STRESS KERATIN 17 (K17) EXPRESSION AS A PREDICTOR OF RESPONSE TO IMMUNE CHECK-POINT BLOCKADE (ICB) TREATED ANAL, VULVAR, AND CERVICAL CARCINOMA

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Abstract 215

Background The over-expression of stress keratin 17 (K17) has been identified as an immune evasion mechanism in a human papillomavirus (HPV) infection mouse model.1 K17 protein expression has also shown to be a prognostic factor in several HPV-associated cancer types, including head and neck (HNC) and cervical cancer.2, 3 Our studies suggest K17 is inversely correlated with CD8 T cell infiltration and response to immune check-point blockade (ICB) in HNC.4 We investigated the expression of K17 and markers of immune activation and response to ICB in anogenital cancers.

Methods Pre-ICB archival FFPE tissue specimens from anogenital cancer patients undergoing ICB-based therapy at the University of Wisconsin-Madison (UW-Madison, n=15) and University of Alabama-Birmingham (n=13) were stained by immunohistochemistry using a validated K17 monoclonal antibody (Abcam, ab109725). Cases were categorized into K17high vs. K17low, as previously described.4 Study endpoints were progression-free survival (PFS), time to treatment failure (TTF) and overall survival (OS). A tissue microarray (TMA) consisting of archival pretreatment tissue samples from 7 ICB-treated anogenital cancer patients from UW-Madison was subject to multiplex single-cell immunophenotyping on the Akoya Biosciences Phenocycler-Fusion using a 30-plex antibody panel (table 1) which included a custom K17 antibody compatible with the Phenocycler platform requirements (Novusbio, NBP2-47684). Analysis was performed inQuPath v.0.4.3 using the StarDist (arXiv:1806.03333) nuclear segmentation algorithm and phenotyping using artificial neural network training. Correlations between the expression of markers and clinical outcomes were assessed using the Spearman’s coefficient, independent t-test and log rank test.

Results Altogether, 28 patients were included in this study (table 2). Fifteen tumors (53.6%) had K17high expression, and 13 tumors (46.4%) had K17low expression (figure 1). K17 status was significantly correlated with TTF (p=0.03), but not PFS or OS (figure 2A). Among patients receiving pembrolizumab-based therapy (n=21), there were 12 (57.1%) K17high vs. 9 (42.9%) K17low tumors. K17 status was again associated with TTF (p=0.007), but not PFS or OS (figure 2B).

Altogether, single cell immunophenotyping data from 11 TMA cores from 7 patients (table 3) revealed OS at 6 months was significantly correlated with K17 expression (K17+PanCK+/PanCK+, p=0.032), CD68 (CD68+PanCK+/PanCK+, p=0.016) and PD-L1 on tumor cells (PD-L1+PanCK+/all cells, p<0.001), (figure 3). There was no correlation between K17 and individual markers.

Conclusions Our findings suggest an inverse trend between K17 expression and clinical outcomes, pending further validation in an expanded patient cohort.

REFERENCES

Ethics Approval This study was approved by the Institutional Review Boards at UW-Madison (IRB 2018–1510, subproject 2021–012) and University of Alabama-Birmingham (IRB-300007835).

Abstract 215 Figure 1 Representative stress keratin 17 (K17) staining patterns in pembrolizumab-treated carcinomas of the cervix. (A) Tumor exhibiting strong (3+) cytoplasmic staining in 20% of the invasive carcinoma component, considered K17high. (B) One-percent strong expressing tumor, considered K17low. (C) Forty-percent strong expressing tumor, considered K17high. (D) Tumor with <5% strong positive staining, considered K17low.
**Abstract 215 Figure 2** Time-to-event endpoint analysis in the combined anogenital cohort (panel A, n=28) vs. pembrolizumab-treated cancers only (panel B, n=19). TTF – time to treatment failure, PFS – progression-free survival, OS – overall survival.

