Comparison of Immuno-Oncology Biomarkers in Adenocarcinoma, Urothelial Carcinoma, and Squamous Cell Carcinoma of the Bladder

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ABSTRACT

Background: Given the rarity of the disease, the genomic features of urinary bladder adenocarcinoma (ABC) are poorly characterized to date. In this comparative comprehensive genomic profiling (CGP) study, the genomic alterations (GA) in ABC are contrasted with those found in classic bladder urothelial carcinoma (UBC) and squamous cell carcinoma pf the bladder (SCCB).

Methods: 143 cases of ABC, 2,142 cases of UCB, and 83 cases of SCCB were subjected to CGP using a hybrid-capture based commercial CGP assay to evaluate the four classes of genomic alterations. Tumor mutational burden (TMB) was determined on 0.83-1.14 Mbp of sequenced DNA and microsatellite instability (MSI) was determined on 114 loci. PD-L1 expression was determined by IHC (DAKO 22C3 antibody).

Results: As seen in the Table, the ABC patients were significantly younger and less often female than the UBC patients (P<0.0001). Possibly reflecting the higher tobacco exposure in these patients, UBC had a higher GA/tumor than ABC. The most frequently altered untargetable (CR) GA varied with TP53, KRAS and APC GA more frequent in ABC and TERT, CDKN2A/B and DNA-repair genes (ARID1A and KDM6A) more frequently altered in UBC. Targetable MTOR pathway GA (PIK3CA, TSC1, PTEN) were more frequent in UBC as were targetable kinase alterations (FGFR3 and ERBB2). Targetable kinase GA in ERBB2, MET and EGFR were noteworthy in ABC. The UBC group had a significantly higher TMB than ABC (P<0.0001) including mean TMB and TMB>20 mut/Mb (P<0.0001). MSI high status was very uncommon in both tumor types.

