CHARACTERIZATION OF A DATA INDEPENDENT ACQUISITION MASS SPECTROMETRY-BASED WORKFLOW IN PLASMA

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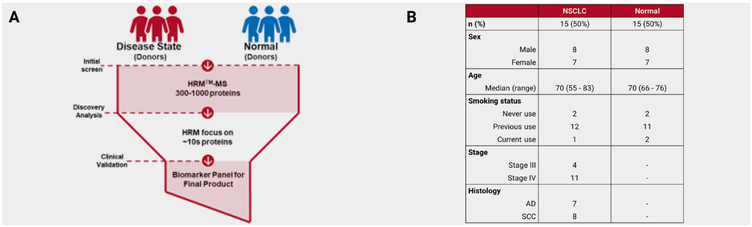
INTRODUCTION

Measurement of circulating biomarkers in cancer has proven utility for early detection, differential diagnosis, and predicting pre-treatment response to therapy. Recently, circulating proteomic biomarkers have received additional attention due to the heterogeneous responses to immunotherapies. To develop a greater understanding of the circulating plasma proteome we have optimized a depleted plasma proteomic workflow, based on label-free data-independent acquisition mass spectrometry (DIA-MS), and applied it to plasma from subjects with late stage NSCLC. This unbiased approach identified proteins of potential diagnostic value for lung cancer.

Figure 1: Outline of study design and rational (A) and details of sample set used (B)



B

	NSCLC	Normal
n (%)	15 (50%)	15 (50%)
Sex		
Male	8	8
Female	7	7
Age		
Median (range)	70 (55 - 83)	70 (66 - 76)
Smoking status		
Never use	2	2
Previous use	12	11
Current use	1	2
Stage		
Stage III	4	-
Stage IV	11	-
Histology		
AD	7	-
SCC	8	-

CONCLUSIONS

Hyper Reaction Monitoring Mass Spectrometry (HRM™-MS) provided unbiased characterization of plasma proteome:

- No depletion >410 proteins, with depletion >1300 proteins quantified across all samples.

Multiple potential biomarkers are identified to be dis-regulated in NSCLC vs Normal subjects:

- Multivariate analysis separated diagnostic subgroups based on proteomic signature.
- 25 most variable proteins appeared to be linked to host immune response to tumor.
- Univariate tests revealed 162 significantly dis-regulated proteins, while functional analysis showed enrichment in acute phase response.
- ROC analysis of top candidates showed diagnostic value of proteins linked to acute phase response (**CRP, C9, SAA1/2 and HPT**), immunomodulation (**S100A8/9**), signaling and metastasis (**LRG1**), and nutrition (**TTR**).

RESULTS

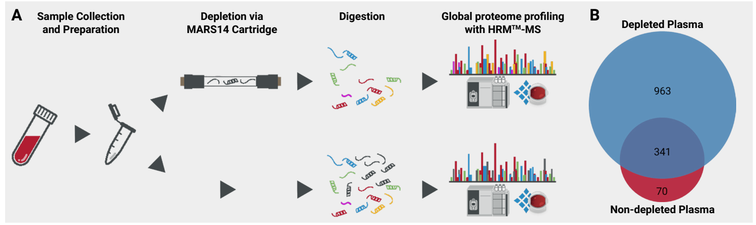
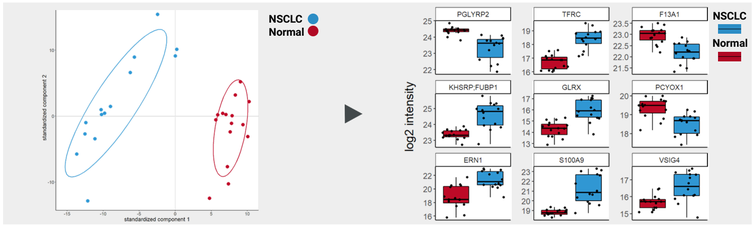


Figure 2: Overview of Assay Workflow (A) and Depletion Optimization (B)
(**A**) Use of commercial cartridges (MARS 14) allows for a standardized and reproducible sample depletion. (**B**) Subsequent DIA acquisition of samples after depletion allowed for quantification of 1,304 proteins (reference library had 1,827 unique spectra). An improvement of >3 times more proteins quantified than without depletion.

Figure 3: Multivariate Analysis of Proteomic Signature in Normal and NSCLC Subjects
Multivariate analysis based on Partial Least Squares Discriminant Analysis (PLS-DA) was able to separate both subject groups. Large variability within disease profiles is observed. Selected proteins from top 25 with biggest effect on the separation are depicted including potential new biomarker candidates like **F13A1**[1] involved in macrophage activation, and known targets with immunomodulatory function such as **S100A9**[2].



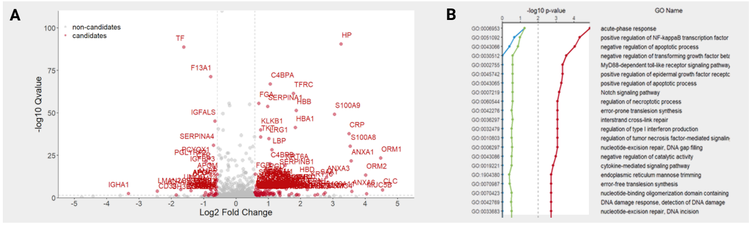
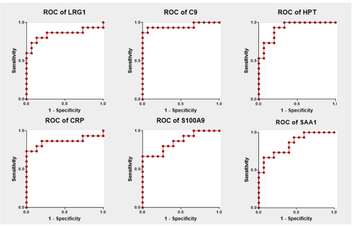


Figure 4: Significantly disregulated proteins (A) and enrichment of biological processes (B)
(**A**) Univariate statistical testing identified 162 dis-regulated proteins (125 up-regulated and 37 down-regulated; q-value > 0.05, log2 fold change > 0.58). (**B**) Analysis of biological processes of all disregulated proteins showed highest enrichment in acute phase reactions followed by other immune responses (including complement pathway).

Figure 5: Receiver Operating Characteristic (ROC) curve (based on d_{min} between 0 and 1)
Potential diagnostic power was evaluated for some of top dis-regulated proteins. Among these acute phase response proteins **SAA1/2**[6,9], **C9**[3], **CRP**[4,7] and **HPT**[4] have been reported as potential prognostic biomarkers for NSCLC. In case of **C9**[3] and **SAA1/2**[9] *in vitro* studies showed their role in macrophage regulation. Calcium binding proteins **S100A8/9**[2], known targets with immunomodulatory effects in NSCLC subjects, as well as **LRG1**[5] which is highly expressed in tumor derived exosomes and linked to metastasis, together with **TTR**[8] (not shown), a nutritional biomarker were also found to have a good diagnostic value.



References: **1.**Kubach et al., Blood 2007, 110:1550-1558 **2.**Shen et al., Cancer Immunol Res. 2015, 3(2):136-148 **3.**Li et al., Cell Death Discov. 2018, 4:63 **4.**Calabrese et al., J. Clin. Med. 2019, 8:414 **5.**Alipoor et al., Front. Immunol. 2018, 9:819 **6.**Liu et al., Biomed Res. Int. 2018, 9815806 **7.**Walker et al., EBioMedicine, 2015, 2:841–850 **8.**Shimura et al., Mol. and Clin Onc. 2019, 10:597-604 **9.**Dakubo et al., 1st ed., Springer, 2017