Identifying drivers of clinical response to immune checkpoint blockade through ex vivo characterization of immune phenotypes in live tumor fragments

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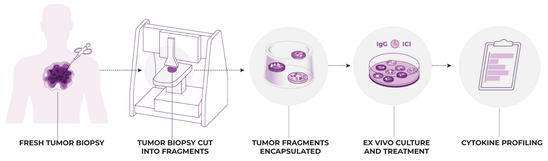
Introduction

- The tumor microenvironment plays a critical role in immunotherapy response, but pathways driving clinical response remain poorly elucidated.[1]
- Ex vivo cytokine profiling accurately predicts response but requires large amounts of tumor tissue.[2]
- The elive™ platform functionally profiles immune response to immune checkpoint inhibitors (ICIs) in live tumor fragments from minimal tissue and predicts clinical outcomes,[3] enabling the identification of drivers of response.

Methods

Cytokine profiling and clinical concordance: Patients with ICI-eligible solid tumors were enrolled in ongoing, prospective observational trials (NCT05478538, NCT05520099, NCT06349642, NCT07327489). Biopsies were cut into live tumor fragments (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 displaying 4 distinct immune phenotypes (response subclusters).

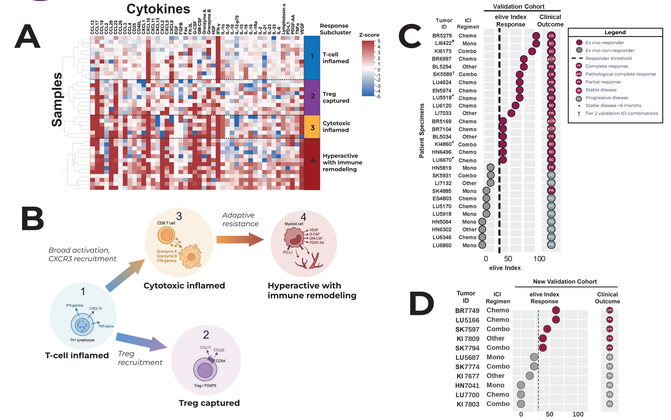
In a validation cohort (n=39), LTFs were treated ex vivo with the same ICI regimen received in the clinic, and ex vivo response was compared with clinical outcomes.



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. The agent also developed a biology-driven methodology for mapping ex vivo responders in the validation cohort (with corresponding clinical data) to response subclusters, enabling investigation of the correlation between clinical outcomes and ex vivo response.

