Better Health, Brighter Future

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

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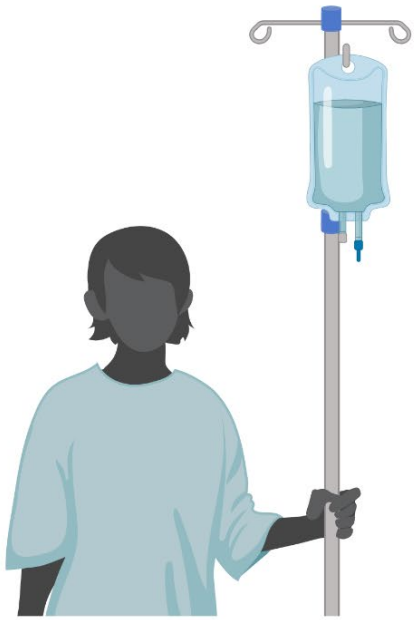
Outline

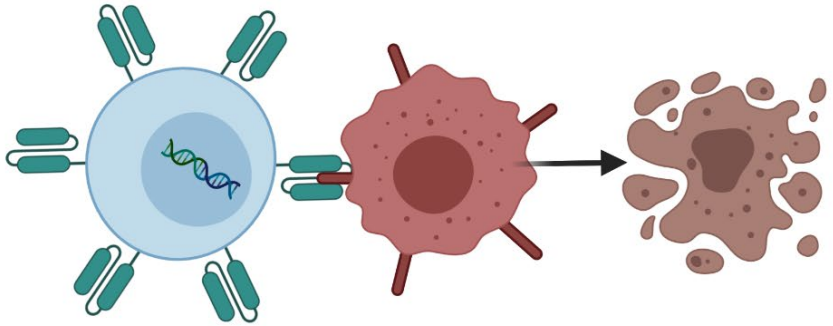
- ❑ Cell therapy and iPSC platform overview
- ❑ MS-based proteomics to tackle challenges in cell surface marker characterization
- ❑ Analytical Strategies and Objectives
- ❑ Protein-level differences revealed in various cell therapy products
- ❑ Conclusion

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

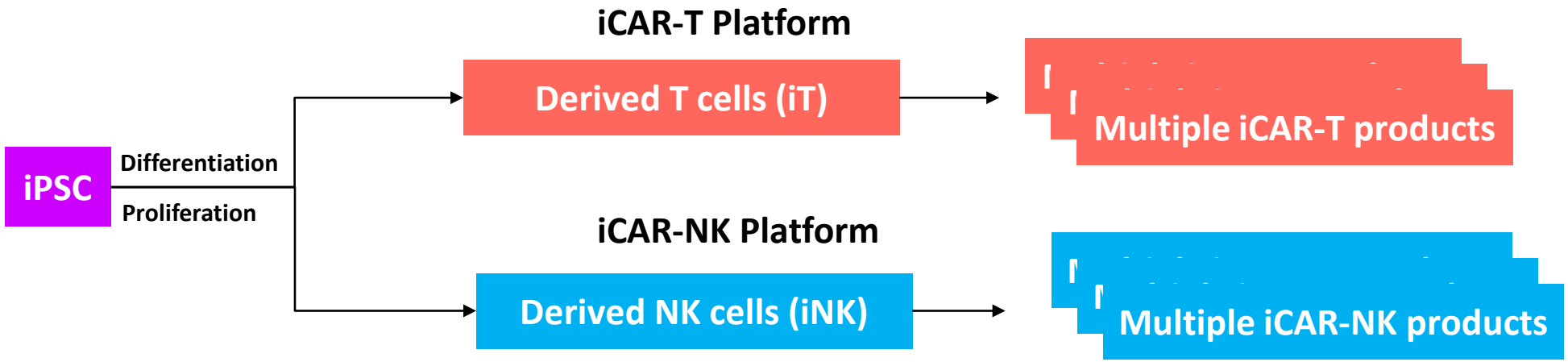
- Current CAR-T Approaches and Associated Risks
  - **Autologous** (Patient Derived)
    - T-cell disfunction
    - Harvest/manufacture failure
    - Disease progression during manufacturing
    - Cost & supply chain
  - **Allogeneic** (Healthy Donor)
    - Rejection