**Abstract 215 Figure 3** Single-cell immune phenotyping analysis. (A) differentially expressed markers relative to clinical outcomes (overall survival (OS) >6 months). (B & C) representative examples of cell phenotyping using artificial neural networks for selected markers. (B) immunofluorescent multi-channel view. (C) cell phenotypes view after artificial neural network training.
### Abstract 215 Table 1

Antibody panel included in multiplex immunofluorescent staining experiment on the Akoya Phenocycler platform.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>N (%)</th>
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<tr>
<td>DAPI</td>
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<td>CD31</td>
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<td>FOXP3</td>
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<td>CD34</td>
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<td>CD163</td>
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<tr>
<td>HLAA</td>
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<td>CD8</td>
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<tr>
<td>SMA</td>
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<tr>
<td>PDL1</td>
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</tr>
<tr>
<td>CD21</td>
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</tr>
<tr>
<td>Pan-Cytokeratin</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Beta Catenin 1</td>
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<td>CD45RO</td>
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</tr>
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<tr>
<td>Granzyme B</td>
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<tr>
<td>HLADR</td>
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<td>ICOS</td>
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<tr>
<td>HIF1A</td>
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<tr>
<td>Cytokeratin 17</td>
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### Abstract 215 Table 2

Patient and tumor characteristic and correlation with stress keratin 17 (K17) status. There were no differences in clinicopathologic characteristics between K17$^{\text{high}}$ vs. K17$^{\text{low}}$ expressing tumors.

<table>
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<th>Characteristic</th>
<th>All (N = 28)</th>
<th>K17$^{\text{high}}$ (N = 15)</th>
<th>K17$^{\text{low}}$ (N = 13)</th>
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<tr>
<td>Age at ICB, median (years, range)</td>
<td>47.0 (29-76)</td>
<td>47.0 (29-71)</td>
<td>47.0 (32-76)</td>
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<tr>
<td>Female gender</td>
<td>26 (92.9)</td>
<td>14 (93.3)</td>
<td>12 (92.3)</td>
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<td>Anatomic location</td>
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<td></td>
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<td>anal</td>
<td>6 (21.4)</td>
<td>2 (33.3)</td>
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</tr>
<tr>
<td>cervical</td>
<td>19 (67.9)</td>
<td>11 (73.3)</td>
<td>8 (61.5)</td>
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<td>vulvar</td>
<td>3 (10.7)</td>
<td>2 (13.3)</td>
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<td>p16 status</td>
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<td>positive</td>
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<td>adenoid cystic</td>
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<td>nivolumab</td>
<td>7 (25.0)</td>
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<td>4 (30.8)</td>
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<td>pembrolizumab</td>
<td>18 (64.3)</td>
<td>11 (73.3)</td>
<td>7 (53.8)</td>
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<td>pembrolizumab + CHF + bevacizumab</td>
<td>3 (10.7)</td>
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<td>Primary</td>
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<td>5 (38.5)</td>
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<td>Lymph node/distant metastasis</td>
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<td>Prior radiation to study tissue</td>
<td>11 (39.2)</td>
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<td>PD-L1 expression (CPS)</td>
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<td>&lt;1</td>
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<td>≥1</td>
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<td>not available</td>
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<td>Median TTF, months (95% CI)</td>
<td>3.44 (1.44-5.44)</td>
<td>2.98 (1.86-4.10)</td>
<td>3.74 (2.54-12.14)</td>
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<td>Median PFS, months (95% CI)</td>
<td>4.75 (1.95-7.55)</td>
<td>4.23 (2.05-8.31)</td>
<td>5.67 (3.23-8.106)</td>
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<tr>
<td>Median OS, months</td>
<td>6.96 (6.15-11.81)</td>
<td>8.56 (4.92-12.20)</td>
<td>19.41 (NC)</td>
</tr>
</tbody>
</table>

### Abstract 215 Table 3

Clinical outcomes data for the TMA cohort undergoing single cell immunophenotyping on the Akoya Phenocycler platform. Seven patients undergoing immune-checkpoint blockade (ICB) therapy at UW-Madison had sufficient tissue available for construction of a TMA and subsequent analysis.

<table>
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<td>Female</td>
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<td>Histology</td>
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<td>ICB regimen</td>
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<td>5 (71.4)</td>
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<tr>
<td>pembrolizumab</td>
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<td>Median TTF, months (95% CI)</td>
<td>3.96 (2.47-5.44)</td>
</tr>
<tr>
<td>Median PFS, months (95% CI)</td>
<td>9.10 (3.95-14.26)</td>
</tr>
<tr>
<td>Median OS, months (95% CI)</td>
<td>6.14 (0.98-11.29)</td>
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</table>

ICB - immune checkpoint blockade, PD-L1 - programmed death ligand 1, CHF - chemotherapy.