Validation of the CD8+/CD68+ Cell Ratio as a Prognostic Biomarker in Stage II-III Melanoma

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Background

- Cutaneous melanoma is an aggressive dermatologic malignancy with recurrence rates of stage II-III disease between 27-46%[1-2]
- Analysis of the tumor microenvironment (TME) in cutaneous melanoma is a promising avenue to identify patients who would benefit most from adjuvant immunotherapy
- Our previous research established the potential of CD68+ macrophage and CD3+CD8+ cytotoxic lymphocyte (CTL) densities, as well as the TME CTL/macrophage ratio, for prognostic biomarker use in metastatic progression and disease-specific survival (DSS)[3]
- We assessed the CTL/macrophage ratio in a second cohort of stage II-III melanoma patients from Geisinger Health System (GHS) to further test the prognostic accuracy of this biomarker

Objective

- The objective of this work was established on the use of the GHS patient data as a validation cohort of our previously described CTL/macrophage biomarker

Methods

- We utilized quantitative multiplex immunofluorescence (qmIF) to analyze 43 samples from patients with stage II-III melanoma
- A trainable machine learning program (inForm) was employed to analyze data on cell phenotypes and densities in the combined tumor + stroma ("total TME")

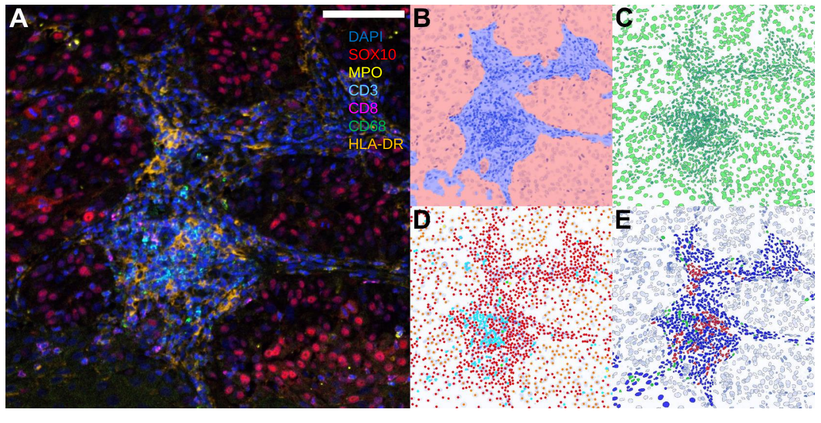


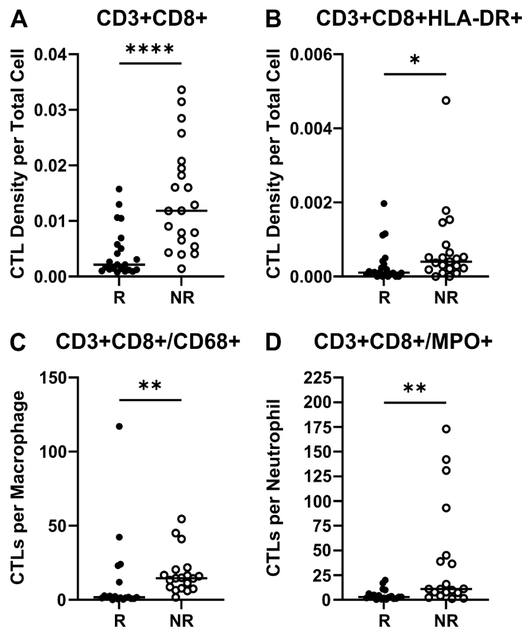
Figure 1: Analysis of tissue sample acquired with Vectra pathology workstation of melanoma specimen using inForm software. A) qmIF of melanoma specimen **B)** Tissue Segmentation **C)** Cell Segmentation **D)** Phenotype - CD68 (green), CD3 (cyan), Tumor (orange), and Other (red). **E)** Scoring – scoring for CD8 positivity shown here in stroma only. White bar represents 100um.

