Development and characterization of a cytokine-based classifier to predict response to immunotherapy using an ex vivo live tumor fragment platform

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Abstract #54

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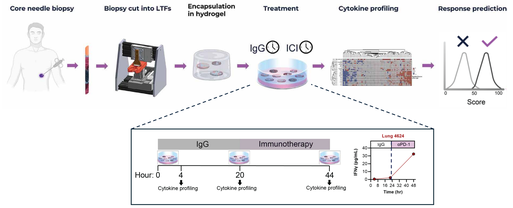
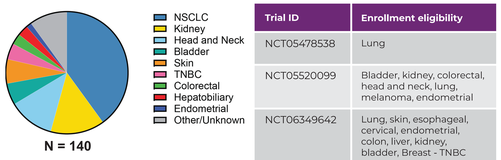
Introduction

- Existing biomarkers for immune checkpoint inhibitor (ICI) response are inaccurate, resulting in ineffective treatment
- A landmark 2021 paper showed the feasibility of accurately predicting ICI response by profiling cytokines from live tumor fragments (LTFs) treated ex vivo with ICI (Voabil et al. 2021)[1]
- However, this approach requires large amounts of tissue to mitigate tumor heterogeneity, making the use of standard of care core needle biopsies (CNBs) challenging
- We developed a novel sequential treatment method that mitigates this challenge, enabling the use of CNBs (Ramasubramanian et al. 2025)[2]
- Clinical data from patients enrolled in 3 observational trials across 14 sites were used to demonstrate, for the first time, the feasibility of accurately predicting ICI response using a single CNB

Methods



The Elephas platform measures cytokine response to ICI in LTFs generated from CNBs.[2] A sequential treatment strategy (black box) in which IgG control followed by ICI is added to the same tissue in a single well, mitigates the challenge of tumor heterogeneity.

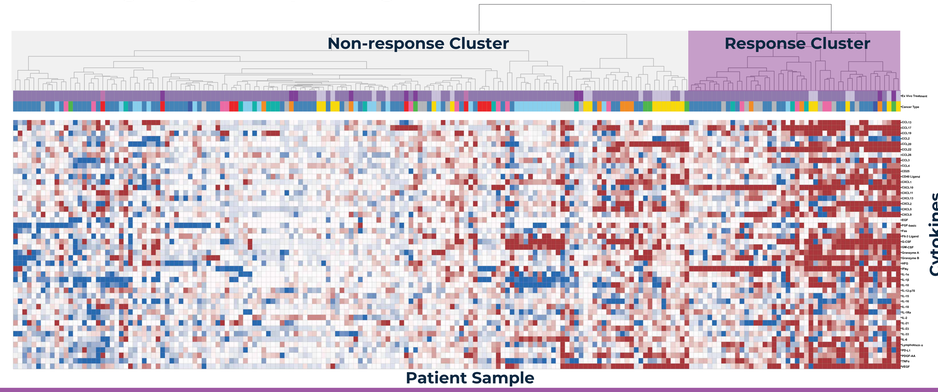


Trial ID	Enrollment eligibility
NCT05478538	Lung
NCT05520099	Bladder, kidney, colorectal, head and neck, lung, melanoma, endometrial
NCT06349642	Lung, skin, esophageal, cervical, endometrial, colon, liver, kidney, bladder, Breast - TNBC

Distribution of specimens across tumor types represented in Elephas clinical trials.

Results

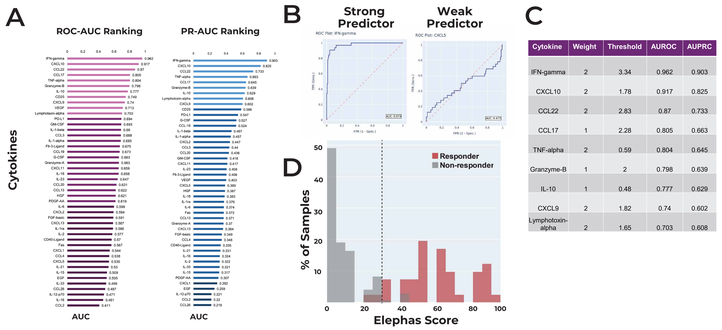
1 Clustering of cytokine profiling data from 140 patients reveals a subset of samples enriched for response



Unsupervised hierarchical clustering of cytokine profiling data from 140 patients was used to create a heatmap depicting changes in cytokine production rates (ICI-IgG) for 46 cytokines.

Two major clusters emerge, showing 24% of samples are enriched for cytokine response to ICI (right). Changes in cytokine production rate are displayed as delta modified *z scores*.

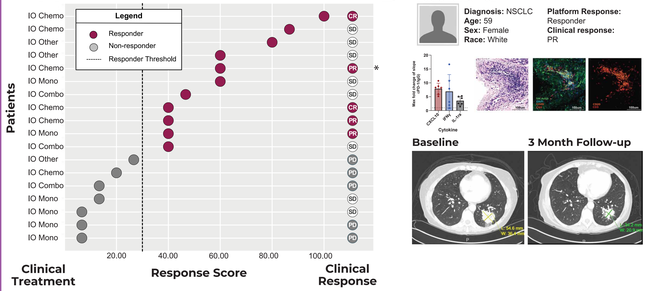
2 Discriminatory cytokines that accurately predict heatmap responders and non-responders are used to create a score to predict response



Cytokine	Weight	Threshold	AUROC	AUPRC
IFN-gamma	2	3.34	0.962	0.903
CXCL10	2	1.78	0.917	0.825
CCL22	2	2.83	0.87	0.733
CCL17	1	2.28	0.805	0.663
TNF-alpha	2	0.59	0.804	0.645
Granzyme-B	1	2	0.798	0.639
IL-10	1	0.48	0.777	0.629
CXCL9	2	1.82	0.74	0.602
Lymphotoxin-alpha	2	1.65	0.703	0.608

A) Area under the curve (AUC) analysis of receiver operator characteristic (ROC) and precision-recall (PR) curves identifies 9 discriminatory cytokines (n=46). **B)** Examples of strong and weak predictors of heatmap responders. **C)** Nine most discriminatory cytokines. **D)** The Elephas score differentiates heatmap responders from non-responders. Dashed line indicates score threshold for responders.

3 Elephas score predicts real world clinical response to ICI



A) Clinical response (measured by RECIST v1.1 or pathologic response) in 18 patients correlates with the Elephas platform response prediction. Asterisk indicates patient highlighted in 3B.

B) Patient vignette of a platform responder. Patient recieved pembrolizumab, pemetrexed, and carboplatin. LTFs were treated ex vivo with aPD-1.

Conclusions

- We show for the first time the feasibility of measuring cytokine response to ICI from CNBs
- Clustering of cytokine data from 140 patients yields distinct heatmap responder and non-responder clusters
- Training on the heatmap yielded a weighted classifier of 9 discriminatory cytokines (AUC 0.968)
- Validation of the classifier on a cohort of 18 patients accurately predicted each clinical responder and progressor ($P < .001$)

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2. Ramasubramanian TS, Adstamongkonkul P, Scribano CM, et al. A live tumor fragment platform to assess immunotherapy response in core needle biopsies while addressing challenges of tumor heterogeneity. *J Transl Med.* 2025 (in press).

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