Plasma Proteomics as a Systemic Monitoring Approach in NSCLC Immunotherapy: Comparative Analysis with ctDNA

Yehuda Brody[1], Michal Harel[1], Yehonatan Elon[1], Shani Raveh Shoval[1], Adam P. Dicker[2], Young Kwang Chae[3], David R. Gandara[4], Ronan J. Kelly[5]

(1) OncoHost LTD, Binyamina, Israel; (2) Thomas Jefferson University, Philadelphia PA, USA; (3) Northwestern University, Robert H Lurie Comprehensive Cancer Center, Chicago IL, USA; (4) University of California Davis Comprehensive Cancer Center, Sacramento, USA; (5) Baylor University Medical Center Charles A. Sammons Cancer Center, Dallas TX, USA

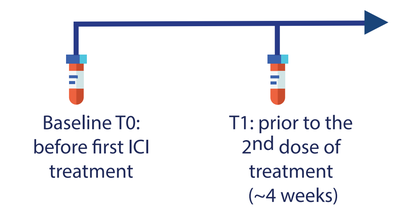
1 Background

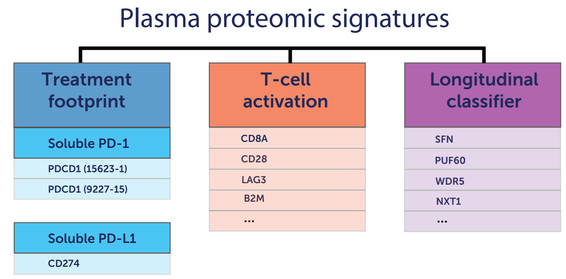
Immune checkpoint inhibitors (ICIs) have revolutionized NSCLC management, yet patient responses remain heterogeneous, with many developing primary or acquired resistance. Real-time, minimally invasive monitoring is essential for optimizing immunotherapy outcomes in cancer treatment. While circulating tumor DNA (ctDNA) provides a direct molecular measure of tumor burden, plasma proteomics offers complementary systemic insights into tumor biology, immune activation, and other treatment dynamics.

There is an unmet need for real-time, minimally-invasive tools capable of dynamically monitoring immunotherapy response and resistance. Plasma proteomics offers a promising avenue to address this challenge.

2 Proteomics signature generation

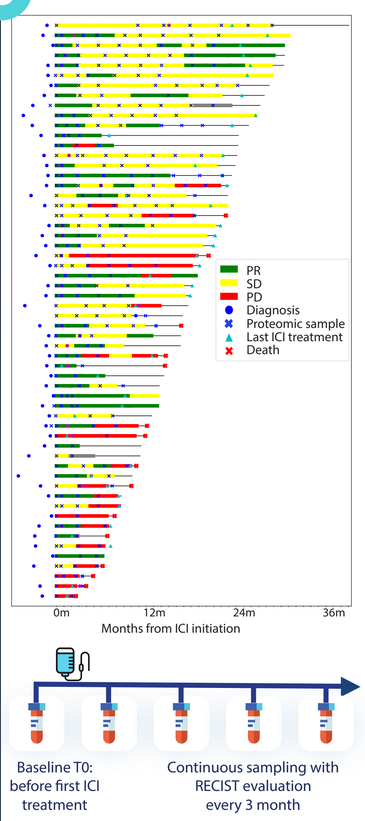
In a previous study (Bar et al., 2024), we identified three plasma proteomic signatures linked to drug engagement, T-cell activation, and other treatment dynamics in metastatic NSCLC patients treated with PD-(L)1 inhibitors. The longitudinal classifier signature, derived by aptamer-based profiling of paired pre- and early on-treatment samples (n = 225), emerged as a key marker of treatment dynamics and clinical outcome.





