Characterization of Microsatellite Instability (dMMR/MSI-H) and Mutational Landscape in a Large Contemporary Cohort of Upper Tract Urothelial Cancer (UTUC) Patients.

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Background/Introduction

- UTUC is a rare genitourinary malignancy characterized by a higher frequency of *FGFR3* and *HRAS* mutations, as shown in 2 series involving a total of 550 patients with upper tract urothelial (1,2).
- In one of these series, which compared 479 patients with UTUC to 1984 patients with bladder cancer, MSI-high was found in 3.4% (0.8% in bladder cancer) (2). In another series of 424 patients with urothelial carcinoma, 3% had MSI-H; 71% of patients with MSI-H had upper tract disease with a response rate of 100% in 5 patients treated with checkpoint inhibitors (3).
- Although the ATLAS trial of PARP inhibitors in urothelial carcinoma has failed to reach primary end point results, predictive biomarkers continue to be defined (4).
- Thus, insights from profile of mutational landscapes of patients with UTUC and MSI can guide drug development. We describe here the largest series of patients with molecular profiling with advanced UTUC and MSI to date.

Cellular pathways relevant to this presentation

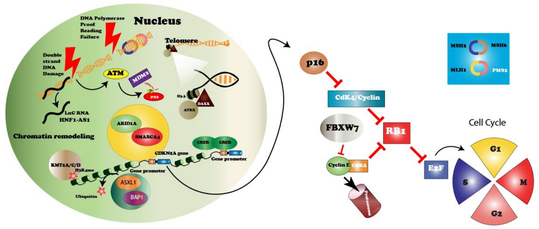

Figure 1. Cellular pathways in Upper Tract Urothelial Cancer. In bold are proteins transcribed by gene mutations associated with dMMR/MSI in patients with UTUC.

Methods

- UTUC tumor samples were analyzed using next generation sequencing (NGS) (NextSeq, 592 gene panel) or whole exome sequencing (WES) (NovaSeq) (Caris Life Sciences, Phoenix, AZ).
- Mismatch repair status (deficient [dMMR] or proficient [pMMR]) and MSI (MSI-high [MSI-H] or stable [MSS]) were detected by immunohistochemistry (IHC), fragment analysis, and NGS.
- Tumor mutational burden (TMB) was measured by counting all somatic mutations found per tumor (high cutoff ≥ 10 mutations per MB). PD-L1 expression was tested by IHC using PD-L1 antibody clones 22c3 (Agilent; positive cutoff CPS ≥ 10) and SP142 (Ventana; positive cutoff ≥ 5% IC).
- Pathogenic fusion events were detected using whole transcriptome sequencing (NovaSeq). Statistical significance was determined using the Chi-square test and adjusted for multiple comparison.
- Immune cell fraction was calculated by QuanTIseq (Finotello 2019, Genome Medicine).

Results

- 538 patients were included – median (range) age was 71.5 (30-89) years, 37.5% were female and 62.5% male (Table 1)
- Prevalence of dMMR/MSI-H was 3.9% (21/538) and TMB-high was 22.7% (96/423).
- Significant molecular differences were not detected in primary vs metastatic disease or in male vs female cases.
- dMMR/MSI-H tumors had higher frequency of TMB-high compared to pMMR/MSS tumors (100% vs. 19.5%, p=0.00003).
- PD-L1 positivity was identified in 33.2% (133/400) cases tested by 22c3 antibody and 28.4% (89/313) cases tested by SP142 antibody.
- No difference was seen in PD-L1 positivity between dMMR/MSI-H vs. pMMR/MSS tumors.
- dMMR/MSI-H tumors had a higher frequency of mutations in genes involved in chromatin remodeling (*ASXL1* 82.4%, *CREBBP* 60%, *SMARCA4* 40%, *KMT2D* 95%, *ARIDIA* 100%, *KMT2A* 20%, *KMT2C* 35.3%, *NSD1* 20%) and pathways targeted by drugs in the clinic or in clinical trials, such as DNA-damage repair (*FANCG* 10%, *ATM* 45%, *ATRX* 40%), regulators of WNT (*RNF43*, an E3 ligase of the Frizzled receptor, 10%), sonic hedgehog (*PTCH1* 21.4%), and MAPK pathways (*CIC* 15%), and other biologic pathways (*ERBB3* 30%, *CDKN2A* 25%, *TSC2* 15%,) All adjusted p<0.05 (Figure 3).
- Pathogenic fusions were detected in 3.8% (17/443) cases, with *FGFR3* fusion being the most common, occurring in 2.7% (12/443) cases.

