Molecular profiling of infiltrating urothelial carcinoma of the bladder

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ABSTRACT #311

Background: Infiltrating urothelial carcinoma (UC) is the most common variant of urinary bladder cancer. The prognosis for muscle infiltrating or metastatic UC of the bladder is poor with no major advances made in the last 20 years. We investigated a large cohort of such patients for specific genetic/biomarker alterations and compared them to other, less common urothelial malignancies.
Methods: We reviewed 602 cases; 518 cases (86%) were locally advanced or metastatic UCs of the bladder and the remaining 84 cases (14%) were non-bladder UCs. Multiple methodologies for optimal assessment of biomarker expression (Caris Molecular Intelligence™, Caris Life Sciences, Phoenix, AZ) were employed: Mutation analysis (Next-generation sequencing, Sanger, pyrosequencing, qPCR, RFLP), *in situ* hybridization (fluorescent and chromogenic), immunohistochemistry, and RNA fragment analysis.
Results: Bladder UC showed slightly higher rates of HER2 gene amplification (12% in bladder vs. 6% non-bladder, p=0.32) and EGFR gene amplification (22% vs. 12%, p=0.25). HER2 and *EGFR* protein expressions were more common in the bladder than in non-bladder sites (10% vs. 1%, p=0.03, and 77% vs. 60%, p=0.5, respectively). Pathogenic mutations in *HER2* and *EGFR* were rare. Although cKIT and cMET receptor kinases were more frequently overexpressed in bladder than in non-bladder cancers (10% vs. 6% and 25% vs. 8%, respectively), activating mutations were also rare. *PIK3CA* and/or *PTEN* mutations were more frequently observed in non-bladder (27%) than in bladder UCs (21%). Non-bladder UC harbored high *FGFR3* gene mutation (33%), which was not observed in any of the UC of the bladder (p=0.02). *TP53* gene mutations were frequently identified in both bladder and in non-bladder cancers (49% vs. 27%, respectively, p=0.15), while *KRAS* was frequently mutated in the bladder adenocarcinomas (56%, p<0.001). Other therapeutically targetable biomarkers over-expressed in bladder UC compared to non-bladder UC included androgen receptor (16% vs. 8%, p=0.07), and MGMT (63% vs. 47%).
Conclusions: Comprehensive molecular profiling of urothelial carcinoma identifies a number of potentially actionable targets, which can be managed by the novel treatment modalities.

Demographics

Category	Total Cases	Gender distribution		Primary vs. Metastatic distribution		Age Distribution		IQR
UC, bladder	463	74%	Male	59%	Primary	66.3	mean	60-74
		26%	Female	41%	Met	66.9	median	
UC, Non-bladder	74	65%	Male	63.5%	Primary	68.5	mean	62-76
		35%	Female	36.5%	Met	69.5	median	
Adenocarcinoma	27	48%	Male	59.3%	Primary	60.7	mean	53-68
		52%	Female	40.7%	Met	62.6	median	
Small cell, Neuroendocrine	17	71%	Male	58.8%	Primary	71.3	mean	65-81
		29%	Female	41.2%	Met	67.2	median	
Squamous cell carcinoma	11	64%	Male	45.5%	Primary	64.6	mean	60-71
		36%	Female	54.5%	Met	65.2	median	

Table 1. UC – urothelial carcinoma; IQR – interquartile range.

