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Methylation TIL predictor associates with prognosis in primary melanoma

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Background: Cutaneous melanomas are known to be immunogenic with significant variation of tumor infiltrating lymphocytes (TIL). TIL grade, scored as brisk, nonbrisk, and absent, is routinely measured in primary tumors but, currently, is not utilized in melanoma staging. While brisk TIL grade predicts improved melanoma-specific survival (MSS), nonbrisk TIL grade is intermediately associated with survival often lacking prognostic significance (Thomas, JCO 2013; Fu, Oncoimmunology 2019; Maibach, Front. Immunol. 2020). Many stage II and III melanomas are scored as non-brisk TIL grade; and, thus, TIL grade does not provide distinguishing information for these tumors. Our goal is to predict TIL or T cell estimates in primary melanoma from DNA methylation data using immunofluorescence CD3+ staining as ground truth.

Patients and Methods: Using a cohort of primary cutaneous melanomas (n=80) from patients spanning all AJCC8 stages and a median Breslow thickness of 1.6mm (0.4-17.0mm), previously analyzed for multiplex-immunofluorescence (CD3, CD8, S100) and whole-genome DNA methylation analysis, we performed elastic net modeling the percentage of CD3+ T lymphocytes to select features and build a prediction model for TILs in primary melanoma called EPI-TIL. Validation was performed in two melanoma multi-genomic studies, TCGA (n=352) and InterMEL (n=399).

Results: Patients with tumors predicted to have a low (lower tertile) EPI-TIL score (ANOVA, $p<0.001$) show significantly worse melanoma-specific survival (MSS) upon Kaplan-Meier (KM) analysis compared to middle and upper tertile scores. Additionally, EPI-TIL significantly adds prognostic value to clinical factors (age, sex, stage) for MSS (ANOVA LRT, $p=0.012$). Validation in TCGA shows a high (upper tertile) EPI-TIL score associates with better MSS using KM analysis (ANOVA, $p<0.009$) and adds prognostic value to clinical factors (age, sex, stage) for MSS (ANOVA LRT, $p<0.002$).

We applied our EPI-TIL prediction model to the recently published DNA methylation analysis in the InterMEL study (Conway et al., JCO Precision Oncology 2024). The InterMEL study is a large retrospective case-control study composed of AJCC8 stage II and III primary cutaneous melanoma with 850K methylation data (n=399) and the majority of the melanomas (84%) scored as nonbrisk TIL grade. EPI-TIL validation in the InterMEL study quantifies TILs and performs significantly better than TIL grade alone, providing improved measures of TIL variability. EPI-TIL shows significant improvement for 5-year MSS for patients with a high EPI-TIL prediction score. Logistic regression analysis found that EPI-TIL significantly predicts improved melanoma survival greater than 5 years compared to clinical factors, even after adjusting for age, sex, AJCC8 stage and TIL grade when higher T lymphocytes infiltrate into the tumor measured as high EPI-TIL compared to a low EPI-TIL score (OR=4.5, CI 2.5-8.6, $p=2.38e-06$).

Conclusion: EPI-TIL performs as an accurate TIL quantifier and is a prognostic predictor for AJCC8 stage II and III primary melanoma.