Deep plasma proteome profiling to discover drug treatment related novel biomarkers in non-small cell lung cancer

Cui Liu[1]; Dongsheng Yue[2]; Bin Zhang[2]; Ruiqi Huang[3]; Wenting Du[4]; Xiaopeng Ma[1]; Cunjing Yu[1]; Wenhao Li[1]; Xikun Wu[3]; Yongchun Tong[1]; Lanjun Xu[1]; Si Mou[1,*]; Changli Wang[2,*]; Zhirong Shen[1,*]

[1]Department of TDRM, BeiGene (Beijing) Co. Ltd. [3]Department of GSDS, BeiGene (Shanghai) Biotechnology Co. Ltd. [4]Department of TDRM, BeiGene (Shanghai) Biotechnology Co. Ltd.
[2]Department of Lung Cancer, Tianjin Lung Cancer Center, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin's Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China
[*]correspondence: zhirong.shen@beigene.com; wangchangli@tjmuch.com; si.mou@beigene.com

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Introduction

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of various cancers. A better understanding of ICI induced protein dynamics and response / resistance mechanisms may contribute to the optimization of treatment strategies.

In early-stage non-small cell lung cancer (NSCLC) cohort with neoadjuvant chemoimmunotherapy, we utilized the plasma proteomics to deepen the understanding of disease biology and explore potential response and resistance biomarkers. 180 plasma samples from 92 individuals were profiled, and in total 4244 blood protein were identified. 2303 proteins were analyzed, of which 103 upregulated proteins and 77 downregulated proteins were observed in both treatment arms post neoadjuvant therapy. Pathway enrichment of altered proteins revealed that neoadjuvant chemoimmunotherapy promoted immune activation significantly.

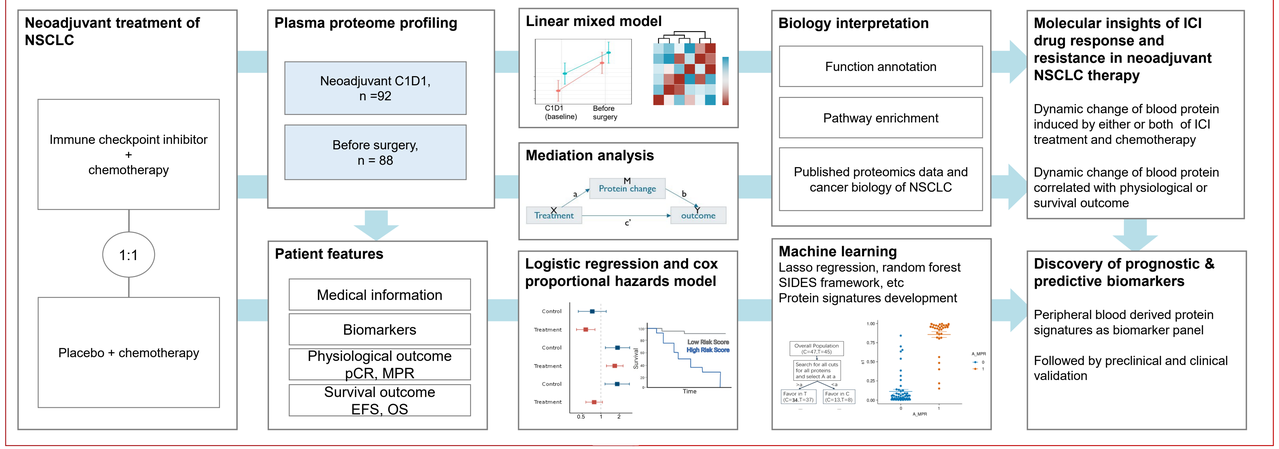
The study reveals the reliability and capability of large-scale and unbiased plasma proteome profiling in understanding the dynamic and mechanism of immune checkpoint inhibitor therapy in oncology.

Study design

Retrospective plasma samples were analyzed at two sampling time, the neoadjuvant C1D1 (baseline) and before surgery. ICI drug treatment and Placebo were allocated at ratio 1 : 1.

