1040

Blinded clinical validation of LiquidTME, a cell-free DNA assay for predicting response to immunotherapy by noninvasively profiling the tumor microenvironment

Aadel A. Chaudhuri[1], David Y. Chen[2], Tucker Hansen[2], Mirna Jarosz[3], Vincent A. Miller[3], Aaron M. Newman[4]

[1]Mayo Clinic, Rochester, MN; [2]Washington University School of Medicine, St. Louis, MO; [3]LiquidCell Dx, San Carlos, CA; [4]Stanford University, Stanford, CA

Background

Combination immune checkpoint inhibitor (ICI) therapy represents a standard-of-care for patients with metastatic melanoma. However, >40% of patients do not respond to treatment and severe immune-related toxicity affects up to 60% of patients. Accordingly, we need more precise ways to select patients for combination ICIs.

To address this, we developed LiquidTME, a liquid biopsy method based on Spatial and Liquid EcoTyper[1] that leverages a deep learning approach to noninvasively characterize the tumor microenvironment from cell-free DNA (cfDNA) methylation data. LiquidTME was previously trained to predict ICI response using samples from 78 patients with advanced melanoma treated at Yale University. Here we performed a blinded validation of LiquidTME in coordination with clinicians at Washington University in St. Louis (WashU).

Blinded validation framework

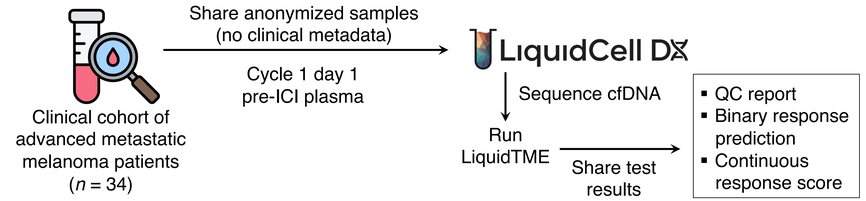

Figure 1. Schematic overview of the blinded validation process for this study. QC, quality control.

Clinical cohort

The WashU cohort consisted of pre-ICI plasma from 34 patients with advanced melanoma, each treated with ipilimumab and nivolumab (IPI/NIVO) or relatlimab and nivolumab (RELA/NIVO). ICI response was classified as durable clinical benefit (DCB) or no durable benefit (NDB) by a board-certified oncologist. Median follow-up time of the cohort was 21.6 months.

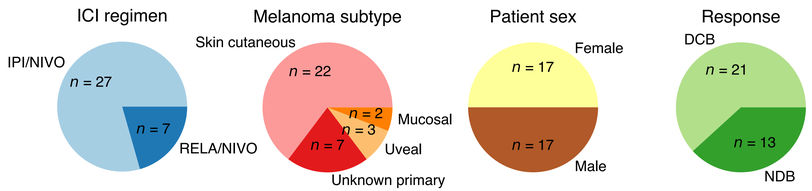

Figure 2. Clinical cohort characteristics, including ICI therapy, disease subtype, patient sex, and ICI response. Median patient age was 67 years.

Methods

Tumor mutational burden (TMB) was determined for 27 patients using commercial CLIA-certified assays and analyzed as nonsynonymous mutations (mt) per megabase (Mb), using the FDA-approved 10 mt/Mb cutpoint for TMB-high and -low groups. Plasma samples for the full cohort (34 patients) were sent to a scientific team that was blinded to all clinical and TMB data.

Cell-free DNA was extracted from 2 mL plasma per patient and subjected to enzymatic methyl-seq (EM-seq) at a median depth of 15x. LiquidTME was performed on the resulting profiles, yielding a binary response prediction and a continuous response score for each sample. All samples passed standard LiquidTME quality control measures. Test results were locked down and returned.

Both assays were compared by area under the receiver operating characteristic curve (AUC) and two-sided Wilcoxon rank-sum tests for response classification, and Kaplan-Meier analysis for progression-free survival (PFS).

LiquidTME

- The tumor microenvironment (TME) underpins therapy response.
- Tools for profiling the TME with high spatial resolution are complex, expensive, and require tissue biopsies.
- LiquidTME bridges this gap by profiling nine spatial TME biomarkers, termed spatial ecotypes (SEs), noninvasively from a clinically practical blood draw using cfDNA.

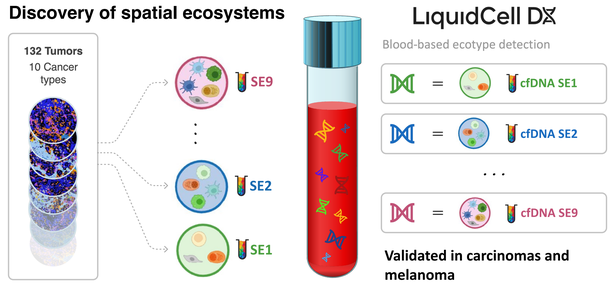

Figure 3. LiquidTME for clinical profiling of TME spatial ecosystems.

