Ex vivo cytokine profiling of live triple negative breast cancer patient specimens from core needle biopsies illustrates proof of concept to assess tumor response to immune checkpoint inhibition

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Introduction

- For a subset of triple negative breast cancer (TNBC) patients, immune checkpoint inhibitors (ICIs) in combination with chemotherapy are associated with improved outcomes[1]
- While PD-L1 expression is not used for patient selection in early TNBC, patients with metastatic/advanced TNBC must be positive for PD-L1 (CPS ≥ 10) to receive ICI[1,2]
- However, PD-L1 has shown limited predictive accuracy
- A landmark 2021 paper demonstrated that cytokine profiling of ex vivo live tumor fragments (LTFs) from resections predicted response to ICI with high accuracy (Voabil 2021),[3] but this method requires large amounts of tissue to address tumor heterogeneity and is not amenable to using tissue from core needle biopsies (CNBs), which are standard of care for TNBC patients
- Using a sequential treatment strategy, in which IgG control followed by ICI is added to the same tissue in a single well, allows for the measurement of cytokine response to ICI in settings where tissue is heterogeneous and of limited amount, such as CNBs[4]
- Here, we show proof of concept that cytokine responses to ICI can be measured ex vivo in live tumor fragments generated from TNBC CNBs

Methods

- CNBs were collected from patients enrolled in one of two prospective observational clinical trials (**NCT05520099** and **NCT06349642**)
- CNBs were cut by an automated, proprietary cutting instrument to generate LTFs of 300 µm in thickness and encapsulated in proprietary hydrogel
- LTFs were treated using a sequential treatment strategy in which control followed by ICI treatment were applied to the same tissue in a single well
- Cytokine production rates were measured longitudinally using a multiplex assay
- Cytokine profiling data from 129 patients was used to generate a heatmap and identify differences in cytokine production rates among heatmap responders and non-responders
- A classifier developed from 9 discriminatory cytokines was used to create a score to predict ICI response
- Current efforts are evaluating how clinical outcomes correlate with platform responses

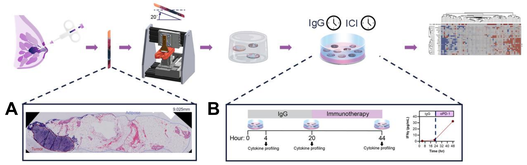


Figure 1. An overview of the ex vivo profiling platform. **A)** H&E showing tumor heterogeneity in a breast tumor biopsy. **B)** A sequential treatment strategy in which IgG control and ICI are added to the same well mitigates the challenge of tumor heterogeneity in settings of limited tissue. Longitudinal cytokine profiling is performed at indicated time points and fold changes in cytokine production rates are calculated to determine response to ICI.

TNBC platform responder and non-responder

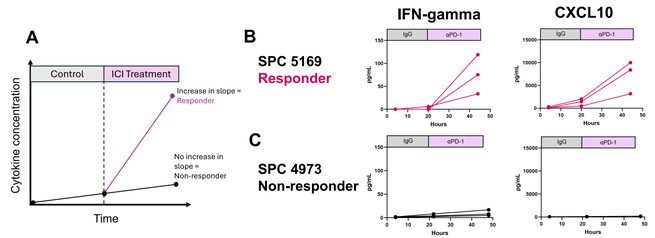


Figure 2. **A)** Schematic showing the change in cytokine production rate (slope) from IgG control phase to treatment phase. Examples of TNBC platform responder **(B)** and non-responder **(C)** cytokine production rates (slopes) during IgG (control) and treatment phases for IFN-gamma and CXCL10.