A comprehensive statistical analysis framework was adapted to gain molecular insights of biomedical mechanisms and to explore baseline predictive and prognostic biomarkers for neoadjuvant therapy. Alterations in blood protein levels were summarized into different dynamic patterns under chemotherapy with or without ICI. Furthermore, we investigated the pathways from treatment to clinical efficacy outcomes through possible protein markers' changes. Machine learning approaches were used to promote multi-variable modeling with advancement in controlling for overfitting and multiplicities. All data-driven findings required the interpretation for their biological functionalities, and validations in published NSCLC studies or by orthogonal techniques (**Figure 1**).

Figure 1. Statistical analysis framework for exploring predictive and prognostic biomarkers and understand drug response



Clinical plasma proteomics platform

An unbiased mass spectrometry-based plasma proteomics platform enable large-scale proteomic measurement of blood proteins of clinical patient cohort (**Figure 2**), revealing dynamics of immuno- and cancer-related proteins in circulation (**Figure 3**).

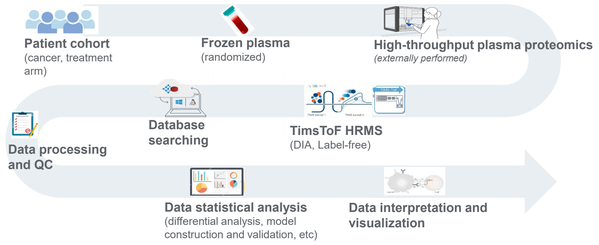


Figure 2. Schematic workflow of clinical plasma proteomics

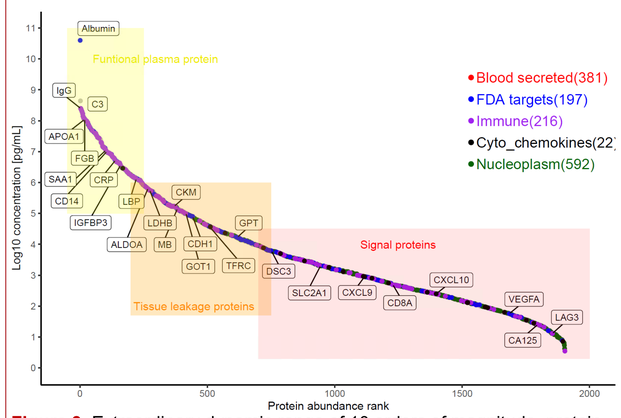


Figure 3. Extraordinary dynamic range of 10 orders of magnitude, protein were ranked on their concentrations matched with those of healthy individuals provided by HPA

Data consistency with medical features

Inflammatory biomarker CRP elevated in line with the neutrophil counts in the respective patients, demonstrating the plasma proteome relative-quantitative measurement are correlated with the physiological functions (**Figure 4a**). The quantitative abundance of gender-specific proteins are concordant with the gender of each patient (**Figure 4b**).

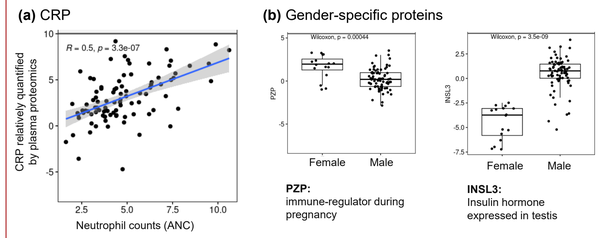


Figure 4. proteomics measurement are consistent with established technique or gender characteristics

Neoadjuvant therapies activate immune response

CXCL9 and CXCL10 were two representative chemokines released to peripheral blood upon neoadjuvant therapy, indicating immune cell migration, differentiation, and activation. A more significant increase in chemokines was observed in the patient group receiving ICI treatment (**Figure 5c**).

