 cell pellet samples
- 3 cell types
- 6000+ plasma membrane fraction protein ID
- 7000+ total protein ID

Qualitative proteomic differences revealed for distinct cell products
Label-free quantitation statistics highlighting membrane protein differences

Cytosolic

Nucleus

• Random distances among data points
• Low protein expression difference

Organelle

Plasma Mem

• Data points forming groups
• High protein expression difference
Proteomics analysis distinguishing iPSC-derived T cells from donor-derived T cells

[Graphs and diagrams showing data analysis for different donor samples and T cell receptors]
Proteomics analysis confirming expression of knock-in gene, highlighting plasma membrane protein expression differences.

- **Wildtype vs. Knock-in T cells from the same source**

- **Donor-derived T cells vs. iT**

- **Source 1 vs. Source 2 T cells**

- **Estimated -Log10(RawPValue)**

- **Colors**:
  - Red: Plasma Membrane
  - Blue: Cytoplasm
  - Purple: Nucleus
  - Green: Extracellular
  - Gray: Other
Conclusion

- A working subcellular fractionation-assisted proteomics profiling platform has been established in house.

- This proteomics approach
  - Adds massive value to the multi-platform characterization of cell therapy products.
  - Leads to improved cell therapy product understanding.
  - Support research for better cell therapy design.
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