Abstract n.4581. Comprehensive genomic profiling (CGP) of upper-tract (UTUC) and bladder (BUC) urothelial carcinoma reveals opportunities for therapeutic and biomarker development

Andrea Necchi[1], Sumanta Pal[2], Jeffrey Ross[3,4], Russell Madison[3], Neeraj Agarwal[5], Guru Sonpavde[6], Monika Joshi[7], Yin Ming[8], Vincent A. Miller[3], P Grivas[9], Jon Chung[3], Siraj M Ali[3]

[1]Fondazione IRCCS - Istituto Nazionale dei Tumori, Milan, IT, [2]City of Hope Comprehensive Cancer Center, Duarte, CA USA [3]Foundation Medicine, Inc. , Cambridge, MA, USA [4]Upstate Medical University, Syracuse, NY, USA, [5]Huntsman Cancer Institute, Salt Lake City, Utah, USA,[6]Dana Farber Cancer Institute, Boston, MA, USA, [7]Penn State Health Milton S. Hershey Medical Center, Hershey, PA, USA, [8]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA, [9]University of Washington, School of Medicine, Seattle, WA, USA

2019 ASCO ANNUAL MEETING

BACKGROUND

UTUC and BUC represent distinct tumor entities that may deserve dedicated therapeutic strategies, in particular with the availability of several clinical studies of targeted therapies or immunotherapy. To understand the genomic landscape and inform the therapeutic development of UC, 2463 cases (479 UTUC and 1984 BUC) were analyzed by CGP for genomic alterations (GAs) and for genome wide signatures.

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 [1,2]
- Tumor mutational burden (TMB) calculated from 1.14 Mb sequenced DNA [1,2]
- Hybrid capture-based genomic profiling of cell-free DNA (cfDNA) was performed on ≥20 ng of cfDNA and sequencing was performed on up to 70 genes (FoundationOne Liquid) [3] to a mean unique coverage depth of >8,000X.
- For comparison of paired tissue and circulating tumor DNA (ctDNA) samples, concordance was evaluated for baited regions common to both CGP assays.
- Targetable GA and signatures were assessed according to the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) [4]

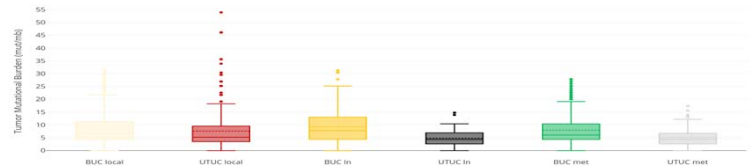
RESULTS

61%/58% primary [PT] and 18%/25% metastatic tumors [MT] from unmatched pts were analyzed. 39% of UC pts overall harbored ≥1 tier 1-2 GA suggesting benefit from approved or investigational targeted therapies (TT). Additionally, 29% had a tier 3 GA that provides a strong rationale for clinical trial consideration. Non-*FGFR3* kinase fusions were observed in 1% of pts (0.6% UTUC v 1.1% BUC), including *BRAF/RAF1* fusions in 0.5%. *BRAF* mut/fusions were observed in 2% (49/2463) of cases and were mutually exclusive with *FGFR3* GA (p=0.002).

In comparing UC from anatomic sites, there were no differences of TMB-H (≥20 mut/mb)/MSI-H for PT and MT but UTUC was enriched for MSI-H (3.4%) relative to BUC (0.77%, p<0.001, all TMB-H). Excluding MSI-H pts, UTUC has lower median TMB (4.35 mut/mb) than BUC (6.96 mut/mb). *FGFR3* GA (26% v 19%, p <0.05) and specifically short variants (SV) (20% v 13%) were enriched in UTUC vs BUC. *HRAS* SV were also enriched in UTUC vs BC (7.3% v 3.0%), attributed to an enrichment in renal pelvis UC (10.1%) v ureteral UC (1.8%, p <0.05). *RB1* GA were more frequent in BUC vs UTUC (21% v 7.8% p <0.001).

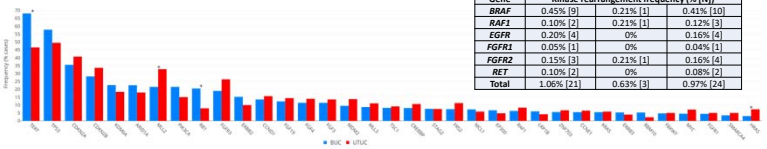
	BUC (n = 1984)	UTUC (n = 479)
M:F	2.82	1.65
Age Median	67	68
TMB Median	6.96 mut/mb	5.23 mut/mb
TMB Mean	9.96 mut/mb	8.29
% MSI-H	0.77%	3.4%
% Local	57.7% (1,146)	61.1% (293)
% Metastatic	25.1% (498)	18.0% (86)
% Lymph Node	9.4% (187)	8.4% (40)
% Unknown	7.7% (153)	12.5% (60)