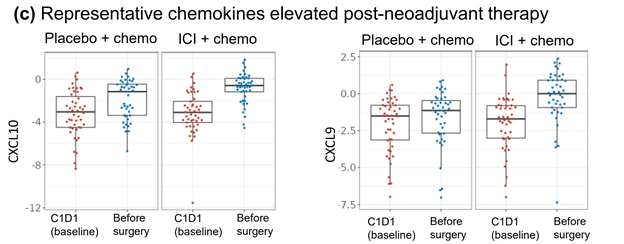
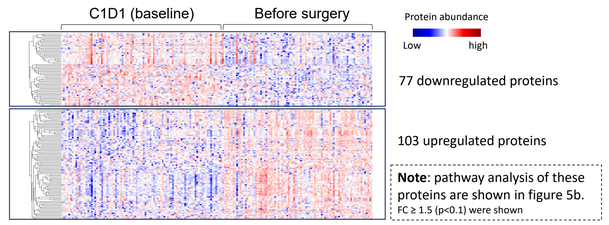


Figure 5. Differentially expressed blood proteins and their involved pathways post neoadjuvant treatment

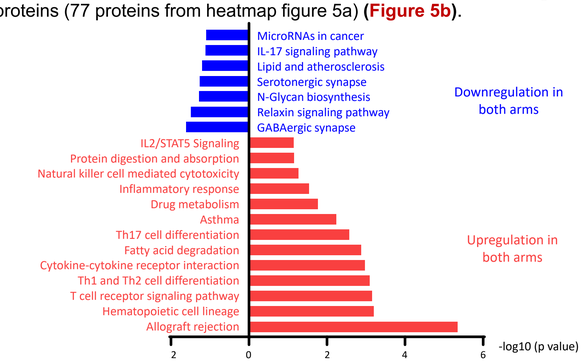
Neoadjuvant therapies activate immune response

We investigated the differentially expressed blood proteins between neoadjuvant C1D1 (baseline) and before surgery, in patients undergoing neoadjuvant chemotherapy with or without ICI immunotherapy. In both arms, 103 upregulated and 77 downregulated proteins were observed post treatment (**Figure 5a**).



(a) Unsupervised hierarchical clustering analysis of upregulated and downregulated proteins in both neoadjuvant treatment arms

In both arms, pathways of T cell activation and differentiation, cytokine receptor signaling and cellular cytotoxicity were significantly enriched for upregulated proteins (103 proteins from heatmap figure 5a). Functions relevant to tumorigenesis and metastasis, were enriched for downregulated proteins (77 proteins from heatmap figure 5a) (**Figure 5b**).



(b) Biological functions altered in both neoadjuvant treatment arms

Association of peripheral plasma with TME

Signatures of plasma proteome were calculated and associated to a tumor microenvironment (TME) indicator. The indicator was evaluated in tumor tissue and categorized into 3 expression levels. High level of TME indicator (score > 50) in tumor was associated with higher level of exhausted T cell signature in circulation, and lower level of extracellular matrix (**Figure 6**). This suggested the association of peripheral plasma proteome with TME.

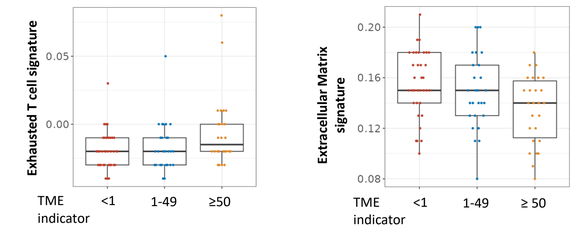


Figure 6. Peripheral levels of exhausted T cells and extracellular matrix in subgroups of patients stratified by a TME indicator

Conclusions

A clinical plasma proteomics platform enables the detection of changes in blood protein abundance with extensive coverage, reliable quality, and no bias.

Clinical plasma proteome analysis of neoadjuvant treatment for NSCLC reveal that the neoadjuvant therapy leads to immune activation, and more significant activation was observed when applying chemotherapy together with ICI drugs. Further analyses are anticipated to explore the dynamics and mechanisms underlying NSCLC neoadjuvant therapy.

Plasma proteome is associated with the tumor microenvironment, assessed by linking a tumor microenvironment indicator to functional signatures in blood.

In perspective, in-depth proteomic analysis of patient plasma is advantageous for identifying biomarkers in peripheral blood, which can indicate ICI drug responsiveness and aid in patient stratification.

Reference
Tognetti, Marco et al. "Biomarker Candidates for Tumors Identified from Deep-Profiled Plasma Stem Predominantly from the Low Abundant Area." *Journal of proteome research*, vol. 21,7 (2022): 1718-1735.