The versatile iPSC platform

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

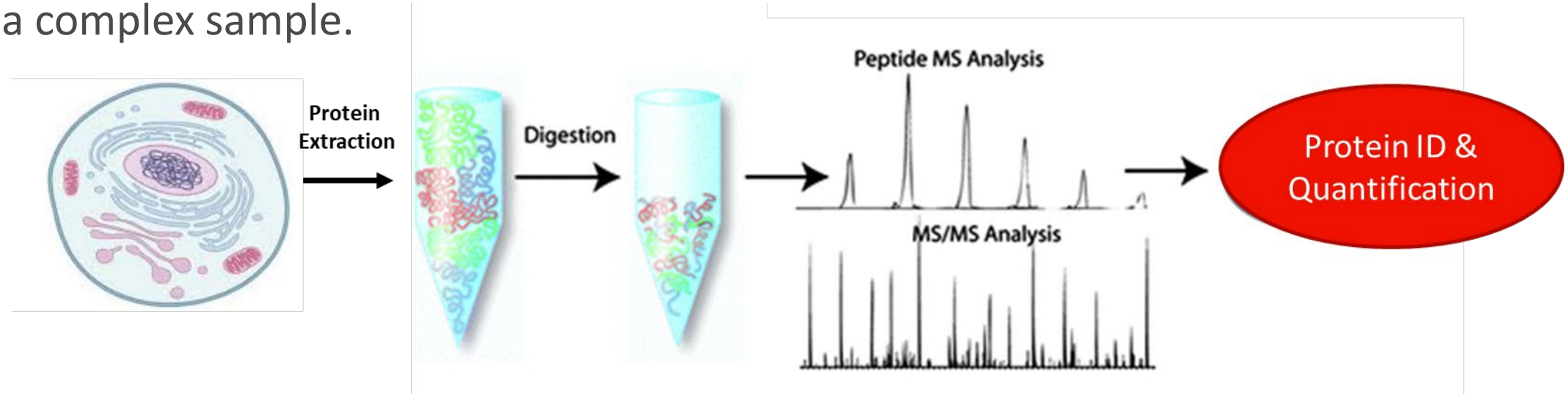


- Why iCAR-T/iCAR-NK?
  - Versatile platform
  - Improved patient access
  - Higher consistency, better quality
  - Affordability

- 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

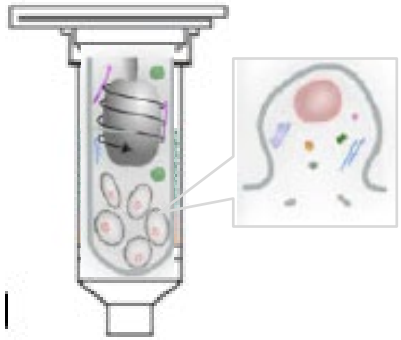
- Bottom-up proteomics is a powerful approach to determining the protein make-up of a complex sample.



