Characterization of immune phenotypes associated with ex vivo response to immune checkpoint inhibitors in live tumor fragments from core needle biopsies

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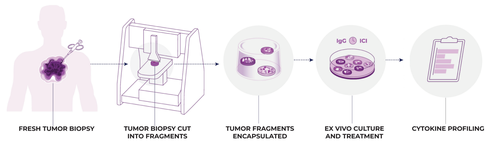
Abstract #435

Introduction

- Characterization of tumor microenvironment (TME) phenotypes associated with clinical response has relied on transcriptomic and imaging analyses of tumor specimens, either pre-treatment or on-treatment, providing a static snapshot of the TME that lacks functional context.[1]
- The elive™ platform measures cytokine responses to immune checkpoint inhibitor (ICI) treatment in live tumor fragments (LTFs) to provide a dynamic readout of the TME that accurately predicts clinical outcomes to ICI treatment in patients.[2]
- Here, we evaluate 4 distinct immune phenotypes observed across ex vivo ICI responders on the elive platform.

Methods

Cytokine profiling and response classification: Patients with ICI-eligible solid tumors were enrolled in ongoing, prospective observational trials (NCT05478538, NCT05520099, NCT06349642, NCT07327489). Biopsies were cut into LTFs, encapsulated in hydrogel, and treated sequentially with isotype-matched IgG antibody control followed by ICI. Cytokine production was assessed by a multiplexed bead-based immunoassay at multiple time points during ex vivo treatment (see platform workflow schematic below). Unsupervised hierarchical clustering of cytokine profiling data from a training cohort (n=124) identified an ex vivo response cluster and non-response cluster.[2]

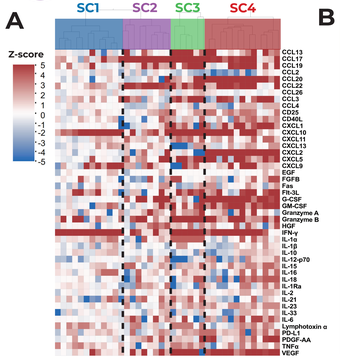


Literature-based characterization of immune phenotype biology: An AI bioscientist agent (Cellformatica) generated a literature-supported interpretation of the biology reflected in each of the response subclusters, including immune pathway assignment.

Multiplex immunofluorescence and spatial proteomics: Encapsulated LTFs were formalin-fixed and paraffin-embedded and sectioned for multiplex immunofluorescence (mIF) or spatial proteomics (performed by Singular Genomics [Figure 3C] and Standard BioTools Hyperion imaging mass cytometry [Figure 4]).

Results

1 Cytokine profiling data cluster into 4 phenotypes (subclusters) of ex vivo immune response

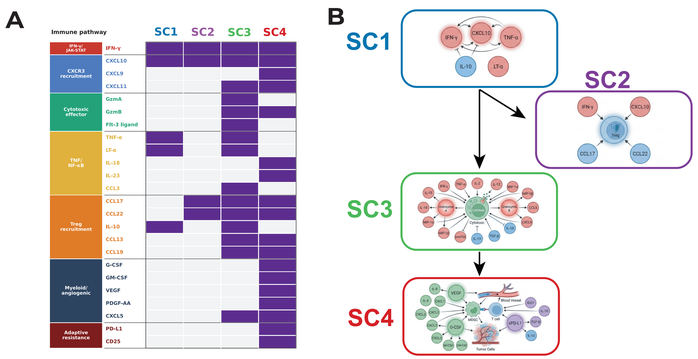


B

Subcluster	Cytokines (#)	Upregulated cytokines
SC1	T-cell Inflamed (5)	IFN-γ, CXCL10, TNF-α, IL-10, Lymphotoxin-α
SC2	T-cell Inflamed with Regulatory Counterbalance (4)	IFN-γ, CXCL10, CCL17, CCL22
SC3	Polyfunctional Cytotoxic-Inflamed (15)	IFN-γ, CXCL10, TNF-α, IL-10, CXCL5, CCL17, Granzyme A, Granzyme B, CCL22, CCL3, CCL13, CXCL11, CCL19, Flt-3 ligand, Lymphotoxin-α
SC4	Hyperactivated with Immune Remodeling (18)	IFN-γ, CXCL10, CXCL9, Granzyme B, CCL17, CCL22, G-CSF, GM-CSF, CD25, PD-L1, VEGF, CCL13, PDGF-AA, CXCL5, CCL19, CXCL11, IL-18, IL-23

