#4163

Clinical Proteomics for Patient Stratification - A GCLP Quantitative Mass Spectrometry Workflow for Multiplexed Measurement of Protein Biomarkers in FFPE Tissues

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CAPRION

INTRODUCTION

- **Precision oncology and the shift towards biomarker-driven trial designs**
  - Biomarker profiling of individual patients may be useful in guiding patient selection
  - Identification of patients with the target biomarker profile may serve as an enrichment strategy for clinical trials
- **Antibody-based assays for biomarker measurement may not be suitable or available**
  - Lack of specific antibodies, insufficient characterization, false +/- signals, limited multiplexing, etc.
- **Mass spectrometry MRM assays for precise accurate quantitation of biomarkers, proteins of interest and novel markers can be quickly developed and validated**
  - Assay development based on knowledge of the target protein sequence(s)
  - Reliability of MRM biomarker measurements demonstrated through CPTAC analytical validation
- **Feasibility of adopting MRM biomarker assays in clinical trials**
  - Clinician-researchers require ad-hoc, 'sample analysis ready' biomarker panels, often within a short timeframe
  - Sample analysis must be performed in a GCLP environment

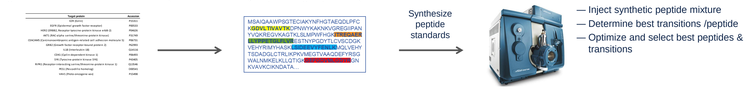
GOALS / OBJECTIVE

- **Demonstrate rapid assay development and analytical validation of up to 12-plex cancer protein biomarker panels in FFPE tissue**
- **Generate robust sample analysis protocols for accurate and precise MRM analysis of limiting amounts of clinical FFPE tissue**
- **Perform clinical sample analysis in a GCLP compliant environment**

METHODS

1. Assay Development

- List of proteins
  (e.g. sci literature, 'omics' studies)
- Select surrogate peptides
  (≤5 per target protein)
- MRM assay development



Synthesize peptide standards

- Inject synthetic peptide mixture
- Determine best transitions /peptide
- Optimize and select best peptides & transitions

2. Pre-validation in FFPE Tissue

- Assay Performance Assessment
- Client-Specific SOP
- Assay Validation Plan
- QA audit of SOP and Validation Plan
- Analyst Qualification

3. Assay Validation in FFPE Tissue

CPTAC Guidelines:

1. Response curve
2. Repeatability
3. Selectivity
4. Stability
5. Reproducible endogenous detection

QA Audit:

1. In-life audit
2. Raw data & summary tables
3. Validation report

CPTAC GUIDELINES

4. Clinical Sample Analysis

Reference slide
— H&E evaluation
— Marking of tumor area

Sequential section(s)
— Deparaffinization
— Dissection of tumor areas

Removal of cross-links
— High temperature cleavage of methylene bridges and protein denaturation

Homogenization
— Rapigest buffer
— Probe sonicator

Total protein yield
— BCA assay
— 4 µm→ ~1 to 2 µg/mm²
— 10 µm→ ~2 to 5 µg/mm²

Digestion to peptides
(Trypsin enzyme)

Desalting
(MCX)

Mass Spectrometry

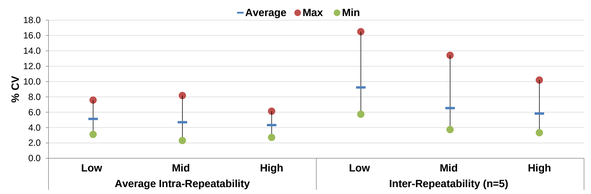
RESULTS 1: Analytical Validation of a 12-plex MRM Assay

CPTAC Experiment 1: Response Curves

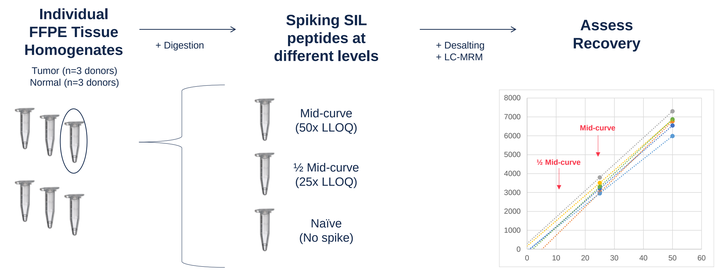
- Sensitivity: LOQ = 0.132 to 1.013 fmol/µg FFPE total protein
- Linearity: r-score > 0.99 (for all curves); Median = 0.9988

