Rapid and autonomous multi-agent augmented analysis of immune checkpoint inhibitor responses from diverse datasets

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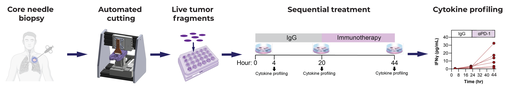
Abstract #5695

Introduction

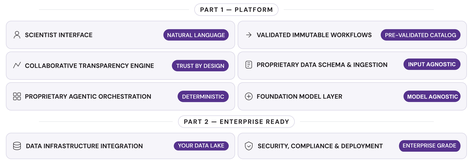
- Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, but objective response rates vary widely by tumor type[1]
- Current FDA-approved biomarkers do not capture the dynamics of ICI response within the tumor microenvironment (TME)[2,3]
- The Elephas elive™ platform utilizes ex vivo cytokine profiling[4,5] to capture dynamic changes in cytokines and chemokines
- To gain a deeper understanding of the active immune pathways in ICI responders, Mithrl — the agentic AI Scientific Decision Engine (SDE) — integrated elive™ platform cytokine profiling data with transcriptomic datasets[6-14] from patients treated with ICIs without manual pipeline engineering to generate novel insights within hours

Methods

Ex vivo cytokine profiling: The elive™ platform measures cytokine responses to ICIs in live tumor fragments (LTFs) from human solid tumor biopsies. Biopsies were cut using an automated cutting device. LTFs were encapsulated in proprietary hydrogel and treated using a sequential treatment strategy in which IgG control followed by ICI was added to the same tissue in a single well. Concentrations of 46 cytokines were measured longitudinally using Luminex multiplex assays.



Mithrl orchestrates a multi-agent system under rigorous scientific control to autonomously design and execute multi-step analyses and biological contextualization of results while being model-agnostic and maintaining data privacy.



Results

1 Mithrl SDE integrates Elephas cytokine profiling data and transcriptomics

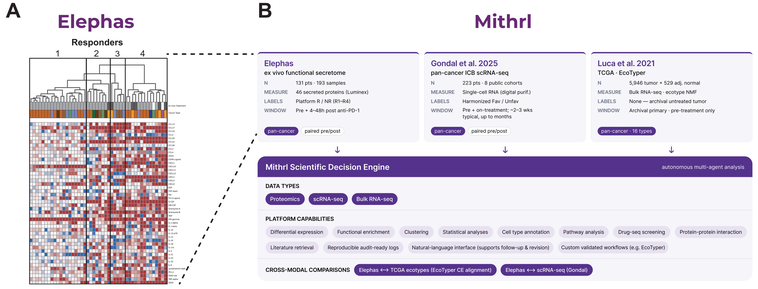


Figure 1. A) Unsupervised hierarchical clustering of cytokine profiling data generated from 131 human tumor specimens identified 4 responder subclusters representing 4 different immune phenotypes (24% of all samples; ICI-non-responder samples not shown). **B)** Mithrl SDE integrated Elephas cytokine profiling data with transcriptomics data rapidly and autonomously to generate novel insights in immune pathways upregulated in ICI responders.

2 EcoTyper immune phenotypes predictive of ICI response validate Elephas responder subclusters

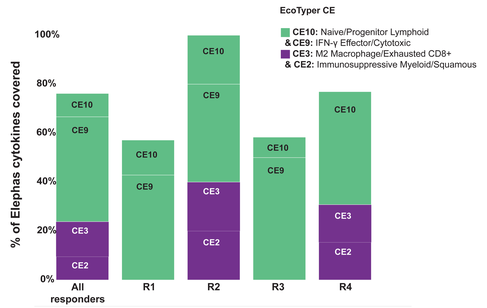


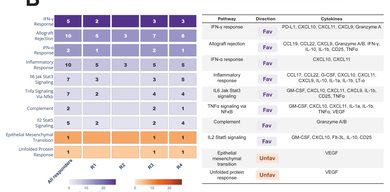
Figure 2. EcoTyper carcinoma ecotypes (CE) CE10 and CE9, which represent the most immunogenic ecotypes enriched for CD8⁺ T cells and were shown to correlate with positive response to ICI,[15] map to each cytokine responder subcluster (R1-4), and make up the majority of each subcluster, compared with immunosuppressive ecotypes CE3 and CE2.

3 Elephas predictive cytokines map to top immune pathways and cell types represented in ICI favorable responders from Gondal et al, 2025

A

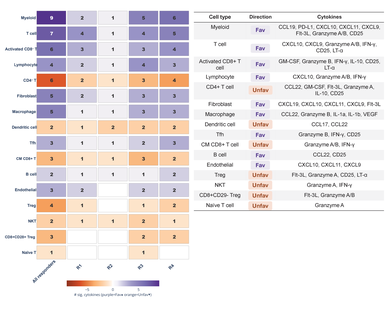
Cluster	Description	Cytokines	#
All responders		IFN-γ, CXCL9, CXCL10, CXCL11, TNFα, Granzyme A, Granzyme B, CCL19, LT-α, Fit-3L, IL-10, CCL17, CCL22, VEGF, CD25, G-CSF, PD-L1 (soluble), GM-CSF, IL-1β, CCL3, IL-1ᵃ	21
Subcluster R1	T cell inflamed with regulatory counterbalance	IFN-γ, CXCL9, CXCL10, TNFα, Lymphotoxin-α, CCL22, IL-10	7
Subcluster R2	Mixed regulatory: Treg and myeloid	IFN-γ, CCL17, CCL22, CCL19, G-CSF	5
Subcluster R3	Cytotoxic inflamed with Treg and VEGF counterbalance	IFN-γ, CXCL10, Granzyme A, Granzyme B, CCL17, CCL22, CCL19, CCL3, IL-10, CXCL11, TNFα, VEGF	12
Subcluster R4	Polyfunctional activated with myeloid-angiogenic remodeling	IFN-γ, CXCL10, CXCL9, Granzyme B, CCL17, CCL22, G-CSF, GM-CSF, CD25, Fit-3L, PD-L1, TNFα, VEGF	13