Conclusions: Deep sequencing reveals that ABC features a widely different genomic profile from UBC and SCCB. CGP uncovers less frequent opportunities for both targeted therapies and immunotherapies in ABC than UBC, and less frequent opportunities for targeted therapies in SCCB than UCB Nonetheless, ABC does feature potential kinase targets such as FGFR3 and ERBB2. In addition, there are biomarkers predictive of immunotherapy benefit in a subset of ABC cases albeit less frequent that that identified in UBC cases.
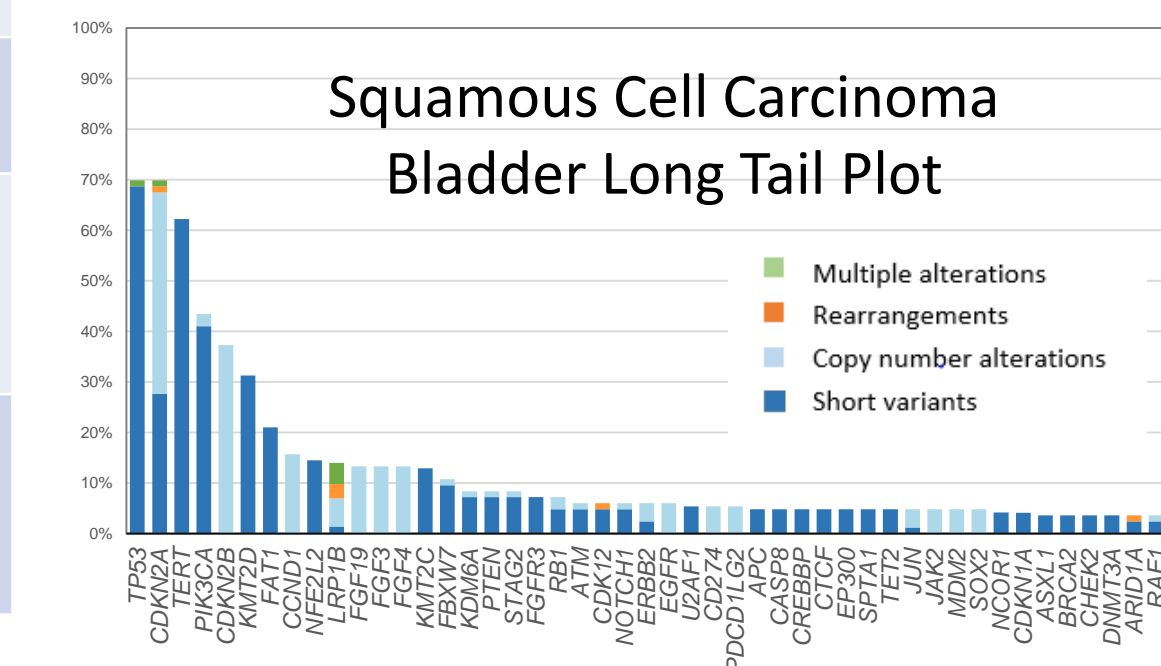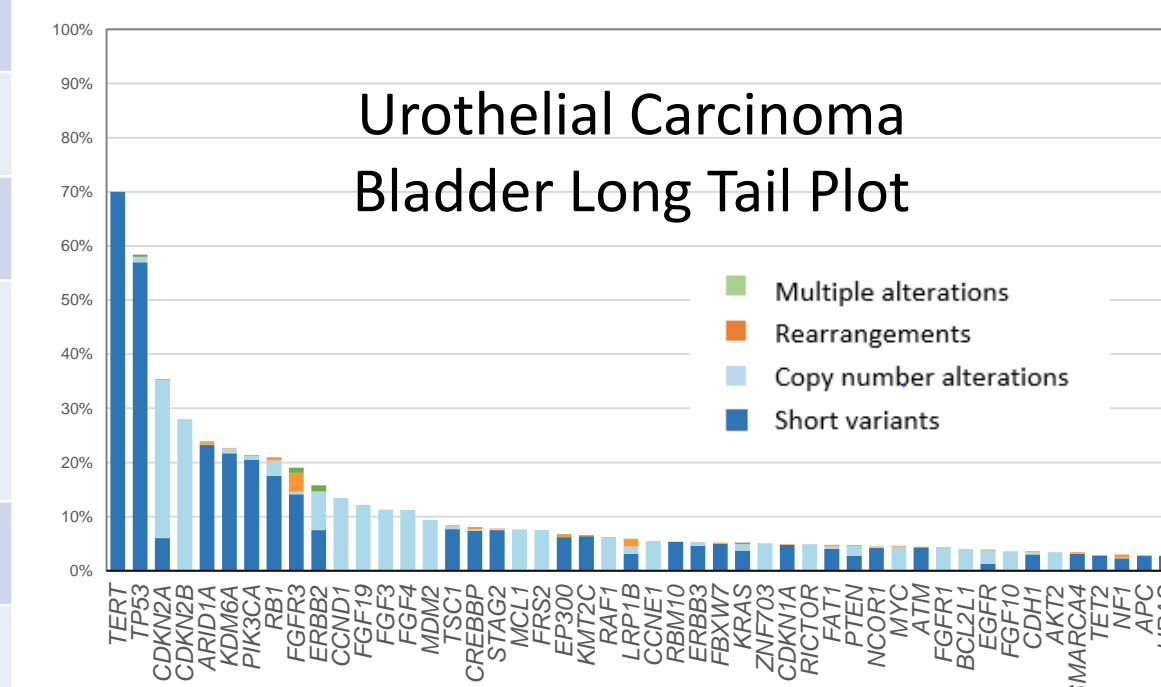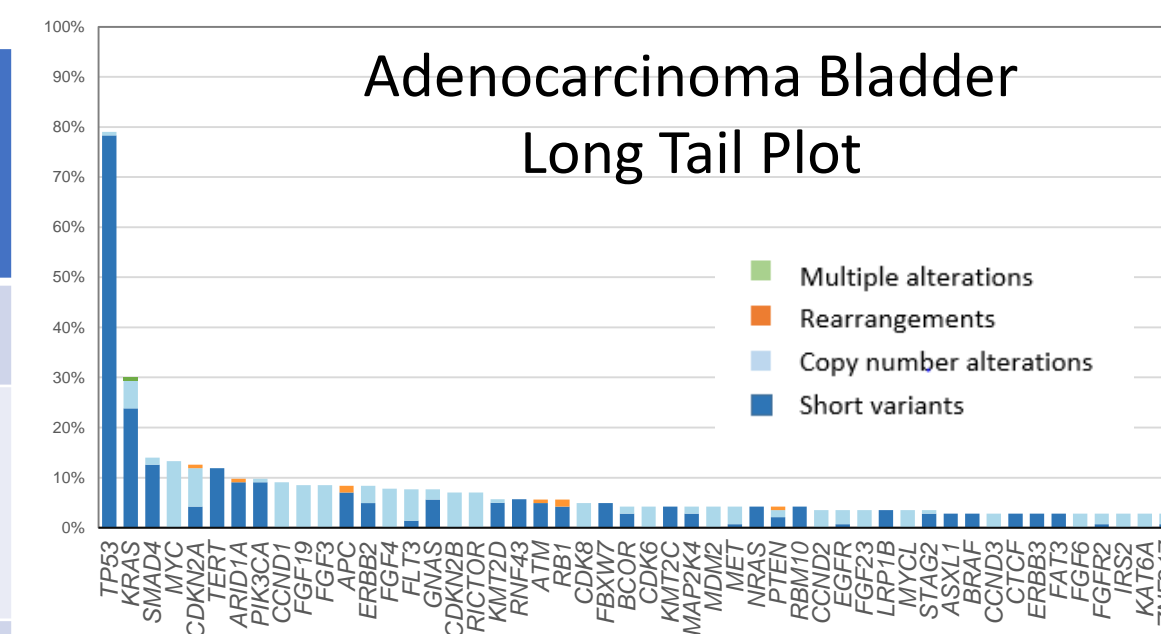
