Molecular profiling of non-urothelial bladder cancer: adenocarcinoma and squamous cell carcinoma

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Abstract#423

Background: Adenocarcinoma (ADA) and squamous cell carcinoma (SCC) are rare and often aggressive histologic subtypes of bladder cancer. For advanced disease, no clear standard therapies exist and NCCN guidelines suggest only fluoropyrimidines, paclitaxel and fotemustine as possible options. Thus, novel therapy based on underlying tumor biology is needed. The purpose of this study was to identify potential targets and therapeutic options for these histologic subtypes, utilizing multiplatform tumor profiling.

Methods: 49 ADA and 24 SCC specimens were tested via a multiplatform profiling service (Carlis Life Sciences, Phoenix, AZ) consisting of gene sequencing (Sanger or next generation sequencing (NGS), gene amplification (CISH or FISH), and protein expression (immunohistochemistry (IHC). Tissue from a metastatic site was included in 21 of the cases.

Results: Both ADA and SCC exhibited high rates of TP53 aberrations (82.4% and 72.7%, respectively). Sequencing revealed mutations in BRCA1 (24.4%), MADAD (12.5%), PTEN (11.8%), KRAS (11.8%), NRAS (11.8%), and FLT3 (0.3%) in ADA. In addition, PIK3CA (21.4%), HRAS (18.2%), BCRA (16.7%), BRCA2 (16.7%), and FBXW7 (9.1%) mutations were detected in SCC. Amplification in EGFR (27.3%) and ERBB2/HER2 (16.7%) were found in ADA, whereas only one ERBB2 (6.3%) amplification was found in SCC using ISH.

Conclusions: The sequence of gains and losses, amplification, and protein expression were found between ADA and SCC, and TP53 was the most altered gene. These data may be useful in the future analysis of these malignancies.

Results (continued)

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<th>Biomarker</th>
<th>Percent</th>
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<tr>
<td>BRCA1</td>
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<td>3.2%</td>
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<tr>
<td>BRCA2</td>
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<td>0.5%</td>
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<tr>
<td>ERBB2</td>
<td>11.8%</td>
<td>0.5%</td>
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<tr>
<td>MET</td>
<td>16.7%</td>
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**Results (continued)**

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**Table 1 – Patient Specimen Information.** Bladder specimens were from either TURBT or local biopsies. No formal staging data was available. Remaining data from distant metastatic sites. Only one adenocarcinoma specimen was confirmed as urothelial in origin. No information was available regarding prior history of chemoablation in the SCC cohort.

Reference:

1. Source: Fox Chase Cancer Center, Philadelphia, PA; 2Carlis Life Sciences, Phoenix, AZ

**Figure 1 – Protein overexpression of bladder adenocarcinoma.** EGFR overexpression was detected in all specimens analyzed. High protein levels of drug pumps (BCRP, MRP1) may explain resistance to cytotoxic therapies. PD-1, a marker for tumor infiltrating lymphocytes (TIL), was 44.4%, with PD-L1 overexpression being detected in 11.3% of specimens analyzed. Other potentially predictive biomarkers are shown. HER2 overexpression was detected in 8.2%.

**Table 2 – Gene copy number alterations in bladder adenocarcinoma.** EGFR amplification was detected in 27.3% of specimens evaluated. In addition, ERBB2/HER2 (16.7%) was found in 27.3% of specimens evaluated. In addition, ERBB2/HER2 (16.7%) was detected in SCC. Amplification in EGFR (27.3%) and ERBB2/HER2 (16.7%) were found in ADA. Other potentially predictive biomarkers are shown. HER2 overexpression was not detected.

**Table 3 – Gene copy number alterations in bladder SCC.** EGFR amplification was detected in 27.3% of specimens evaluated. In addition, ERBB2/HER2 (16.7%) was detected in 27.3% of specimens evaluated. In addition, ERBB2/HER2 (16.7%) was detected in SCC. Amplification in EGFR (27.3%) and ERBB2/HER2 (16.7%) were found in ADA. Other potentially predictive biomarkers are shown. HER2 overexpression was not detected.