Results: Immunohistochemical profiling

Histotype/ IHC marker	UC, Bladder (%)	UC, Non-bladder (%)	*p*-value	Adeno-carcinoma (%)	p-value	Small cell carcinoma (%)	p-value	Squamous cell carcinoma (%)	*p*-value
AR	16.2	6.2	**0.04**	3.8	0.16	11.8	1.0	9.1	1.0
ER	1.9	1.6	1.0	0.0	1.0	0.0	1.0	27.3	**.002**
PR	2.6	1.6	1.0	4.0	0.50	17.6	**.013**	0.0	1.0
cKIT	10.3	4.8	0.40	11.8	0.69	37.5	**0.05**	0.0	1.0
cMET	25.2	8.3	0.11	44.4	0.24	0.0	0.20	0.0	1.0
EGFR	77.4	50.0	0.25	0.0	0.24	0.0	0.24	100.0	1.0
HER2	10.2	3.1	**.018**	3.8	0.50	0.0	0.39	0.0	0.61
MGMT*	62.9	43.3	**.0030**	69.2	0.68	20.0	**.0017**	40.0	0.19
TLE3	23.9	11.1	0.20	22.2	1.0	0.0	0.20	33.3	0.57
TOPO2α	68.1	71.9	0.64	76.2	0.63	92.3	0.07	57.1	0.68
TUBB3*	39.0	23.5	0.39	80.0	0.15	80.0	0.15	0.0	1.0
PTEN*	63.0	56.7	0.53	37.5	0.26	25.0	0.71	25.0	1.0
PGP	27.2	15.5	0.07	45.5	0.09	7.7	0.2	11.1	0.4
RRM1	32.1	10.8	**<.0001**	33.3	1.0	62.5	**0.02**	22.2	0.7
SPARC	68.7	33.3	**<.001**	19.2	**<.0001**	35.3	**0.01**	9.1	**<.0001**
TOPO1	63.3	38.7	**<.0004**	32.0	**<.003**	81.3	0.2	44.4	0.30
TS	17.0	22.2	0.37	12.5	0.80	47.1	**0.005**	11.1	1.0

Table 2. *Expression of the biomarker below the threshold is considered predictive of response to therapy. TS = Thymidylate synthase; SPARC1 = Osteonectin; RRM1=Ribonucleotide reductase M1; PGP=P-glycoprotein;TLE3=transducin-like enhancer of split 3; MGMT=0-6-methylguanine DNA methyltransferase; TUBB3=tubulin beta 3.

Sequencing (NGS and Sanger)

Gene	UC, Bladder	UC, Non-bladder	Small cell carcinoma	Squamous cell carcinoma	Adenocarcinoma
APC	2/45 (4.4%)	0/16	0/4	0/1	0/5
BRAF	1/120 (0.8%)	0/24	0/9	0/3	0/7
CDH1	3/47 (6.4%)	0/16	0/4	0/1	0/5
cKIT	1/78 (1.3%)	0/20	0/9	0/1	0/6
cMET	1/47 (2.1%)	0/16	0/4	0/1	0/5
EGFR	1/53 (1.9%)	1/19 (5.3%)	1/5 (20%)	0/1	0/5
HER2	1/47 (2.1%)	0/16	0/4	0/1	0/5
FBXW7	2/47 (4.3%)	2/16 (12.5%)	0/4	0/1	0/5
FGFR3	0/21	3/10 (30.0%)*	0/3	0/1	0
HNF1A	0/41	1/16 (6.3%)	0/2	0/1	0/4
HRAS	0/42	1/16 (6.3%)	0/4	0/1	0/5
KDR	1/47 (2.1%)	0/16	0/4	0/1	0/5
KRAS	5/135 (3.7%)	0/24	0/11	0/3	5/9 (55.6%)*
PIK3CA	19/113 (16.8%)	5/22 (22.7%)	0/8	0/3	1/8 (12.5%)
PTEN	4/47 (8.5%)	1/16 (6.3%)	0/4	0/1	0/5
RB1	2/46 (4.3%)	0/16	0/4	0/1	0/5
SMAD4	1/47 (2.1%)	0/16	0/4	0/1	0/5
STK11	1/45 (2.2%)	0/16	0/4	0/1	0/5
TP53	23/46 (50.0%)	3/14 (21.4%)*	4/4 (100%)	0/1	1/4 (25%)

Table 3. Mutation frequency (%) profile of different subtypes of urothelial carcinomas. *Significantly higher (*P*<0.05) in comparison with UC of the bladder.

In situ hybridization

Gene/ISH positivity rate (%)	UC bladder (n=463)	UC non-bladder (n=84)	*p-value 1 vs. 2*	Small cell carcinoma (n=17)	Squamous cell carcinoma (n=11)	Adenocarcinoma, bladder (n=27)	*p-value 1 vs. 5*
HER2	33/284 (11.6%)	1/43 (2.3%)	*0.0640*	0/8 (0%)	0/3 (0%)	2/12 (16.7%)	*0.6401*
EGFR	44/198 (22.1%)	3/31 (9.7%)	*0.1503*	2/7 (28.6%)	1/3 (27.3%)	3/11 (28.6%)	*0.7134*
cMET	1/75 (1.3%)	0/11 (0%)	*1.0*	0/3 (0%)	0/3 (0%)	0/6 (0%)	*1.0*
TOPO2A	2/50 (4%)	0/8 (0%)	*1.0*	0/4 (0%)	0/4 (0%)	0/3 (0%)	*1.0*