MATERIALS AND METHODS
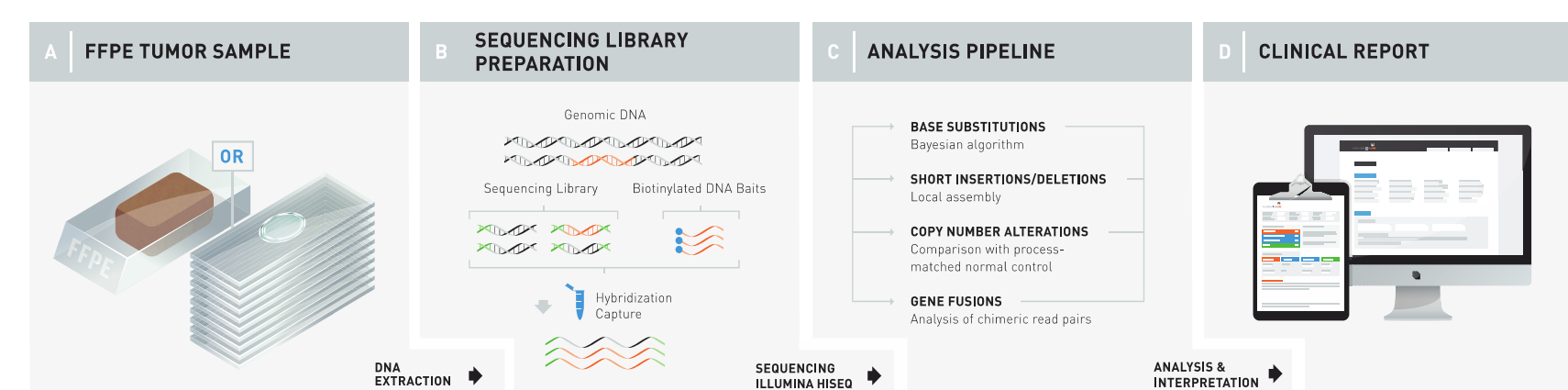
- ≥50 ng DNA extracted from 40 μm of FFPE sections
- Sequencing performed for up to 315 cancer-related genes and introns from 28 genes commonly rearranged in cancer
- Hybrid capture-based sequencing using adaptor ligation-based libraries
- Mean coverage depth >600X
- Base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes were assessed
- Tumor mutational burden (TMB) calculated from 0.83-1.14 Mb sequenced DNA
- PD-L1 expression was determined by IHC (DAKO 223C antibody)
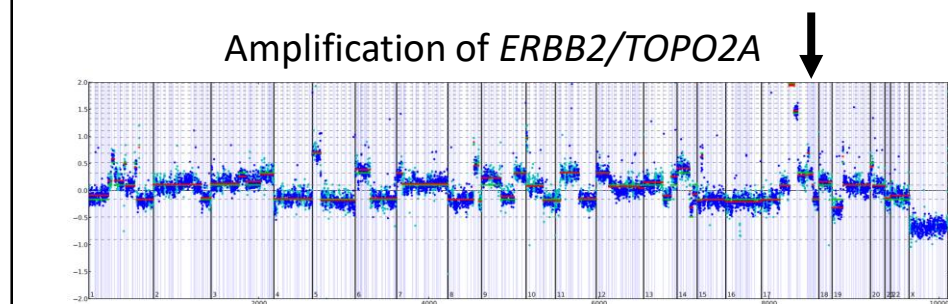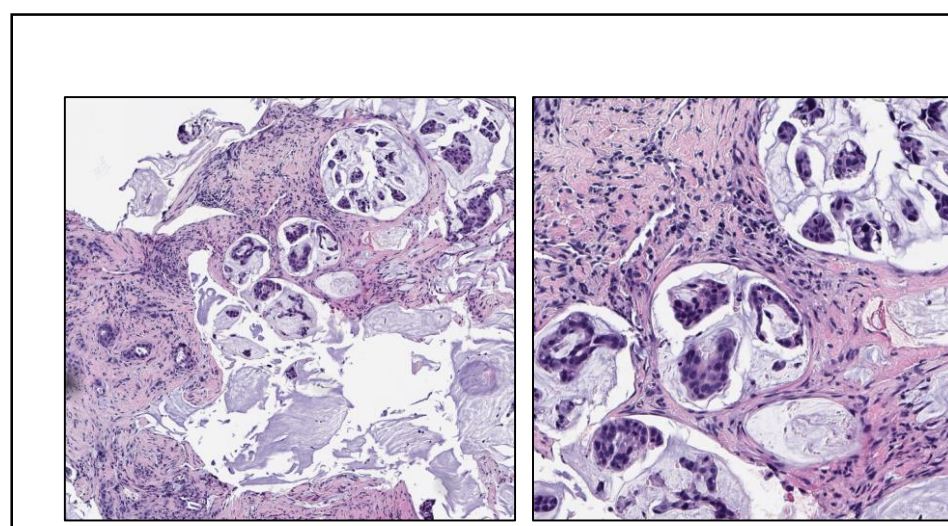
RESULTS