CPTAC Experiment 2: Repeatability, 5 Different Days

- Total variability = 20 % criteria met for all peptides



CPTAC Experiment 3: Selectivity



- % Difference between mid-curve/2 and ½ mid curve: ≤ 11.4%
- % Difference in slope: ≤ 10.9%. Exception: SDLVNEEATGQFR (CEACAM5) in 1 donor was 23.5%

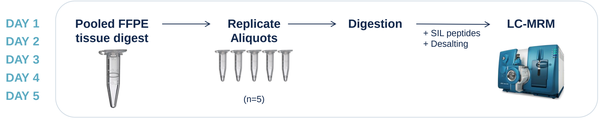
Illustration adapted from CPTAC guidelines. https://proteomics.cancer.gov/sites/default/files/assay-characterization-guidance-document.pdf

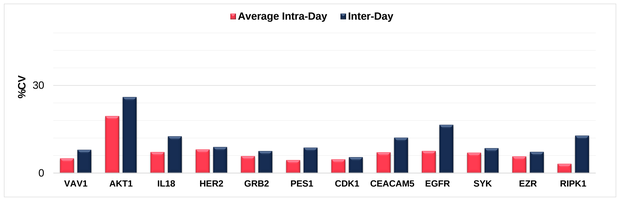
CPTAC Experiment 4: Stability

- All peptides were stable (Wet for 48 h; Dry extract: 2X freeze-thaw, 14 d at -20 °C)

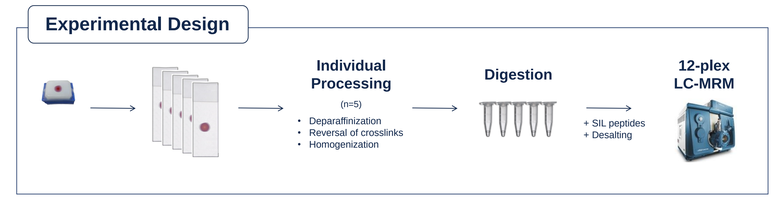
CPTAC Experiment 5: Reproducibility of Endogenous Detection

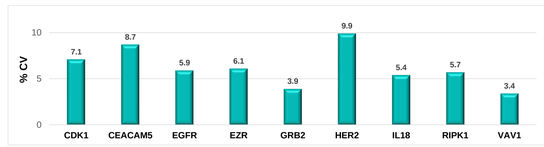
- Median Intra-Day CV 6.5 %; Inter-Day CV: 12.8 %





RESULTS 2: Assay Reproducibility for Endogenous Levels in FFPE

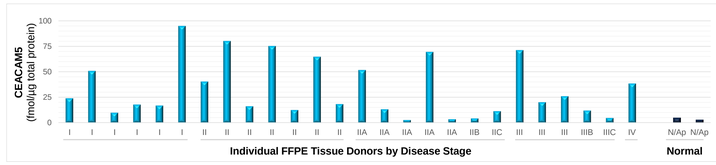




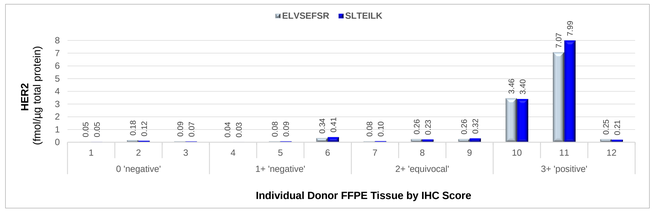
* Not shown: Target biomarker proteins with endogenous levels <LOQ

RESULTS 3: MRM Biomarker Measurements vs Disease Stage

[A] Variability of CEACAM5 in CRC I to IV

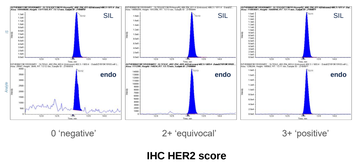


[B] HER2 Concentration by MRM vs IHC Score



* HER2 was detected using both peptides in all 12 study samples with a S/N > 5

- Representative MRM signal quality in FFPE tissues, peptide SLTEIK



SUMMARY / CONCLUSIONS

- **Precise accurate multiplexed quantitation of clinically relevant biomarkers in limiting amounts of FFPE tissue was demonstrated using a custom 12-plex biomarker panel validated to CPTAC guidelines**
- **GCLP-compliant quantitative multiplexed clinical analysis of protein biomarkers by MRM-MS in FFPE tissue is feasible**
- **Approach can be used for patient stratification, optimization of treatment outcomes, drug resistance prediction, and to support clinical development of novel therapies**

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