Prospects for Proteomics by Data-Independent-Acquisition Mass Spectrometry to Advance the Characterization of CAR-T cell Therapies

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The role of NIST in advancing biomanufacturing

NIST Measurement Tools & Reference Materials are central to Innovation & Quality

Measure to Understand and Diagnose
- Cancer
- Genetic Disorders
- SARS COV-2

Measure to Accelerate & Improve Treatment Options
- mAbs
- Gene Therapy & Viral Vectors
- Cell Therapies
- RNA Vaccines

Innovation & Quality
What are CAR T-cells?

- Engineered T-cells to target circulating cancer cells
  - **Autologous**: T-cell from the patient
  - **Allogenic**: T-cell from a donor
- 5 FDA approved therapies (all autologous)

Opportunities and challenges for proteomics on CAR T-cells

- PK & PD evaluation
- Toxicity monitoring
- MoA

- Effect on CAR-T cell biology
- Success rate
- % of CAR expressing cells

- T-cell Purity
- T-cell MQAs

- Gene vector MQAs
- Effect on the T-cell biology
- Success rate of transfection
- % of CAR expressing cells

Lombard-Banek, C. & Schiel J.E., Molecules, 2020
Expanding MAM principles to CAR T-cell therapies

- Secreted
- 1 protein $\rightarrow$ 200 peptides
- Historical product knowledge
- Not limited in quantities
- NISTmAb as system suitability

- Live cell $\rightarrow$ Complex material extraction
- “Active ingredient” = membrane protein
- 10,000+ proteins $\rightarrow$ $10^6$ peptides
- Limited starting material
- No system suitability available
Protein quantification from SWATH® Acquisition

- Low number of missing values
- High dynamic range
- Provides a library for future targeted assays

Implementing DIA/SWATH-MS for Cell Therapies

1. Control Strategy

2. Generating PQPs

3. SWATH-MS Measurements
Establishing an instrument performance qualification

1. Control Strategy
Technical Variability and Devising Method Control

Peptide Mixture

Commercial complex digest (e.g., K562 digest)

Jurkat

Sample Preparation
- Protein extraction
- Protein modifications
- Enzymatic digestion

Separation
- Injection
- Separation performance
- Carry over

Data Processing
- Quantification
- Identifications
- Mass deviation
- Spray stability

Mass Spectrometry
- Dynamic range
- Sensitivity

NIST MATERIAL MEASUREMENT LABORATORY
Instrument and measurement control design scheme

- Use established extraction and digestion method
- # of protein ID/quant.
- Next Steps
  - Refine digest
  - Perform intermediate precision
  - Set control strategy

- Select protein quant.
- PepCal spike in quant.

K562 (D)
- # peptides ID
- # proteins ID

K562 (S)
Lombard-Banek, C., et al., in preparation, 2021
Some specific values for the simple: PepCal Mix

Lombard-Banek, C., et al., in preparation, 2021
Some specific values for SWATH® on K562

Lombard-Banek, C., et al., in preparation, 2021
Extracting information from SWATH-MS data

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2 Generating PQP Libraries
General workflow for SWATH-MS data interpretation

Platform agnostic WF

Compare

Global changes

Specific enrichment

MATERIAL MEASUREMENT LABORATORY

14
Protein identification and quantification from SWATH® data: Generation of PQPs

PeakView® Software
Example of SWATH-MS measurements for a CAR-T model
CAR-Jurkat model to advance analytics

1. Transduced to express anti-HER2 CAR (Gen 2)

2. Future application of this model:
   1. Pre/post transduction
   2. MQAs assessment
   3. In process samples for process development/monitoring
   4. PQAs identification and product control
Example of SWATH-MS measurements of CAR-Jurkat cells

- CAR

Log(Abundance)

Rank

NIST MATERIAL MEASUREMENT LABORATORY
Coverage of the CAR protein with targeted peak extraction

MALPVTALLLPAILLLHAARPDIQMTQSPSSLASSVGDRVTITCRASQDVNTAWAWYQQK
PGKAPKLILYSAFLYSGVPSRFSRGSRGTDFTLTTISSLQPEDFATYCQOHYTTPTFG
QTKEIKGGGGSGGGSGGGGSEVQLVE GGGLVQP GPSLRLSCAA SGFNKDTH HWV
ROAPGKGLEWVARITYPNGTGREYTRYADSVKRFTITسوTISNHTAYLOMNSSLRAEDTAVYCS
RWGGDGKYAMDYWGOGTLVTVSTTTPAPRPPTPAPTIAQPLSLRPEACRPAAAGAVTH
RGLDFACDFWVLVVGGVLACYSSLVTVAFIIFWVRSKRSSLHDYMNMTPRPPGPTRK
HYQPYAPRDFAAAYRSRVKFSR.SADAPAYQGQNQLYNELNLGRREEYDVLDRKGGGRPDPE
MGKKPRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRAGKHGHDGLYQGLSSTATKDTDYDAL
HMQALPPR

37.7% sequence coverage
Conclusions

1. We have established a measurement control strategy to collect large scale proteomic data for cell-based biotherapeutics

2. PQP-based data analysis is similar to MAM to extract information from RAW data

3. SWATH-MS measurements of CAR-Jurkat showed broad dynamic range and possibility to identify/quantify the CAR protein
   - Identification of the CAR protein with ~38% coverage from large scale data.

Disclaimer: Instruments identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology
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