- 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

Frit breaks plasma membrane

2. Slow/short-time spin down

3. Fast/long-time spin down

4. Add strong lysis buffer & spin down

5. Precipitate in PBS

Nuclei fraction

Organelle + Plasma membrane

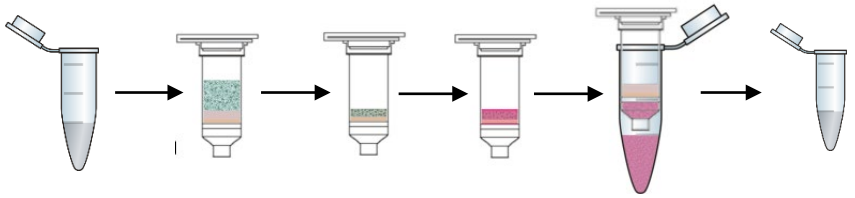
Organelle fraction

Cytosolic fraction

Strong lysis buffer

Plasma membrane fraction

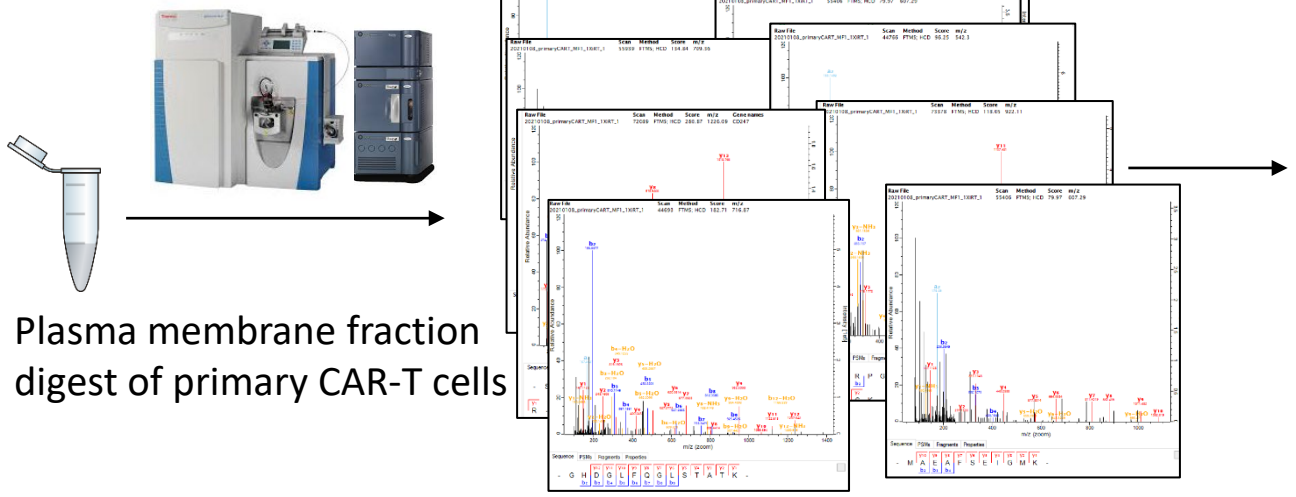
Trypsin digestion of subcellular fractions of interest



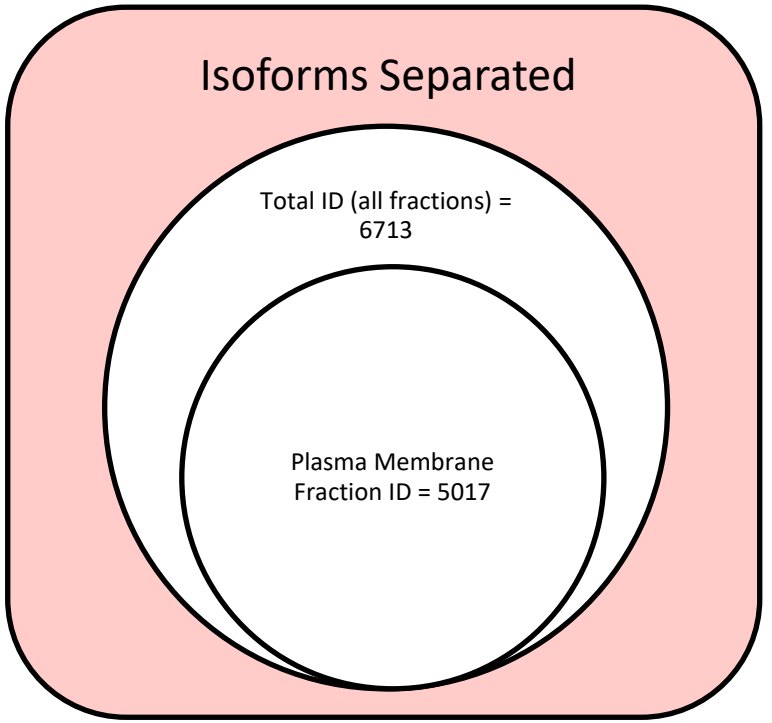
- Analytical goals
  - Discover unique cell surface marker proteins
  - Characterize & quantify CAR construct on transduced CAR-T/NK cells