Heatmap of platform responders and non-responders is used to create a score to predict response

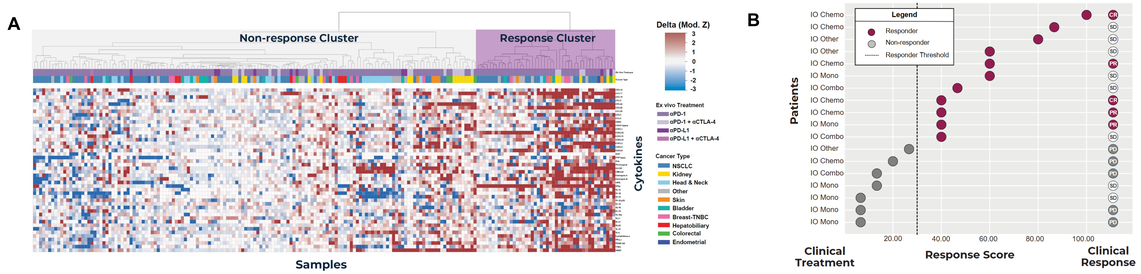


Figure 3. **A)** Heatmap of cytokine profiling data from 193 samples (from 129 patients) show samples fall into a non-response cluster or response cluster. **B)**. Clinical response (measured by RECIST v1.1 or pathologic response) of 18 patients correlates with platform predicted response. *Data shown in SITC 2025 Poster #54, Dana, N., et al.*

TNBC platform responses correlate with clinical response

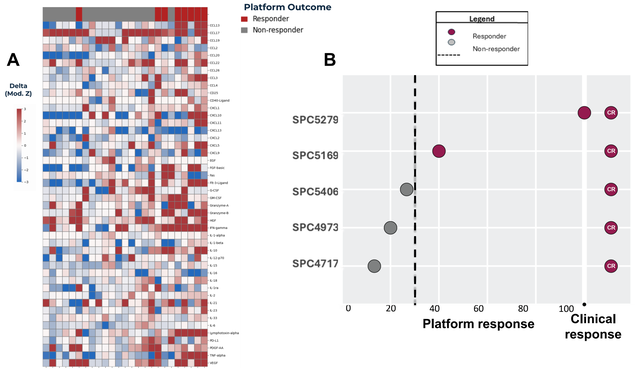


Figure 4. **A)** A heatmap of TNBC specimens tested on the platform showing fold change in cytokine production rates following ICI treatment. **B)** Correlation of platform response with clinical response for 5 TNBC patients. All patients received ICI + chemotherapy and ex vivo LTFs were treated with αPD-1 only.

TNBC platform responder shows complete clinical response within 3.5 months

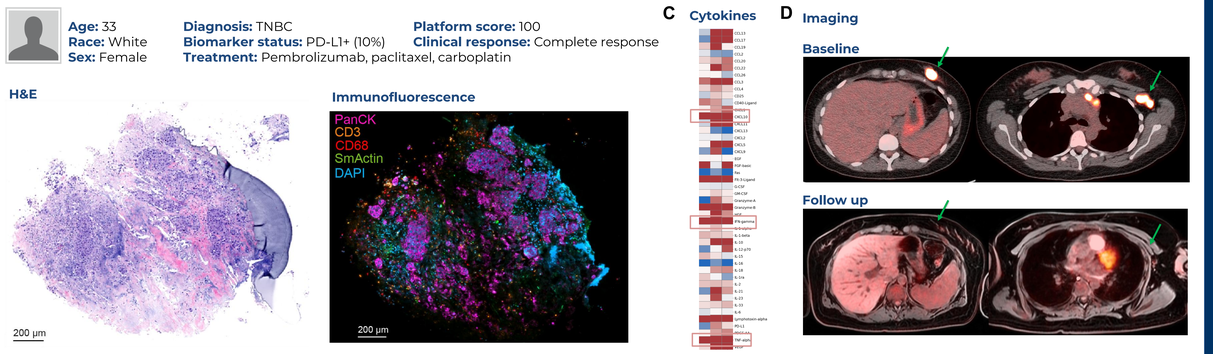


Figure 5. Patient vignette of a platform responder, showing patient demographics **(A)**, H&E and immunofluorescence of a representative LTF **(B)**, cytokine response on the platform **(C)**, and baseline and follow-up PET scans, which show a complete radiological and metabolic response within 3.5 months (green arrows point to lesion) **(D)**.

Conclusions

- We report, for the first time to our knowledge, the ability to detect ICI responses via cytokine profiling in LTFs from TNBC CNBs, illustrating proof of concept
- A weighted classifier of discriminatory cytokines was used to create a score that accurately discriminates heatmap responders and non-responders
- The correlation of cytokine response on the platform to clinical response is under investigation to determine the predictive accuracy of the platform

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Read more about the platform