**Conclusions**

- **Multi-omic profiling can identify differences in the underlying biology of adenocarcinoma and squamous cell carcinoma of the bladder.**
- **In adenocarcinoma, comparatively high ERBB2 and EGFR should be evaluated further in clinical trials with newer pan-HR therapies given previous negative studies using other HER-targeted therapy.**
- **The higher rate of dysregulation along the PIK3CA/AKT/mTOR pathway in bladder SCC warrants further investigation.**
- **Targeting the PD-L1/PD-1 axis in non-urothelial bladder cancer is worthy of clinical trial investigation.**
- **Future analyses of these malignancies should include emerging markers, such as TSCI and FGFR3.**

**Table 4** – Comparison of bladder adenocarcinoma and SCC with urothelial carcinomas. This table shows biomarkers that are significantly different (p < 0.05) between the non-urothelial carcinoma specimens in the cohort and 221 consecutive urothelial bladder carcinomas culled from the Caris Life Sciences database. Most differences were identified using either Sanger or NGS in the IHC panels with the exception of TP53 by IHC in the comparison between bladder adenocarcinoma and urothelial bladder carcinomas. Fewer differences are observed in bladder squamous cell carcinoma, perhaps due to the low overall number of SCC available for comparison.

**Table 5** – Comparison of bladder adenocarcinoma and SCC with urothelial carcinomas. This table shows biomarkers that are significantly different (p < 0.05) between the non-urothelial carcinoma specimens in the cohort and 221 consecutive urothelial bladder carcinomas culled from the Caris Life Sciences database. Most differences were identified using either Sanger or NGS in the IHC panels with the exception of TP53 by IHC in the comparison between bladder adenocarcinoma and urothelial bladder carcinomas. Fewer differences are observed in bladder squamous cell carcinoma, perhaps due to the low overall number of SCC available for comparison.

**Figure 2 – Sequencing analysis using either Sanger or NGS in bladder adenocarcinoma.** A high percentage (82.4%) of TP53 aberrations were detected in the adenocarcinoma cohort. Other aberrations were also detected by sequencing, with some being potentially targeted. In contrast, PIK3CA aberrations were detected in 21.4%, and 8.3% of specimens. Most of the 47 genes analyzed in this cohort showed no aberrations. These included the following: ARB1, AKT1, AKT2, AKT3, AR, BCR, BCR1, BRCA1, BRCA2, CDH1, CSK, CTR, CTNNB1, ERBB2, FBXW7, FGF1, FGFR1, FUS, GNAS, GNAS1, GNAS2, GNAS3, HNF1A, HRAS, IKB1, JAK2, KDR, KRAS, KIT, LST1, MET, MIR15, NOTCH1, NPM1, PDCD1, PTEN, PTPN11, RBL1, RET, SMAD2, SMAD1, SMAD4, STK11, and VHL.

**Figure 3 – Protein overexpression of bladder squamous cell carcinoma.** PD-L1 expression was detected in 44.4%, with PD-L1 overexpression being detected in 22.2% of adenocarcinoma specimens analyzed, which is double the rate of SCC.

**Figure 4 – Sequencing analysis using either Sanger or NGS in bladder SCC.** A high percentage (82.4%) of TP53 mutation rates are observed. In contrast, the higher of aberrations was detected by sequencing, with some being potentially targeted. A high rate of overexpression was also detected. Other potentially targeted aberrations were also found. Most of the 47 genes analyzed in this cohort showed no aberrations. These included the following: ARB1, AKT1, AKT2, AKT3, AR, BCR, BCR1, BRCA1, BRCA2, CDH1, CSK, CTR, CTNNB1, ERBB2, FBXW7, FGF1, FGFR1, FUS, GNAS, GNAS1, GNAS2, GNAS3, HNF1A, HRAS, IKB1, JAK2, KDR, KRAS, KIT, LST1, MET, MIR15, NOTCH1, NPM1, PDCD1, PTEN, PTPN11, RBL1, RET, SMAD2, SMAD1, SMAD4, STK11, and VHL.

**Table 6** – Comparison of molecular profiling in referring urothelial carcinomas of bladder and nonurothelial carcinomas. **Table 5** – Comparison of bladder adenocarcinoma and SCC with urothelial carcinomas. This table shows biomarkers that are significantly different (p < 0.05) between the non-urothelial carcinoma specimens in the cohort and 221 consecutive urothelial bladder carcinomas culled from the Caris Life Sciences database. Most differences were identified using either Sanger or NGS in the IHC panels with the exception of TP53 by IHC in the comparison between bladder adenocarcinoma and urothelial bladder carcinomas. Fewer differences are observed in bladder squamous cell carcinoma, perhaps due to the low overall number of SCC available for comparison.