Evaluation of LiquidTME

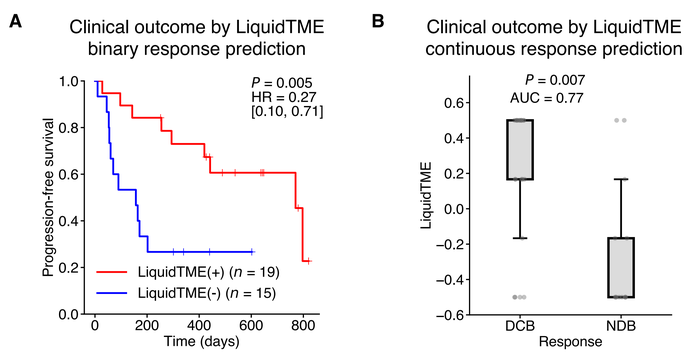

Figure 4. LiquidTME results over the full blinded clinical validation cohort (*n* = 34). **A**, Kaplan-Meier analysis of progression-free survival with patients stratified into responder and non-responder groups by LiquidTME(+) and LiquidTME(-) status, respectively. LiquidTME(+) patients achieved a median PFS of 1.7 years longer than LiquidTME(-) patients. **B**, Box plot showing continuous LiquidTME scores by clinician-annotated response.

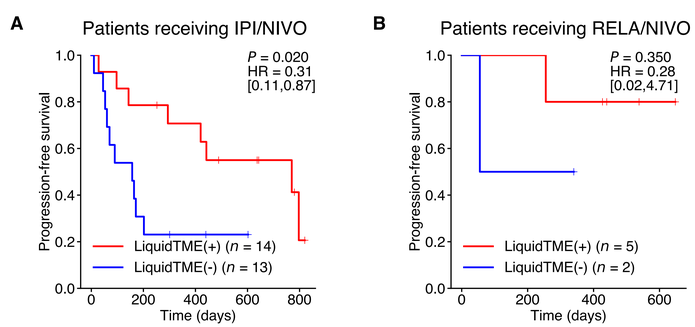

Figure 5. Kaplan-Meier analysis of LiquidTME results by ICI regimen. **A**, Patients receiving IPI/NIVO treatment. **B**, Patients receiving RELA/NIVO treatment. Similar performance is observed across therapies.

Comparison with TMB

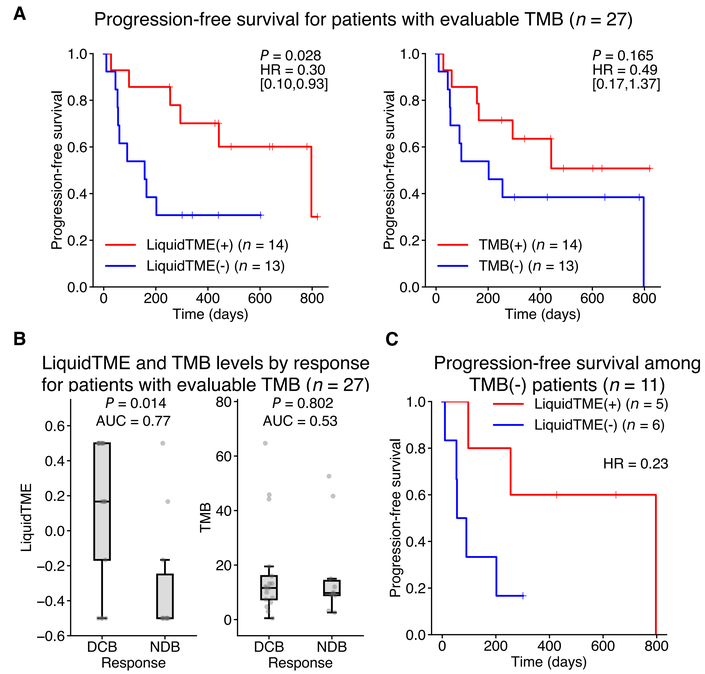

Figure 6. LiquidTME results for patients with evaluable TMB (*n* = 27). **A**, Kaplan-Meier analysis of progression-free survival with patients stratified into responder and non-responder groups. *Left*: Stratification by LiquidTME(+) and LiquidTME(-) status, respectively. *Right*: Stratification by TMB(+) and TMB(-) status, respectively. **B**, Box plots showing continuous LiquidTME scores (left) and TMB (right) by clinician-annotated response. **C**, Same as panel A left but for TMB(-) patients. Patients with TMB < 10 mut/Mb are considered TMB(-), and patients with TMB ≥ 10 mut/Mb are considered TMB(+).

Conclusions and future directions

- LiquidTME identified durable responders to combination ICI from pretreatment plasma in this blinded validation cohort of melanoma patients and successfully stratified responders and non-responders independently of therapy type.
- This study was the first demonstration of LiquidTME stratification of patients treated with RELA/NIVO, implying conserved determinants of ICI response.
- In patients with TMB data, LiquidTME maintained whole-cohort performance. In contrast, TMB high vs. low subsets failed to stratify PFS or response.
- Given its favorable performance, LiquidTME shows promise as a clinical tool to guide personalized immunotherapy decision-making.
- Ongoing and planned studies will characterize the LiquidTME across a broad array of solid tumor types and therapeutic classes.

References

1. Zhang*, Brown*, Usmani*, et al. Non-invasive profiling of the tumour microenvironment with spatial ecotypes. *Nature* (2026), in press. **DOI**: 10.1038/s41586-026-10452-4.