Feasibility study: successful detection of CAR in primary CAR-T cells

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



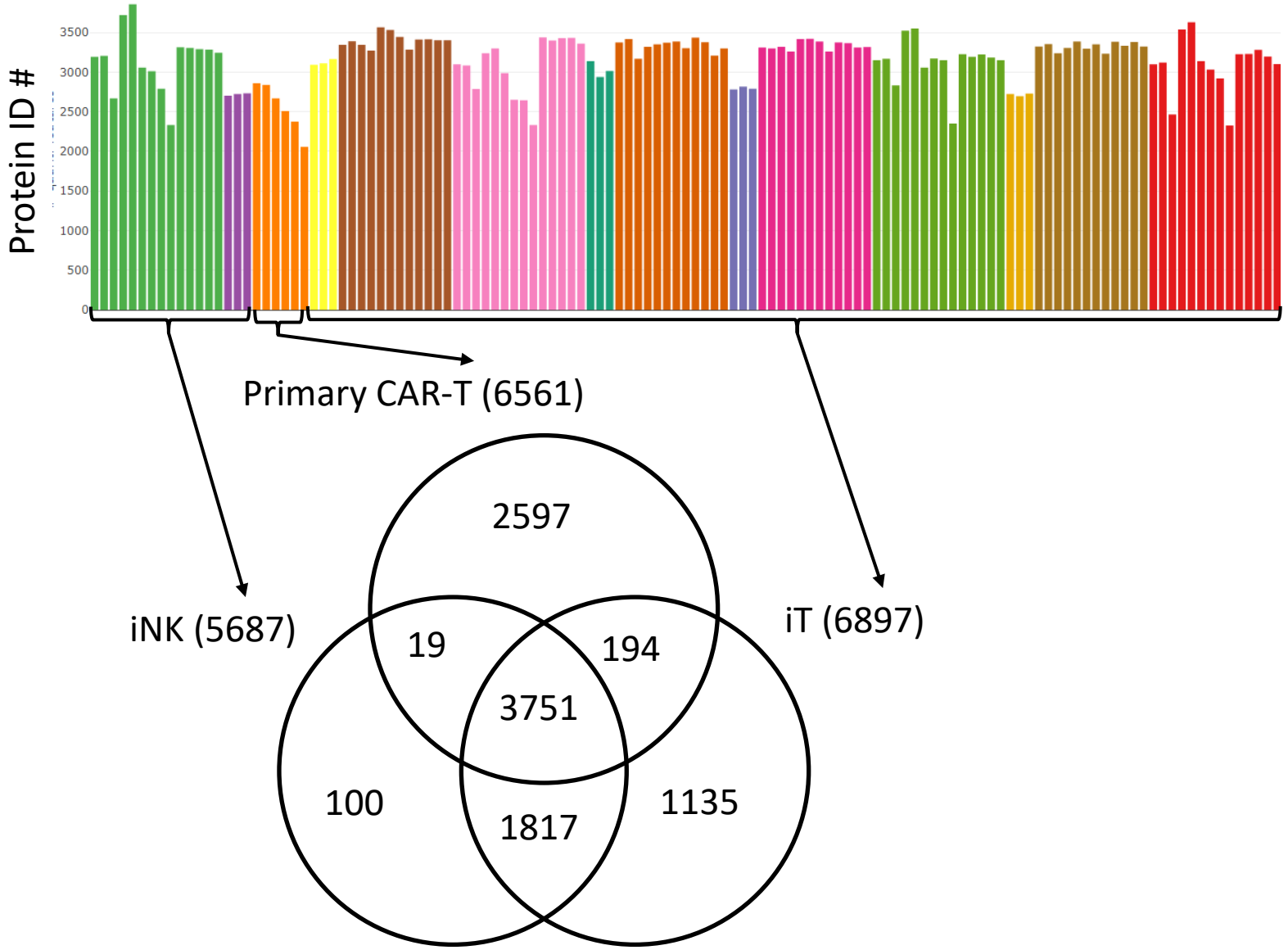
Plasma membrane fraction digest of primary CAR-T cells



- 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

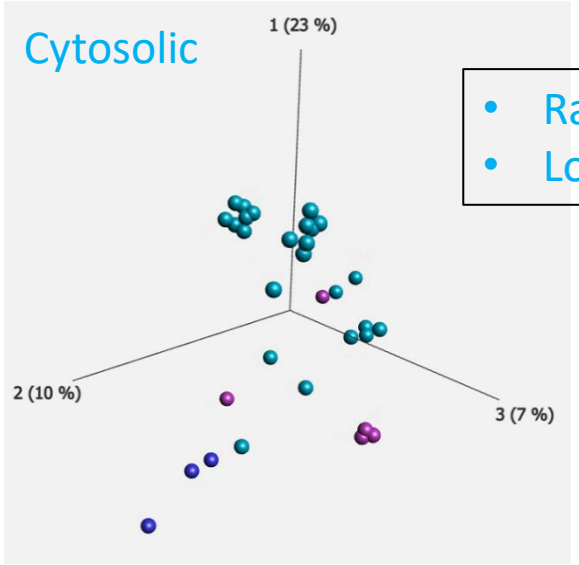
- 14 cell pellet samples
- 3 cell types
- 6000+ plasma membrane fraction protein ID
- 7000+ total protein ID



Qualitative proteomic differences revealed for distinct cell products

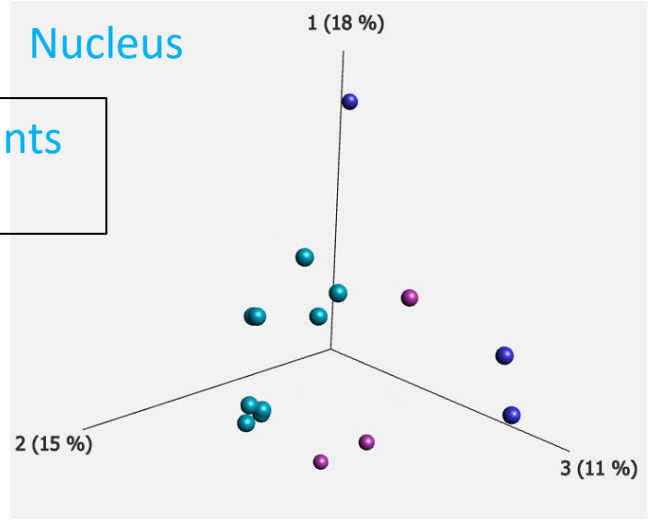
Label-free quantitation statistics highlighting membrane protein differences

