Penile Squamous Cell Carcinoma Tumor Immune Microenvironment Aberrations Captured By Multiplex Immunofluorescence Are Associated With Clinical Outcomes

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Background

- Penile squamous cell carcinoma (PSCC) is a rare and aggressive malignancy.
- Despite encouraging activity of immune therapies in other cancers, the immune environment of PSCC remains poorly characterized.
- In this study, we characterize the immune microenvironment using multiplex immunofluorescence (mIF) and image analysis approaches in 57 patients with PSCC treated at H. Lee Moffitt Cancer Center (Tampa, FL).

Methods

- Formalin-fixed and paraffin-embedded representative tissue microarray blocks were immunostained using the AKOYA Biosciences OPAL TM 7-Color Automation IHC kit (Waltham, MA) on the BOND RX autostainer (Leica Biosystems, Vista, CA) for 10 immune markers: CD20, CD3, CD4, CD8, CD45RO, CD68, CD206, CD163, NKP46, FOXP3 (Figure 1).
- Multi-layer TIFF images were exported from InForm (AKOYA) and loaded into HALO Image Analysis Platform (Indica Labs, New Mexico) for quantitative image analysis. Each tissue core divided into tumor and stroma compartments → densities of cell phenotypes (cells/mm²).
- Maximally selected rank statistics test → cut-points of continuous variables with maximal discriminatory value for clinical outcomes. (Figure 1)
- Survival analyses → Kaplan-Meier plots, Cox regression and Log-rank test.

Results

- Median age at diagnosis was 60 years (interquartile range, 53-73 years)
- Equal distribution of stages I-II (25/57, 44%) and stage III (25/57, 44%); 7/57 (12%) were stage IV
- 34/57 (60%) were HPV-negative
- The density of CD8+ and CD68+CD163+CD206+ (type 2 macrophage, M2) in the tumor and stroma were similar in HPV(+) and HPV(-) PSCC (Figure 2 A and B)
- Using statistically determined binary cut-offs for CD8+ and M2 density (higher vs lower):
  ➢ Higher CD8+ density → shorter median OS (Log-Rank P=0.04)
    • Higher: 36.6 months (95% CI, 19.0-84.1 months)
    • Lower: 161.7 months (95% CI, 16.2-161.7 months)
  ➢ Higher M2 density → shorter median OS (Log-Rank P=0.02)
    • Higher: 13.3 months (95% CI, 1.0-not estimable)
    • Lower: 80.1 months (95% CI, 27.1-161.2; Figure 3)
- Using cell density as a continuous variable and controlling for pathologic stage in Cox regression:
  ➢ M2 density → increased risk of death (Hazard ratio, 1.002; P=0.04)
  ➢ CD8 density → no significant association with risk of death (Hazard ratio, 5.086; P=0.13)

Conclusions

Multiplex image analysis of primary PSCC found no difference in the immune contexture of HPV(+) and HPV(-) patients.

High levels of tumor-associated macrophages and cytotoxic T-cells were associated with inferior survival outcomes.

Further characterization of T-cell subsets to gauge the activated or exhausted status of T-cells are warranted.

FPN 739P
Dr. Chaiboud has no relevant disclosures.