Methods (continued)

- Whole slide scans (H&E) were digitally scored for immune and tumor cell counts in QuPath, where a TIL score (ratio of immune cells to immune+tumor cells) was assigned based on a threshold of 16.6%[4]
- Cell densities and ratios were subsequently analyzed to determine associations with clinical outcomes including both disease recurrence and DSS

Results

Figure 2: Density of phenotype combinations compared on recurrence. A) Overall cytotoxic T lymphocyte (CTL) density ($p<0.0001$) **B)** Activated HLA-DR+ CTL density ($p=0.0165$) **C)** CTL/macrophage ratio ($p=0.0031$) **D)** CTL/neutrophil ratio ($p=0.0005$). R=recurrent, NR=non-recurrent.



The density of CTLs and activated HLA-DR+ CTLs was higher in the total TME ($p<0.0001$, $p=0.0165$, respectively) in those without distant metastatic recurrence (DMR) when compared to those with DMR. The CTL/macrophage ratio in the total TME was greater in those without recurrence ($p=0.0031$). The CTL/neutrophil ratio was higher in the total TME ($p=0.0016$) in those without recurrence.

Survival analysis of 38 patients with known cause of death indicated improved survival in those with a higher CTL/macrophage ratio in the total TME ($p=0.0005$) as well as those with high CTL/neutrophil ratios in the total TME ($p<0.0001$). Cox regression analysis demonstrated a strong association between low CTL/macrophage and CTL/neutrophil ratios in the total TME and an increased risk of death ($p=0.003$, *HR*: 7.24, *CI*: 1.99-26.31; $p=0.003$, *HR*: 22.35, *CI*: 2.87-173.97, respectively). Further survival analysis of 26 stage II tumor patients with known cause of death utilized digital TIL density analysis from a QuPath classifier. TIL score was shown to be significantly predictive of disease-specific survival via Kaplan-Meier survival analysis ($p=0.0228$).

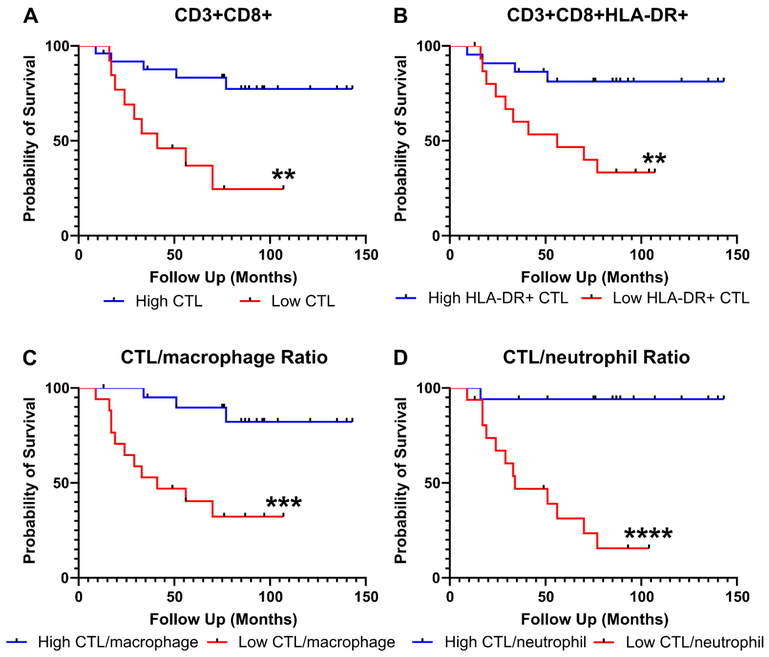


Figure 3: Survival analysis of patients split by total TME densities and ratios. Kaplan-Meier survival curves for patients stratified by **A)** CTL density ($p=0.0011$), **B)** HLA-DR+ CTL density ($p=0.0068$), **C)** CTL/macrophage ratio ($p=0.0005$), **D)** CTL/neutrophil ratio ($p<0.0001$). Patients were stratified into binary High/Low groups for each cell density and ratio by generating a binary threshold values using Receiver Operator Characteristic (ROC) analyses.

Conclusions

- The TME of patients without disease recurrence featured higher ratios of CTLs to macrophages and neutrophils as well as increased infiltration of CTLs and activated HLA-DR+ CTLs
- Improved DSS was also independently associated with elevated CTL/macrophage and CTL/neutrophil ratios in the total TME
- Ongoing validation efforts are underway with the aim of adapting these biomarkers into clinically relevant prognostication tools

References

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Disclosures

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