Cytosolic

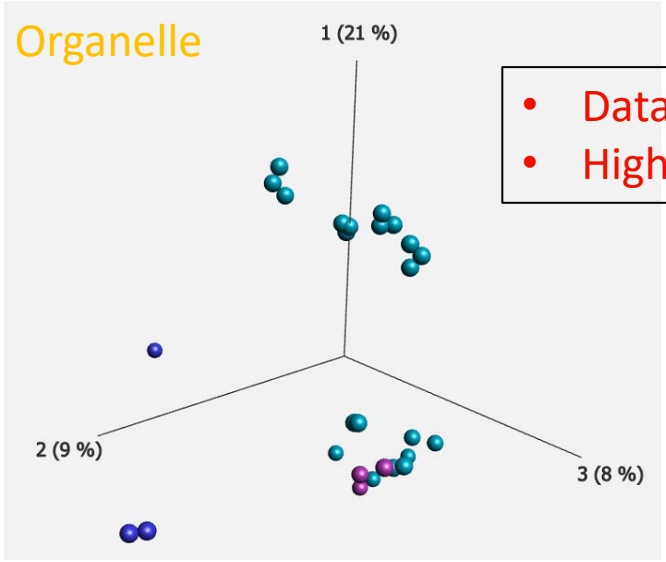


- Random distances among data points
- Low protein expression difference

Nucleus

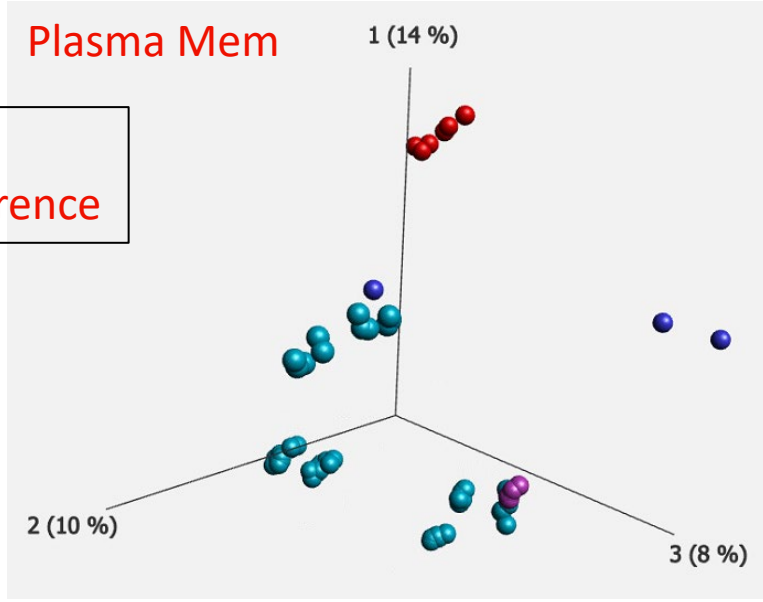