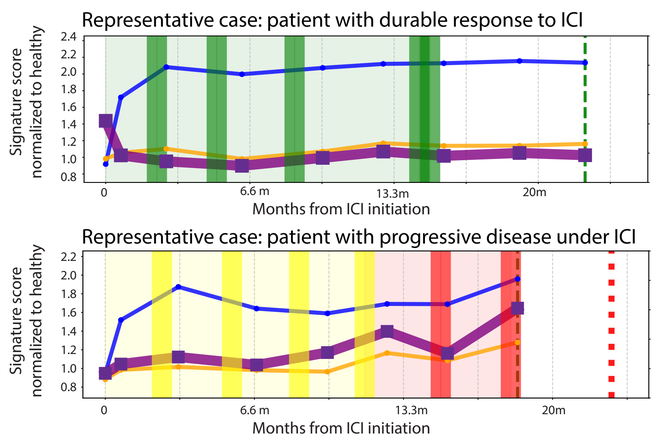
Bar J. et al. Front. Immunol, 2024

3 Monitoring NSCLC immunotherapy response using plasma proteomic signatures



We evaluated the three signatures in an independent longitudinal NSCLC cohort (n = 57) treated with anti–PD-(L)1 therapy. Plasma samples were collected before treatment and every three months, alongside imaging-based response assessments.

Parameters		n(%)
n		57 (100)
Treatment	ICI + Chemo	34 (60)
	ICI	22 (39)
	IPI Nivo	1 (2)
Sex	Male	34 (60)
	Female	23 (40)
ECOG	0-1	43 (75)
	≥2	6 (10)
	NA	8 (14)
Histology	Adenocarcinoma	40 (70)
	Squamous cell carcinoma	11 (19)
	Other	6 (11)



Among 13 patients meeting criteria for matched plasma and clinical evaluations, proteomic dynamics enabled early detection of treatment response or progression—on average 200 days (~6.5 months) before radiological assessment (range: 123–277 days).

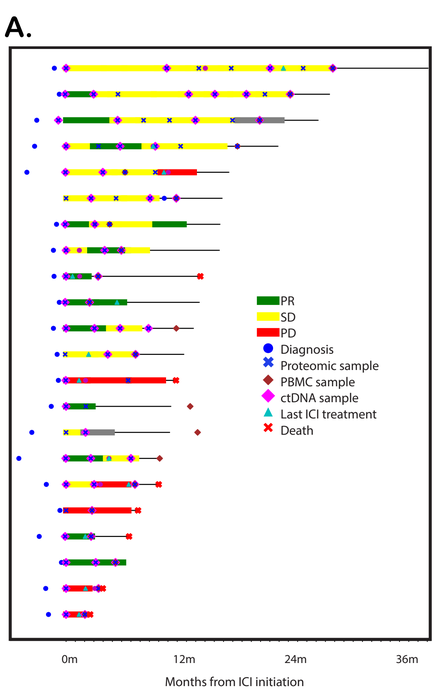
Signature scores were calculated as the mean z-score of constituent proteins and normalized to a healthy-control baseline (n = 30).

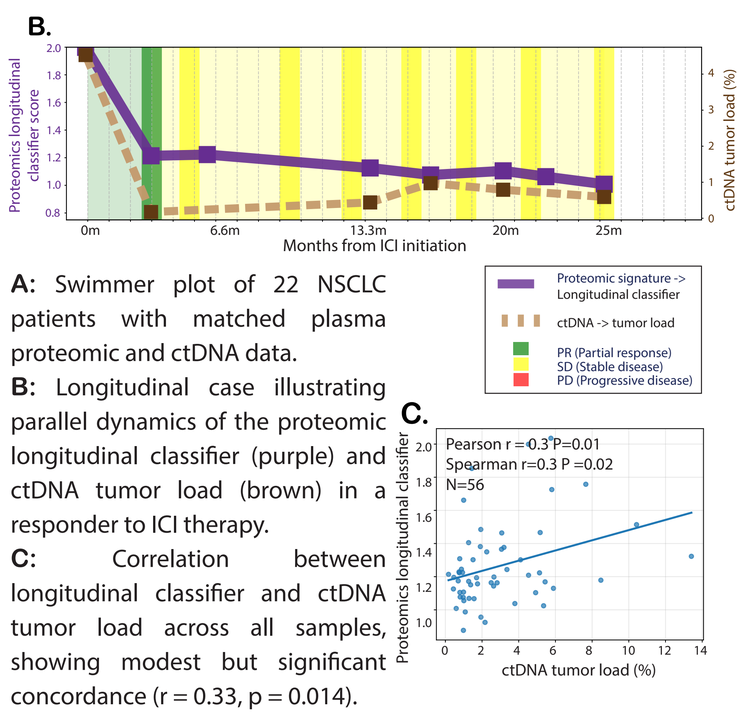
Representative NSCLC monitoring cases:

- Early rise in the soluble PD-1 signature reflects drug engagement and bioavailability.
- The T-cell activation signature emerges at treatment initiation.
- The longitudinal classifier distinguishes clinical outcomes:
  - Responders: sustained decline consistent with tumor regression.
  - Non-responders: delayed increase anticipating RECIST-defined progression.