Table 4. HER2 FISH: HER2:CEP 17 signal ratio of >=2.0 is amplified and <2.0 is not amplified; 1.8-2.2 is equivocal. cMET CISH: >= 5 copies is amplified; TOP2A:CEP17 signal ratio of >=2.0 is amplified; EGFR: ≥ 4 copies in ≥ 40% of tumor cells.

Co-incidences of biomarker alterations in bladder UC

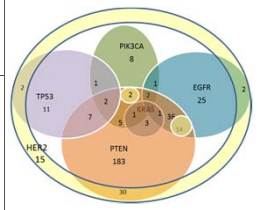


Figure 1. Venn diagram of concurrent relationships in biomarker protein expression, gene amplification, and/or mutations for *PIK3CA, PTEN, HER2, EGFR, KRAS,* and *TP53. HER2* results include ISH amplifications and gene mutations; *PIK3CA* results include FISH amplifications and gene mutations; *PTEN* results include IHC loss and gene mutations; *TP53, KRAS* results include gene mutations; *EGFR* results include FISH amplifications, IHC overexpression, and Sanger/NGS mutations.

Drug association heat map

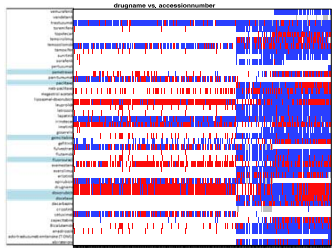


Figure 2. Drug association heat map of UC bladder using Caris Molecular Intelligence recommendation based on biomarker status. Highlighted rows = NCCN recommendation (6 of 43). Red = recommendation for benefit, gray = indeterminate, and blue = recommendation for lack of benefit from indicated therapy.

Patient with response to Caris recommended treatment, based on molecular profiling

Case 1, UC, bladder. HER2 positive by IHC, HER2 amplified. Patient was treated with lapatinib, an oral HER2 inhibitor 1500mg po daily plus MM-111, an intravenous, irreversible HER2 inhibitor at 20mg/kg iv q 3wks (US Oncology protocol, phase 1 trial). Response was mixed with some nodes regressing and others stable for 4 mos.

Case 2, UC, non-bladder. HER2 amplified. Patient was treated with MM111 (567mg weekly; (US Oncology protocol, phase 1 trial), trastuzumab (225mg reduced to 113mg weekly) and Taxol (80mg/m^2 weekly) with an objective response in the lung lesions. CT showed a significant decrease in all lung lesions (fig. 3).

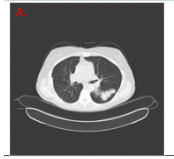
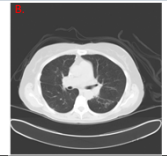


Figure 3. Lung scans, Case 2. A. Before, and B. after treatment.



Conclusions

- Comprehensive molecular profiling of urothelial carcinoma utilizing multiple technologies identifies a number of actionable targets that could lead to personalized therapy using both NCCN recommended therapies AND therapies not currently approved for UC, but approved for other tumor types (6. vs **37** potential *additional* therapies, fig. 2).
- *EGFR* and *HER2* gene mutations were uncommon: the identification of concurrent amplification of *EGFR* and HER2 genes suggests that combination therapy might be utilized to overcome HER2 resistance in urothelial carcinoma.
- *PIK3CA* and/or *PTEN* alterations were commonly seen in both non-bladder and bladder urothelial carcinomas, while *KRAS* mutations predominantly affected the bladder adenocarcinomas.
- cKit and cMet receptor kinases were overexpressed more commonly in bladder than in non-bladder cancers (11% vs. 5% and 25% vs. 8%, respectively); however, activating mutations were rare.
- Other targetable biomarkers significantly overexpressed in bladder UC compared with non-bladder UC included androgen receptor (16% vs. 6%), MGMT (63% vs. 43%), RRM1 (32% vs. 11%), SPARC (69% vs. 33%) and TOPO1 (63% vs. 39%).

References

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