RESULTS



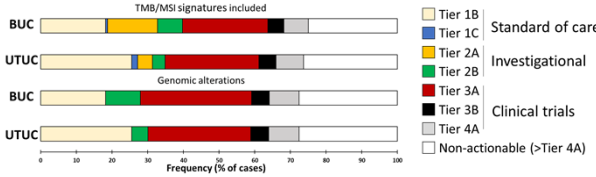
Comparison of TMB in BUC and UTUC by site of biopsy

Gene	BUC (N=1984)	UTUC (N=479)	Total (N=2463)
	Kinase rearrangement frequency (% [N])		
BRAF	0.45% [9]	0.21% [1]	0.41% [10]
RAF1	0.10% [2]	0.21% [1]	0.12% [3]
EGFR	0.20% [4]	0%	0.16% [4]
FGFR1	0.05% [1]	0%	0.04% [1]
FGFR2	0.15% [3]	0.21% [1]	0.16% [4]
RET	0.10% [2]	0%	0.08% [2]
Total	1.06% [21]	0.63% [3]	0.97% [24]



Comparison of genomic alterations in BUC and UTUC

*significant difference between BUC and UTUC; 1% of cases harbored non-*FGFR3* kinase fusions; significant differences between PT and MT were not observed except in *RB1* in BUC (24% v 15%; p = 0.005)

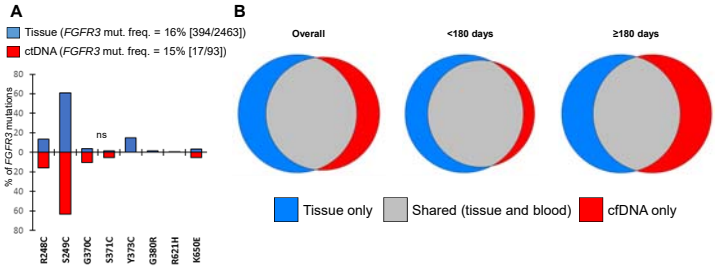


Targetable genomic alterations and signatures identified in BUC and UTUC.

Genomic alterations were ranked using the ESCAT actionability scale. Each case was assigned a tier according to the highest ranked genomic alteration/signature. ESCAT rankings were performed with and without TMB/MSI genomic signatures considered on the actionability scale.

***BRAF* fusions and mutations comprise a potentially targetable genomic subset**

BRAF fusion or mutation was observed in 2% of UC cases and were mutually exclusive with *FGFR3* GAs. Details of *BRAF* fusions and mutations shown.



C

Time interval between tissue/blood collection	Number of cases with matched tissue/blood	Number of tissue only mutations	Number of blood only mutations	Number of shared mutations	Sensitivity (PPA to tissue)	% of all mutations shared	% cases with at least 1 shared mutation
<90 days	7	4	1	13	77%	72%	100% (7/7)
<180 days	10	6	2	16	73%	67%	90% (9/10)
≥180 days	11	9	9	18	67%	50%	55% (6/11)
Overall (median 232 days)	21	15	11	34	69%	57%	71% (15/21)

Comparison of mutations detected by genomic profiling of tissue and blood samples as a function of time.

(A) Unmatched tissue (N=2463) and blood samples with detected ctDNA (N=93) from patients with urothelial cancer were evaluated for *FGFR3* mutation frequency. The distribution of *FGFR3* mutations identified in tissue and blood are shown. ns, not significant.

(B-C) For the 21 patients with matched tissue and blood samples with detected ctDNA, mutations were classified into those found in tissue-only, blood-only, or shared (found in both tissue and blood). Concordance was evaluated as positive-percent agreement (PPA) with tissue as a reference and as % of all detected mutations that were shared.

- Frequency and distribution of targetable *FGFR3* mutations were similar between tissue and ctDNA
- Concordance varied with time interval between tissue and blood collection.
- For samples with a time interval of <180 days between sample collection, there was a 73% PPA to tissue and 90% of cases shared at least 1 mutation.

CONCLUSIONS

- Against a background of 50% actionability in UC with opportunities for immunotherapy, TT, or combinations thereof, the UTUC cohort is enriched for *FGFR3* and *HRAS* SV relative to BUC*HRAS* mutations predominantly in UC of the renal pelvis, that warrants further investigation into the distinct modes of oncogenesis for UC as stratified by anatomic origin.
- Liquid biopsy-based genomic profiling identified targetable *FGFR3* alterations. 73% of mutations present in matched tissue samples were also detected in paired liquid biopsy samples (<180 day time interval).
- These results argue strongly for the routine incorporation of CGP prior to systemic therapy initiation in metastatic UC.

References

1. Frampton GM, Fichtenholtz A, Otto GA, et al. Nat Biotechnol. 2013;31:1023-1031
2. Chalmers ZR, Connelly CF, Fabrizio D. Genome Med. 2017;19;9:34
3. Clark, Chung, Hughes , et al. J Mol Diagn. 2018. PMID: 29936259
4. Mateo J, Chakravarty D, Dienstmann R, et al. Ann Oncol. 2018 Sep 1;29(9):1895-1902