- ABC patients were significantly younger and less often female than UBC patients and SCCB patients (P<0.0001)
- UBC and SCCB had a higher GA/tumor than ABC
- The most frequently altered untargetable GA varied with TP53, KRAS and APC GA more frequent in ABC and TERT, CDKN2A/B more frequently altered in UBC and SCCB
- DNA-repair genes (ARID1A and KDM6A) were most frequently altered in UBC
- Targetable MTOR pathway GA (PIK3CA, TSC1, PTEN) were more frequently identified in UBC and SCCB than ABC
- Targetable kinase alterations (FGFR3 and ERBB2) were most frequent in UBC, but targetable kinase GA in ERBB2, MET and EGFR were still noteworthy in ABC and SCCB
- The UBC and SCCB groups had significantly higher TMBs than ABC (P<0.0001) including mean TMB and TMB>20 mut/Mb (P<0.0001)
- MSI high status was very uncommon in all three tumor types
- UBC and SCCB tumor cells and TILS more frequently express PD-L1 on IHC staining

	Adenocarcinoma (ABC)	Urothelial Carcinoma (UBC)	Squamous Cell Carcinoma (SCCB)
Cases	143	2,142	83
Median Age (Range) In years	58 (24-83)	67 (19-88)	62 (31-88)
Males/Females	57/86	1597/545	45/38
GA/tumor	5.4	7.7	8.2
MSI High	2/106 (2%)	11/1661 (1%)	1/69 (1%)
CD274 (PD-L1) gene amplification	0 (0%)	17 (1%)	4 (5%)
Mean TMB	2.4 mut/Mb	9.9 mut/Mb	10.4 mut/Mb
TMB > 10 mut/Mb	14 (10%)	697 (32%)	26 (31%)
TMB > 20 mut/Mb	4 (3%)	243 (11%)	13 (16%)
PD-L1 IHC Positive Tumor Cells	2/11 (18%)	76/244 (31%)	3/10 (30%)
PD-L1 IHC Positive TILs	0/11 (0%)	74/244 (29%)	3/10 (30%)