B



Pathway	Direction	Cytokines
IFN-γ response	Fav	PD-L1, CXCL10, CXCL11, CXCL9, Granzyme A
Allograft rejection	Fav	CCL19, CCL22, CXCL9, Granzyme A/B, IFN-γ, IL-10, IL-15, CD25, TNFα
IFN-α response	Fav	CXCL10, CXCL11
Inflammatory response	Fav	CCL17, CCL22, G-CSF, CXCL10, CXCL11, CXCL9, IL-10, IL-1α, IL-1b, LT-α
IL6 Jak Stat3 signaling	Fav	GM-CSF, CXCL10, CXCL11, CXCL9, IL-1b, CD25, TNFα
TNFα signaling via NFκB	Fav	GM-CSF, CXCL10, CXCL11, IL-1α, IL-1b, TNFα, VEGF
Complement	Fav	Granzyme A/B
IL2 Stat5 signaling	Fav	GM-CSF, CXCL10, Fit-3L, IL-10, CD25
Epithelial mesenchymal transition	Unfav	VEGF
Unfolded protein response	Unfav	VEGF

C



Cell type	Direction	Cytokines
Myeloid	Fav	CCL19, PD-L1, CXCL10, CXCL11, CXCL9, Fit-3L, Granzyme A/B, CD25
T cell	Fav	CXCL10, CXCL9, Granzyme A/B, IFN-γ, CD25, LT-α
Activated CD8+ T cell	Fav	GM-CSF, Granzyme B, IFN-γ, IL-10, CD25, LT-α
Lymphocyte	Fav	CXCL10, Granzyme A/B, IFN-γ
CD4+ T cell	Unfav	CCL22, GM-CSF, Fit-3L, Granzyme A, IL-10, CD25
Fibroblast	Fav	CXCL19, CXCL10, CXCL11, CXCL9, Fit-3L
Macrophage	Fav	CCL22, Granzyme B, IL-1α, IL-1b, VEGF
Dendritic cell	Unfav	CCL17, CCL22
Th	Fav	Granzyme B, IFN-γ, CD25
CM CD4+ T cell	Unfav	Granzyme A/B, IFN-γ
B cell	Fav	CCL22, CD25
Endothelial	Fav	CXCL10, CXCL11, CXCL9
Treg	Unfav	Fit-3L, Granzyme A, CD25, LT-α
NKT	Unfav	Granzyme A, IFN-γ
CD8+CD29- Treg	Unfav	Fit-3L, Granzyme A/B
Naive T cell	Unfav	Granzyme A

Figure 3. A) Table of upregulated cytokines in each cytokine responder subcluster (R1-4). **B)** Heatmap of top upregulated pathways among scRNAseq favorable responders (purple) and unfavorable responders (non-respondres, orange) mapped to Elephas cytokine responder subclusters. Numbers indicate the number of cytokines represented in each pathway, listed in the table to the right. **C)** Heatmap of immune cell types identified in scRNAseq favorable and unfavorable responders (scRNAseq data filtered for immune cells). Numbers reflect the Elephas responder cytokines that correspond to each immune cell type, listed in the table to the right.

4 Elephas predictive cytokines correlate with scRNAseq favorable responders from Gondal et al, 2025

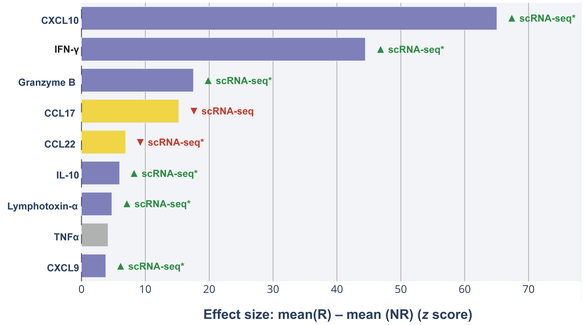


Figure 4. Cytokine effect sizes for the top 9 cytokines identified by Elephas as predictive of ICI response. Purple bars = concordance with scRNAseq favorable responders, yellow bars = discordance, and gray bar = no scRNAseq data available. Arrows indicate log2 fold change direction of scRNAseq data of favorable responders compared with unfavorable responders. * indicates statistical signficance, $p < .05$.

Conclusions

- Immune phenotypes of responders identified from Elephas cytokine profiling analyses were validated by Mithrl SDE using public transcriptomic datasets
- Cytokine profiling captures functional and temporal information not reflected in static transcriptomics, which may account for variation across datasets
- Mithrl SDE rapidly and autonomously integrated cytokine profiling and transcriptomic datasets without manual pipeline engineering, providing a scalable model for AI-accelerated translational research

Acknowledgments

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Learn more about Mithrl SDE

Learn more about ex vivo cytokine profiling to predict ICI response

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