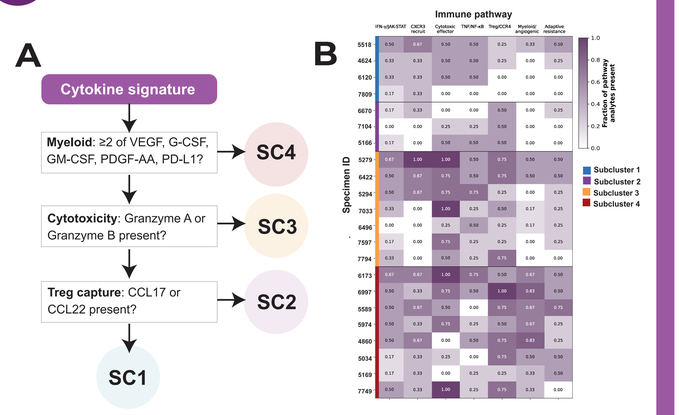
Results

1 The elive tumor profiling platform measures ex vivo immune responses to ICI



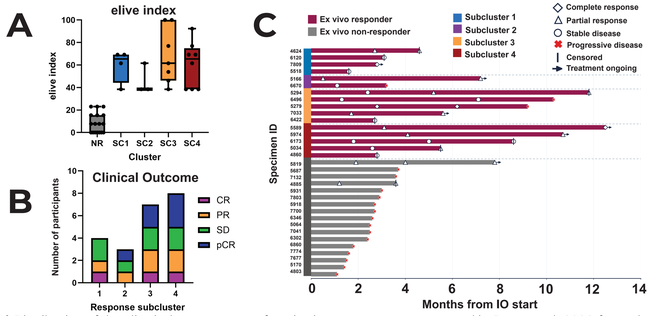
A) Clustered cytokine profiling data in ex vivo responders define 4 immune phenotypes of ex vivo immune response (response subclusters). **B)** Branching model of ex vivo immune phenotypes, showing the evolution of immune response from the minimally T-cell inflamed signature of subcluster 1 (SC1) to cytotoxic inflamed subcluster 3 (SC3), ending in the hyperactive immune remodeling phenotype of subcluster 4 (SC4). The second branch proposes subcluster 1 can alternatively evolve into a Treg-captured state (subcluster 2 [SC2]). **C)** Validation cohort of 28 patients, as published in Braun et al., 2026, showing 93% concordance of ex vivo immune response and clinical outcomes. **D)** The new clinical validation cohort (n=11) demonstrating continued clinical concordance.

2 Mapping ex vivo responders in the validation cohort to response subclusters



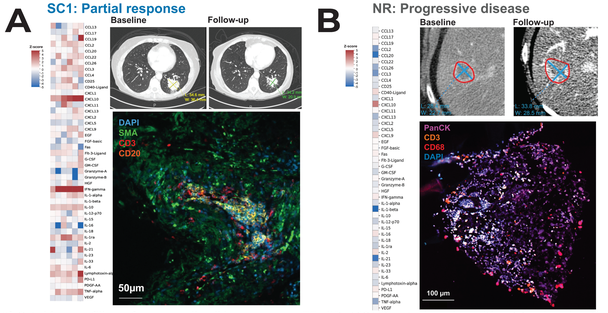
A) Schematic of a biology-driven methodology for mapping validation cohort ex vivo responders to response subclusters that is consistent with the model in Figure 1B, suggesting that subcluster 1 is the initial T-cell inflamed state that evolves to either subcluster 2 (Treg-captured) or subclusters 3 and 4, with subcluster 4 representing a maximally inflamed state that shows evidence of regulatory counterbalance and adaptive resistance. **B)** Heatmap of pathway involvement per validation cohort ex vivo responder, grouped by immune phenotype (subcluster). Numbers indicate the fraction of overlap between a specimen's cytokine signature and the cytokines involved in the designated immune pathway.

3 Distribution of elive index and clinical outcomes by response subcluster



A) Distribution of the elive index, a measure of ex vivo immune response reported in Braun et al., 2026, for each validation cohort specimen across the immune phenotypes (response subclusters, assigned using the methodology outlined in Figure 2A). NR = ex vivo non-responder. **B)** Distribution of clinical outcomes for ex vivo responders in the validation cohort across immune phenotypes (response subclusters). **C)** Swimmer plot of progression-free survival grouped by response subcluster for validation cohort specimens with available PFS data.

4 Molecular characterization of ex vivo response matches inferred biology



A) Cytokine profiling of non-small cell lung cancer LTFs (ID: 4624) treated ex vivo with αPD-1 showing the signature of response subcluster 1 (left). Participant had a partial response to ICI + chemotherapy (top right) at 3-month follow-up. Multiplex IF (mIF) of LTFs showed the presence of a potential tertiary lymphoid structure. **B)** Cytokine profiling of ex vivo non-responder LTFs (ID: 5931, melanoma) treated ex vivo with αPD-1 + αCTLA-4. Participant had progressive disease following treatment with Ipilimumab + nivolumab at 3-month follow-up, and mIF showed low immune cell infiltration.

Conclusions

- Updated clinical validation cohort shows measured ex vivo immune response is 95% concordant with clinical outcomes.
- Four phenotypes of ex vivo immune response are observed following treatment with ICIs, representing phases of evolving immune response.
- Mapping of ex vivo responders in the validation cohort (n=22) to 1 of 4 immune phenotypes showed that favorable responses (CR, pCR, PR) spanned all 4 phenotypes.

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Disclosures: *Pooja Advani is a member of the Elephas Biosciences Medical Advisory Board.*

References: **1.** *Morad G, et al. Cell. 2021;184(21):5309-5337.* **2.** *Voabil P, et al. Nat Med. 2021;27(7):1250-1261.* **3.** *Braun D, et al. Cell Rep Med. 2026; https://doi.org/10.1016/j.xcrm.2026.103114.*

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