Adenocarcinoma Bladder Long Tail Plot

- Multiple alterations
- Rearrangements
- Copy number alterations
- Short variants

Urothelial Carcinoma Bladder Long Tail Plot

- Multiple alterations
- Rearrangements
- Copy number alterations
- Short variants

Squamous Cell Carcinoma Bladder Long Tail Plot

- Multiple alterations
- Rearrangements
- Copy number alterations
- Short variants

CASES

Amplification of ERBB2/TOPO2A

Adenocarcinoma of the bladder in a 79 year old Caucasian female that progressed to stage IV disease. CGP revealed major ERBB2 amplification at 112 copies/cell. TOPO2A was also co-amplified. Additional alterations included short variant mutations in ATM, APC, STAG2, TERT, and TP53. Amplification of ERBB2 has been found in 6.2% of bladder urothelial carcinoma cases. The ERBB2 amplification frequency in bladder adenocarcinoma found in this study and in the literature is similar to that for urothelial carcinoma. In one study, HER2 overexpression was identified in 19% of bladder cancers, with significant enrichment in grade 3 and muscle invasive tumors. Overexpression of HER2 has not been confirmed as an adverse prognostic factor in bladder cancer. As widely demonstrated, ERBB2 amplification may predict sensitivity to therapies targeting HER2, including antibodies such as trastuzumab, pertuzumab, ado-trastuzumab emtansine (T-DM1) and dual EGFR/HER2 kinase inhibitors such as lapatinib, afatinib, neratinib and dacomitinib. In patients with bladder cancer, concurrent PIK3CA or PTEN alterations that activate the PI3K pathway have been associated with resistance to therapies that target HER2.

