Multiplexed Protein and RNA Quantification on a Single Instrument Harmonizes Multi-omic Analyses of Biomarkers for Immunotherapies and Targeted Therapies in Non-Small Cell Lung Cancer

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SUMMARY

- Actionable biomarkers for lung cancer span DNA, RNA, and proteins yet no single technology currently exists that can quantify these analytes in a single workstream.
- Using the Simple Western™ system, we multiplexed, resolved, and co-quantified protein and mRNA from EML4-ALK fusions, PD-L1, and reference genes in cell lines and formalin-fixed paraffin-embedded (FFPE) tumor biopsies.
- The relative mRNA expression levels measured by Simple Western quantitatively agreed with gold standard qPCR analysis.
- This multi-omic technology has the potential to improve diagnosis and treatment in NSCLC, where RNA and DNA variants and protein expression are established in practice guidelines for targeted and immuno-therapies, and in other disorders.

INTRODUCTION

Multi-omic approaches can combine protein, DNA, and RNA analyses to elucidate diagnostic biomarkers and pathways, advancing our understanding of complex diseases. These assays, however, require different technologies and platforms to resolve the distinct physico-chemistries of protein and DNA/RNA. In contrast, single-platform quantification of proteins and nucleic acid markers offers many potential benefits, including reduced sample requirements, decreased inter-assay variability, streamlined and less error-prone workflows, and integrated results reporting.

Here we demonstrate expanded capabilities of an established protein analysis system (Simple Western, ProteinSimple®) to characterize nucleic acids, and show that this system can quantify oncogenic tyrosine kinases, immune checkpoint proteins, RNA translocations, and other mRNA transcripts associated with targeted or immune-based therapies for non-small cell lung cancer (NSCLC).

MATERIALS AND METHODS

The proteogenetic workflow includes the following steps: 1) Isolation of protein and DNA or RNA from cell lines or residual clinical FFPE tissue biopsies; 2) Generation of hapten-labeled amplicons from mRNA using single-tube multiplex RT-PCR; and 3) Co-injection of labeled amplicons and corresponding proteins on a Simple Western instrument, a capillary electrophoresis immunoassay system. This approach enables protein-specific immunoassays and, as shown here, nucleic acid sizing and quantification. Simple Western analysis of nucleic acids was compared to capillary electrophoresis (CE) using a 3500 Genetic Analyzer (Thermo Fisher) and 2100 Bioanalyzer (Agilent) along with qPCR using a 7500 Real-Time PCR system (Thermo Fisher).

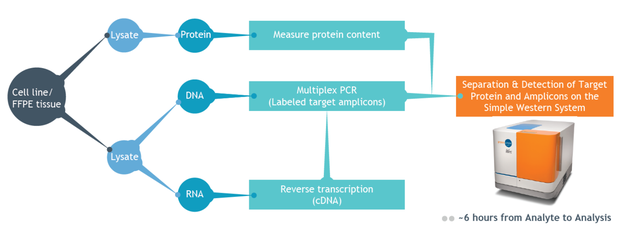

Figure 1. Study Design and Workflow for Co-detection of Nucleic Acids and Protein on the Simple Western System.
Nucleic acids and protein were isolated from lysates of cell lines or FFPE tissue specimens. PCR amplicons were hapten-labeled using multiplex RT-PCR followed by co-injection of labeled amplicons and proteins into a single capillary for detection and quantification on the Simple Western system.

†Research Collaboration Project

RESULTS

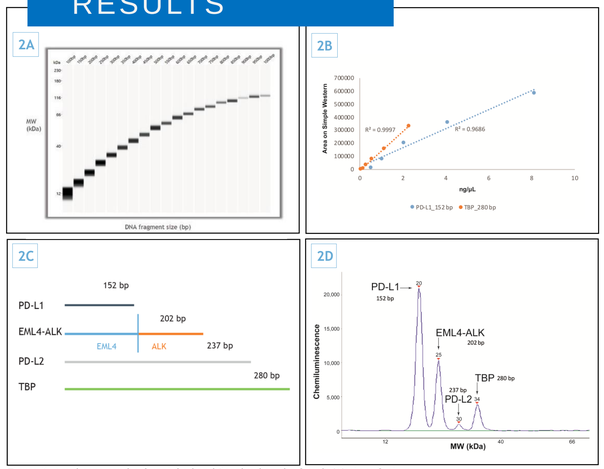

Figure 2. Amplicons can be Quantified and Resolved on the Simple Western System.
A) DNA fragments were separated over a range of 100-1000 bp. **B)** The input range for detection of 152 bp and 280 bp PCR amplicons spanned approximately two orders of magnitude. **C)** Amplicon lengths for PD-L1, EML4-ALK, PD-L2 and TBP transcripts ranged from 152 to 280 bp. **D)** Amplicons shown in C) were resolved within at least 35 bp by Simple Western using standard conditions.