Organelle

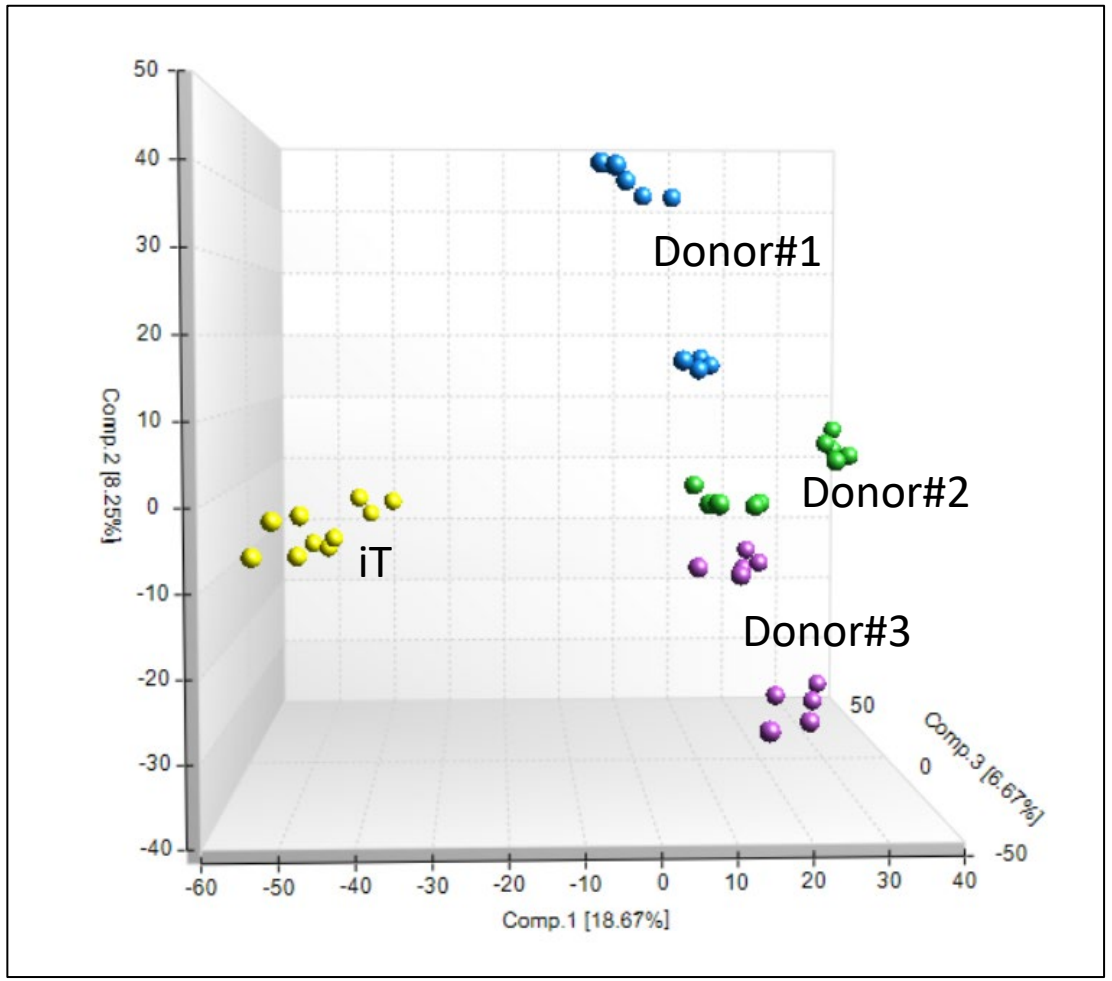


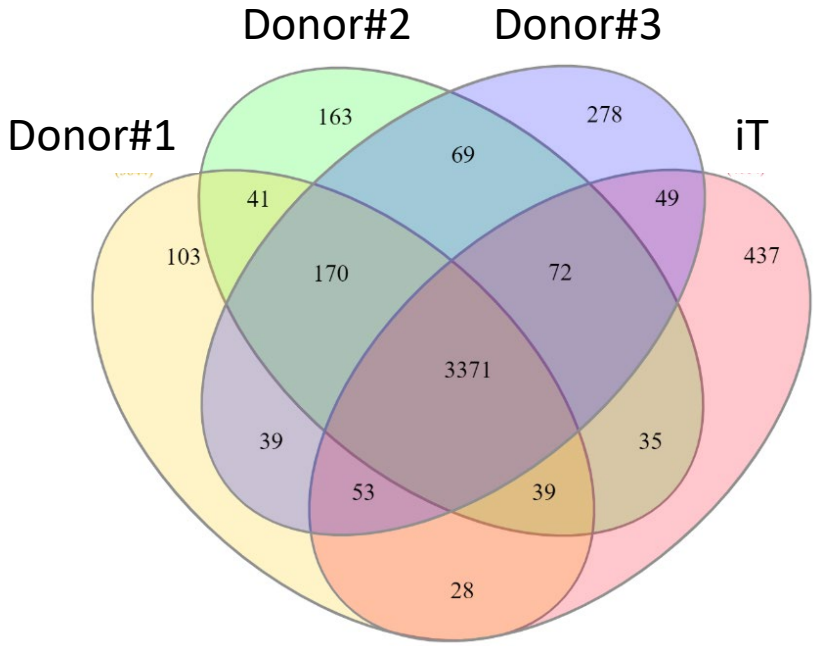
- Data points forming groups
- High protein expression difference