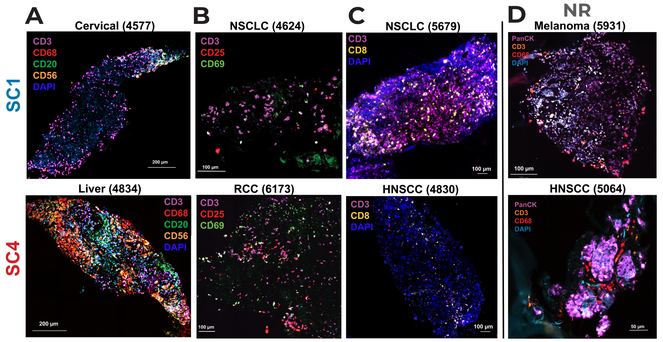
A) Unsupervised hierarchical clustering of cytokine profiling data from ex vivo responders (n = 39 samples from 32 biopsies) divides cytokine signatures into 4 subclusters (SC1-4), or phenotypes of ex vivo immune response. Heatmap colors reflect modified z-scores of the delta in cytokine production rates between the ICI and IgG treatment phases. **B)** A table of the upregulated cytokines in ex vivo responders for each response subcluster highlighting that all 4 subclusters display a core signature of IFN-γ and CXCL10.

2 Immune pathway involvement increases from SC1 to SC4, with SC2 as a separate regulatory branch



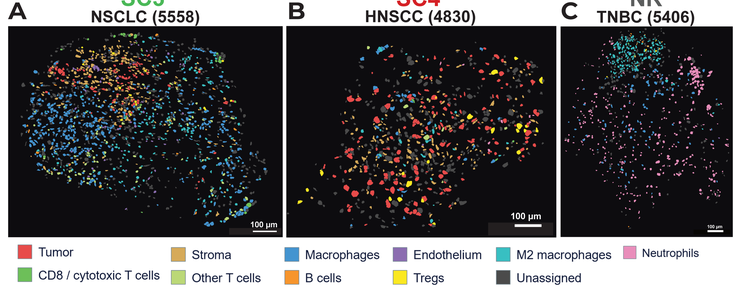
A) An AI-generated literature-based review of the cytokines found upregulated in the response subclusters (compared with the non-response cluster, not shown) identified 7 active immune pathways. Purple boxes indicate cytokines present in each subcluster signature. **B)** Schematics of the representative biology for each subcluster. SC1 reflects a T-cell inflamed phenotype; SC2 reflects a Treg-captured phenotype; SC3 shows an active, cytotoxic-inflamed phenotype; SC4 is maximally inflamed with evidence of adaptive resistance and angiogenic remodeling.

3 Cell types vary across response subclusters



A) mIF of representative LTFs from SC1 and SC4 showing fewer cell types in SC1 than in SC4, consistent with SC1's less complex cytokine signature. **B)** mIF shows the distribution of CD3+CD25+ T cells, consistent with Tregs, in representative LTFs from SC1 and SC4. **C)** Spatial proteomics shows the presence of CD8+ T cells in representative LTFs from SC1 and SC4. **D)** mIF from two ex vivo non-responders (NR) shows the limited immune infiltration compared with responders.

4 Spatial proteomics further illuminates differences in the TME across response subclusters and non-responders



Cell segmentation and cell typing of imaging mass cytometry (IMC) data (>30 markers) generated cell maps of representative LTFs: **A)** LTF from SC3 showing macrophages and cytotoxic T cells. **B)** LTF from SC4 showing Tregs. **C)** Representative ex vivo non-responder (NR) LTF showing M2 macrophages and neutrophils.

Conclusions

- Spatial profiling of LTFs on the elive platform captures TME features consistent with those described in vivo.
- Functional and spatial characterization of 4 distinct immune phenotypes of ex vivo ICI response revealed a continuum of immune states spanning inflamed and immunosuppressive signatures.
- Correlation of clinical outcomes with these immune phenotypes may provide a precision mechanism for matching patients to specific ICI treatment regimens.

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Acknowledgments

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References

1. *Morad G, et al. Cell. 2021;184(21):5309-5337.*
2. *Braun D, et al. Cell Rep Med. 2026. https://doi.org/10.1016/j.xcrm.2026.103114.*