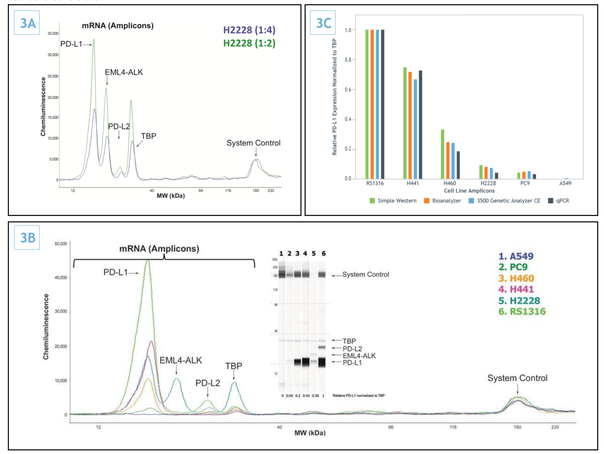

Figure 3. 4-Plex RT-PCR and Simple Western Quantification of Lung Cancer-associated Transcripts.
Lung cancer RNA fusions and other transcripts can be multiplexed and quantified in a dose-dependent manner using a reference gene (TBP). **A)** Quantification of EML4-ALK fusion, PD-L1, PD-L2, and TBP transcripts in NSCLC cell line H2228. **B)** Simple Western results for PD-L1 mRNA quantification of cell lines with known differences in expression were consistent with their underlying abundance as reported in literature[1,2]: A549 < PC9 < H460 < H441. Note that elevated TBP expression was observed in the H2228 cell line; this result is consistent with reports from RNA-seq in databases such as Expression Atlas[3]. **C)** Relative expression levels of PD-L1 on Simple Western for all cell lines were consistent with orthogonal platforms (2100 Bioanalyzer, 3500 Genetic Analyzer and 7500 Real-Time PCR) when normalized to TBP[4]. For each platform, the same RT product and input was used for PCR. Similar trends in PD-L1 expression were observed when the data were normalized to total RNA input.

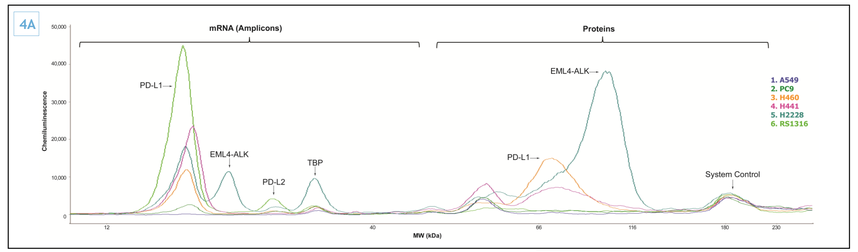

Figure 4. 6-Plex RNA and Protein NSCLC Biomarkers can be Resolved and Quantified in a Single Capillary.
A) Proteogenetic characterization across six different cell lines. Protein levels were consistent with independent literature reports. [5-9] **B)** Proteogenetic characterization across five different FFPE biopsies. Of note, FFPE 1, 3, 4 and 5 were of demonstrably poorer RNA quality compared to FFPE 2. **C)** PD-L1 mRNA expression quantification was consistent across different CE platforms and qPCR. The same RT product was used for each platform comparison. All data were normalized to total RNA only due to extremely low TBP expression in select samples. Note that PD-L1 mRNA expression was highest for FFPE 2, in agreement with PD-L1 protein expression (see 4B).

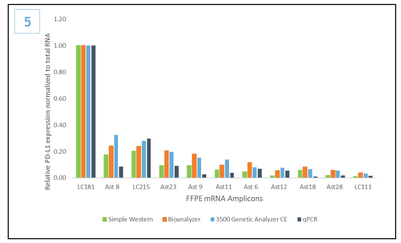

Figure 5. Relative expression levels of PD-L1 on Simple Western for 11 additional FFPE amplicon samples.
Results were compared across orthogonal platforms (2100 Bioanalyzer, 3500 Genetic Analyzer CE and 7500 Real-Time PCR) when normalized to total RNA. Of note, qPCR relative quantification was within two-fold of the value from Simple Western for 7 of 11 samples.

CONCLUSIONS

- Originally developed for protein analysis, Simple Western can separate and quantify nucleic acids. Using this approach, transcript levels were shown to be consistent with results from three orthogonal platforms including two CE systems and qPCR.
- A novel proteogenetic workflow enabled the analyses of at least six different NSCLC RNA and protein biomarkers using a single capillary on a Simple Western system.
- PD-L1 mRNA expression and, separately, PD-L1 protein abundance in well-characterized cancer cell lines were consistent with literature reports. However, matched comparisons of relative PD-L1 mRNA and protein expression were discrepant in several cases. These differences may reflect post-translational regulation of gene expression.
- Futures studies will complete the analysis of the larger FFPE sample set, in order to expand the correlation analysis of the mRNA expression and protein abundance.
- This multi-omic technology may improve the integration of different classes of NSCLC biomarkers, as well as in other disorders where combinations of RNA, DNA and protein biomarkers can accelerate precision medicine.

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