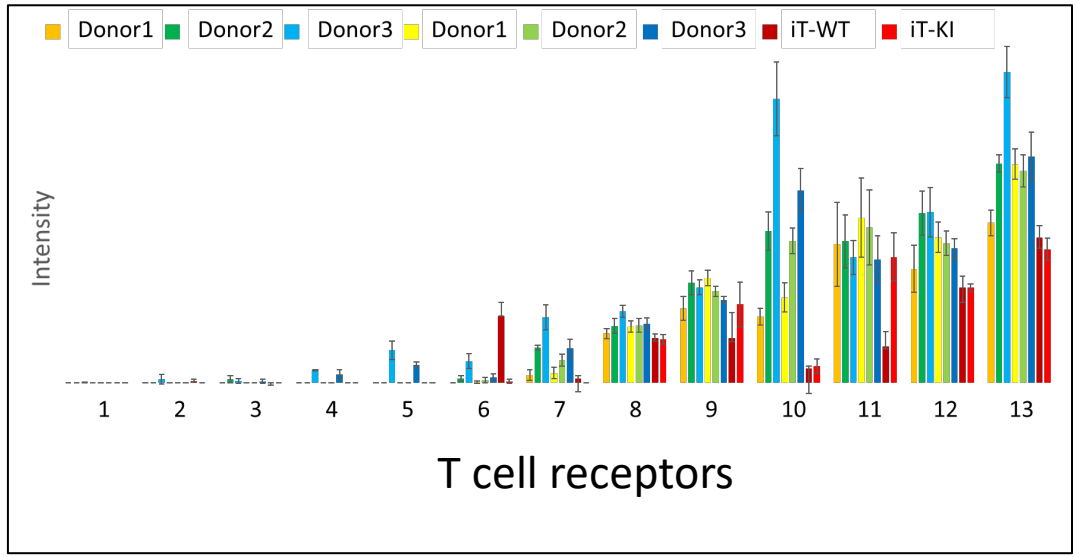
Plasma Mem



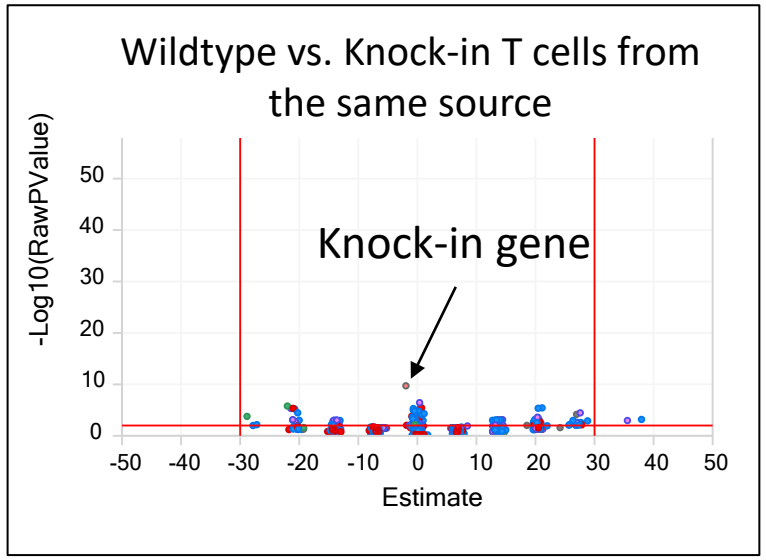
Proteomics analysis distinguishing iPSC-derived T cells from donor-derived T cells