Amplification of MET
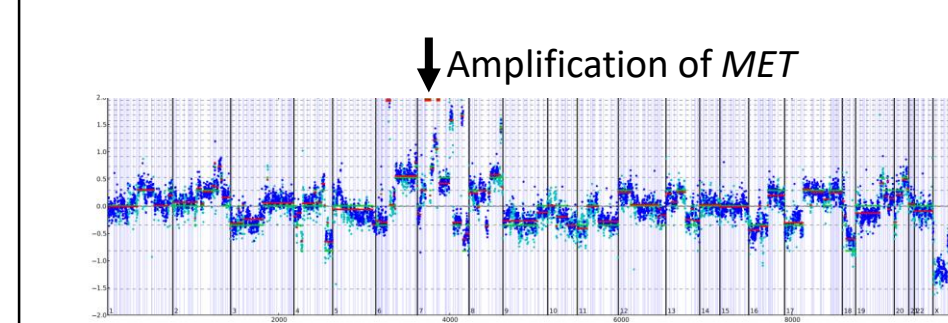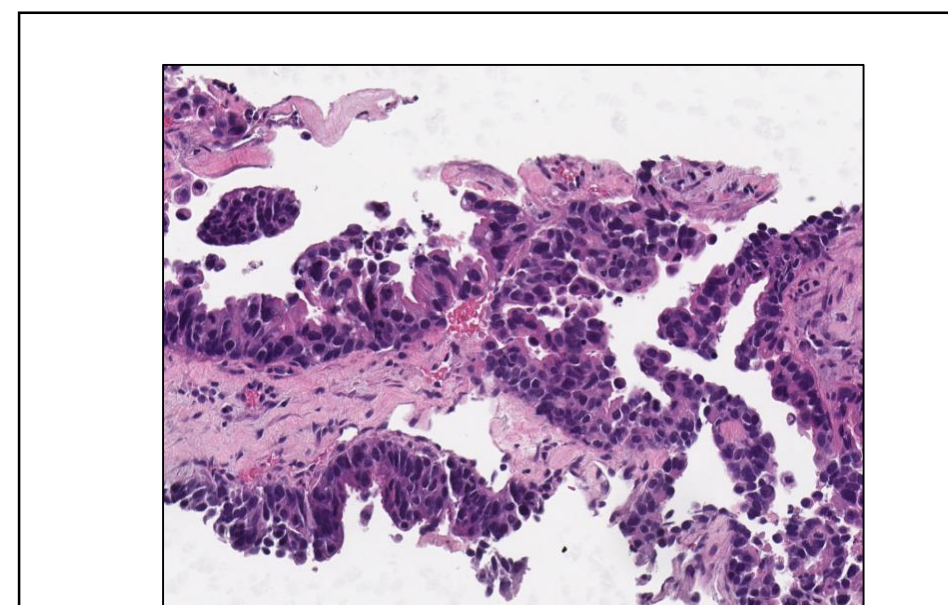
Needle biopsy of the liver in a patient with Stage IV adenocarcinoma of the bladder in a 78 year old Caucasian man. Comprehensive genomic profiling revealed amplification of CCND3, EGFR, MET (13 copies), VEGFA, CDK6 HGF and MYC. Additional short variant variations were found in FOXP1, TBX3 and TP53. MET activation associated with high level gene amplification results in signaling mediated partly by the RAS-RAF-MAPK and PI3K pathways to promote proliferation. High-level amplification of MET has been reported in 2% of bladder carcinomas. Overexpression of Met at the has been reported in 5-45% of urothelial carcinomas. MET overexpression in bladder carcinoma has been shown to be correlated with poor long-term survival, higher pathological stage and tumor grade. Strong evidence suggests that MET activation may predict sensitivity to targeted therapies such as crizotinib and cabozantinib. Strong clinical data also suggest sensitivity of MET-altered tumors to various other MET inhibitors in clinical development.

CONCLUSIONS

- Deep sequencing reveals that ABC features a widely different genomic profile from UBC, while SCCB harbors some similarities and some differences when compared to ABC
- CGP uncovers less frequent opportunities for both targeted therapies and immunotherapies in ABC than for UBC, and less frequent opportunities for targeted therapies in SCCB than UBC
- Nonetheless, ABC and SCCB do feature potential kinase targets such as FGFR3 and ERBB2
- In addition, there are biomarkers predictive of immunotherapy benefit in a high number of SCCB cases and a subset of ABC cases albeit less frequent that that identified in UBC cases