4 ctDNA vs plasma proteomics (matched subset)

In a subset of 22 patients (56 matched samples), ctDNA analysis was conducted alongside proteomic profiling. cfDNA was extracted from plasma with paired PBMC gDNA controls. Tumor load, defined as aggregate variant allele frequency (VAF), showed a modest but significant correlation with the proteomic longitudinal classifier, reflecting partial concordance between tumor burden and systemic signals.





A: Swimmer plot of 22 NSCLC patients with matched plasma proteomic and ctDNA data.

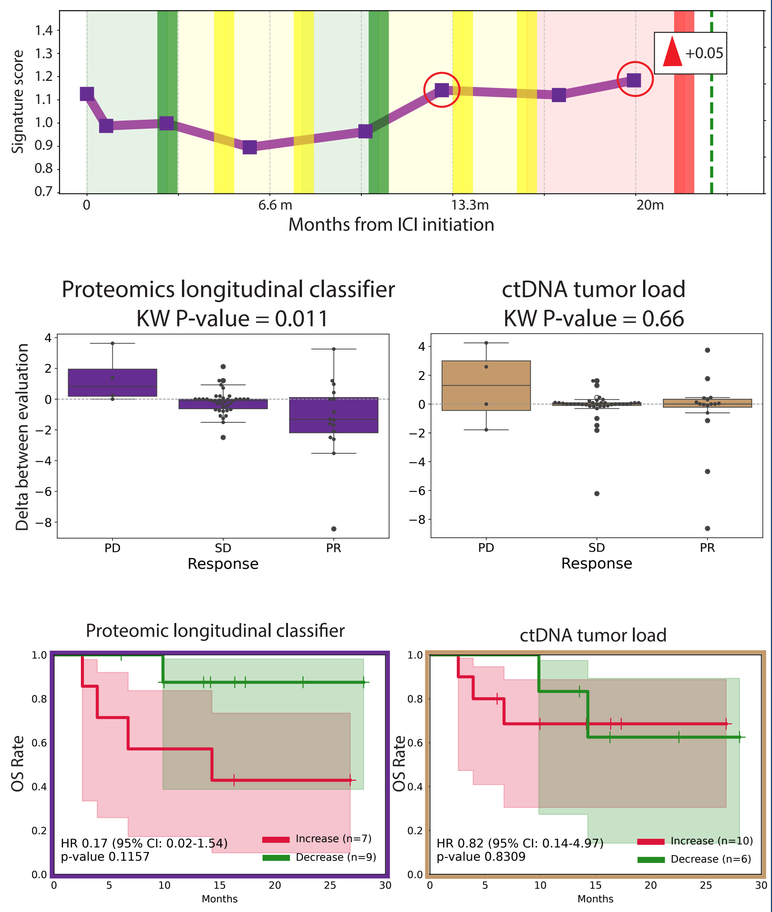
B: Longitudinal case illustrating parallel dynamics of the proteomic longitudinal classifier (purple) and ctDNA tumor load (brown) in a responder to ICI therapy.

C: Correlation between longitudinal classifier and ctDNA tumor load across all samples, showing modest but significant concordance (r = 0.33, p = 0.014).

5 Plasma proteomic-based longitudinal classifier shows significant agreement with RECIST evaluation

Agreement between molecular and radiographic responses was evaluated using the Kruskal–Wallis rank-sum test, comparing the observed change in each signal (Δ signature or ΔctDNA tumor load) with the direction of response defined by RECIST categories. A significant result indicates concordance between plasma-based proteomic longitudinal classifier dynamics and imaging outcomes.

In 16 patients with paired pre- and on-treatment samples containing both proteomic and ctDNA data, Kaplan–Meier analysis showed that a decrease trend in the longitudinal classifier was associated with longer overall survival (proteomics: HR = 0.17; p = 0.12; ctDNA: HR = 0.82; p = 0.8).



6 Conclusions

- Plasma proteomics enables real-time monitoring of NSCLC patients treated with immune checkpoint inhibitors.
- The longitudinal classifier allows early detection of non-response, preceding standard imaging.
- Integrating proteomic and ctDNA analyses may provide a more comprehensive framework for precision immunotherapy management.

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