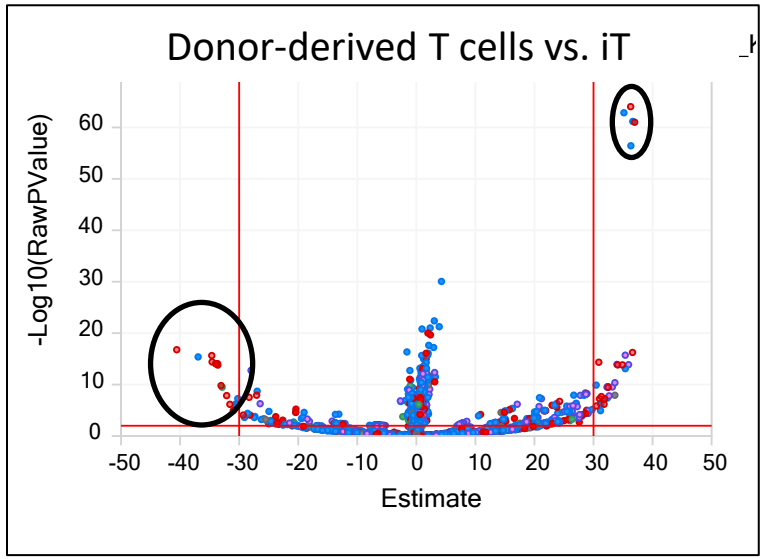


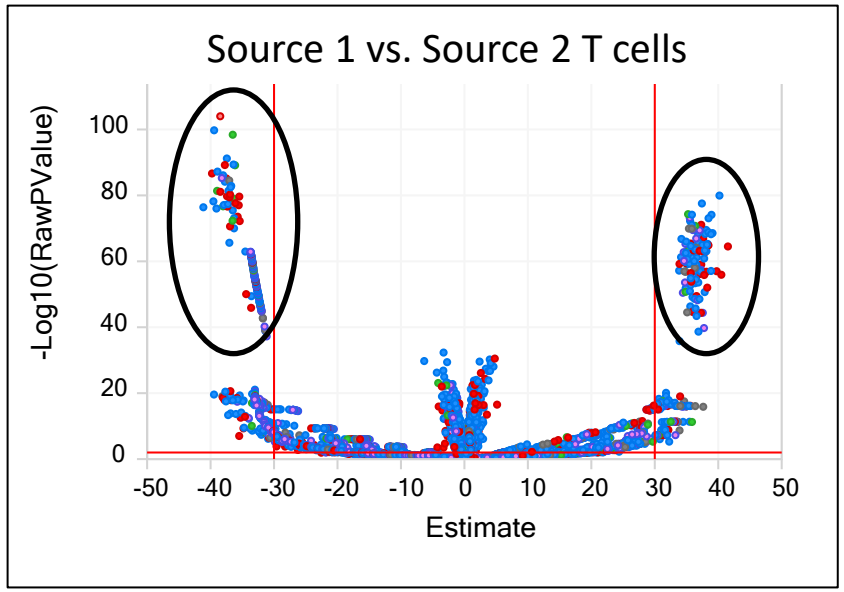


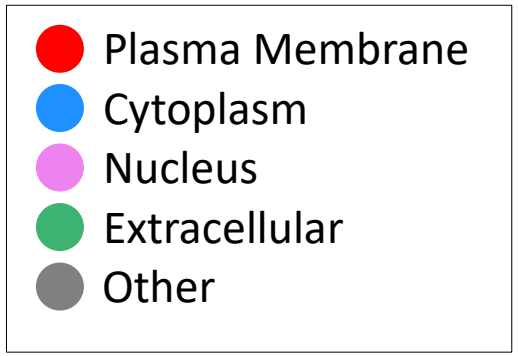


Proteomics analysis confirming expression of knock-in gene, highlighting plasma membrane protein expression differences





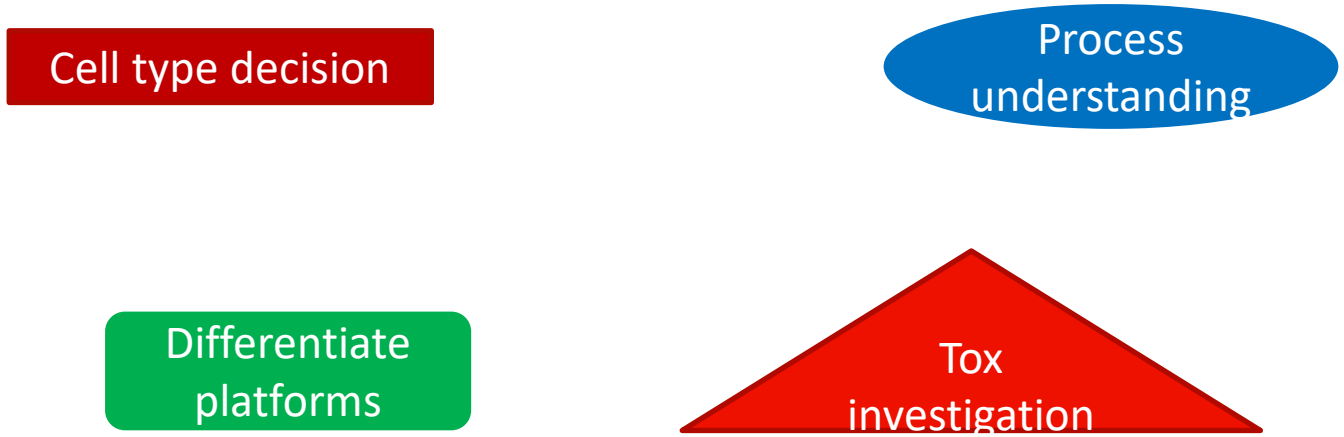




Conclusion

- 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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