Characteristic	Upper Tract Urothelial Cancer (All Cases)	UniTMB ≥ 10 mut/MB	dMMR/MSI-H	IHC-PD-L1 (22c3)	IHC-PD-L1 FDA(SP142)
Total, N cases (% of total)	547	96/423 22.7	21/538 3.9%	133/400 33.25%	89, 313 28.4%
Median Ave, years (SD) -Age Range, years	70 (10.7) 30-98	71.5 (9.4) 30-89	67.9 (11.2) 30-86	70.2 (10.8) 32-89	72.2 (9.7) 45-89
Female/Male, N cases -(%Female/%male)	219/328, 547 (40%/60%)	36/60, 96 (37.5%/62.5%)	7/14, 21 (33.3%/66.6%)	52/81, 133 (38.3%/60.9%)	35/54, 89 (39.3%/60.6%)
Metastatic/Primary + Local recurrence, N cases -(% Metastatic/%Primary)	216/331, 547 (39.4%, 60.5%)	30/66, 96 (31.25%/68.75 %)	6/15, 21 (28.5%/71.4%)	56/77, 133 (42.1%/57.8%)	32/57, 89 (35.9%/64.0%)

Table 1. Upper Tract Urothelial Cancer Patient Demographics and Tumor Characteristics.

Common Genomic Aberrations and Potential Markers of IO therapy Response in UTUC.

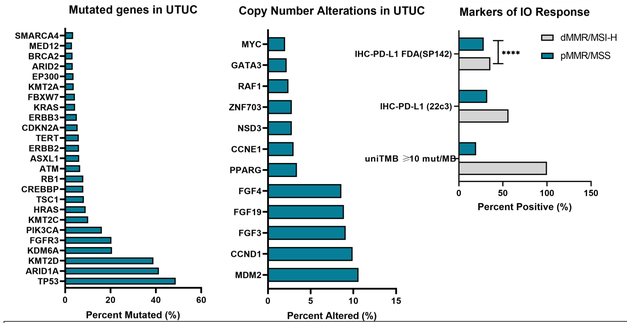

Figure 2: Genomic and transcriptomic landscape of UTUC. Distribution of gene mutations (Left), copy number alterations (Center) and markers of IO therapy response (Right) in UTUC. **** = P-value < 0.00005, but Q-value < 0.00005 determined by χ^2 Test.

Chromatin Remodeling and DNA Damage Repair mutations are significantly associated with dMMR/MSI compared with pMMR/MSS in patients with UTUC

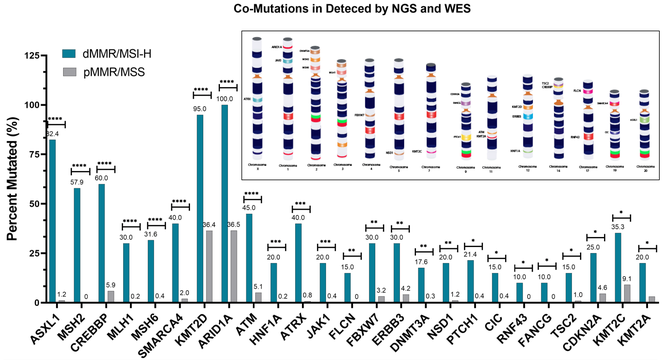

Figure 3. Co-Mutations in dMMR/MSI-H UTUC. Gene Mutations in dMMR/MSI-H (blue) and MSS (orange) UTUC detected by Next Gen Sequencing (NGS). **** = P-value < 0.00005, but Q-value < 0.00005, *** = P-value < 0.0005 and Q-value < 0.0005, ** = P-value < 0.005 and Q-value < 0.005, * = P-value < 0.05 and Q-value < 0.05. P-values determined by χ^2 Test and adjusted to correct for multiple testing using a Benjamin & Hochberg method. Top right: location of genes in the karyotype. Kataegis is a pattern of C-> T hypermutations with characteristic chromosomal distribution associated with the cytidine deaminase APOBEC-3B signature in urothelial carcinoma, to be described in upper tract urothelial carcinoma (5).

M1 Macrophage tumor infiltration elevated in metastatic UTUC

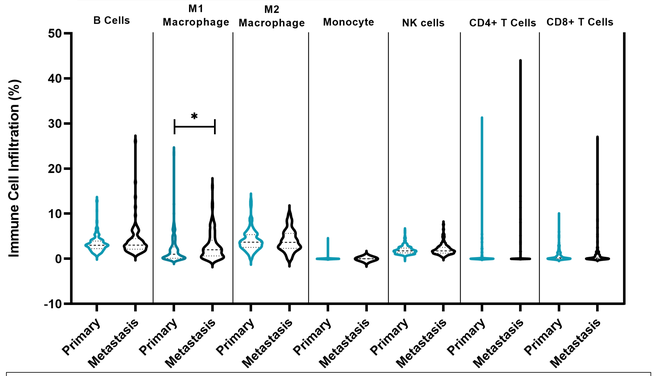

Figure 4. Immune cell tumor infiltration in Primary and Metastatic UTUC. Immune cell gene expression determined by Quantiseq analysis in Primary (blue) and metastatic (black) UTUC. Immune cells queried include B cells, M1 Macrophage, M2 Macrophage, Monocytes, NK cells, CD4+ T cells and CD8+ T Cells. * = P-value < 0.05 determined by non-parametric Wilcoxon Rank Sum test.

Monocyte tumor infiltration elevated in pMMR/MSS UTUC

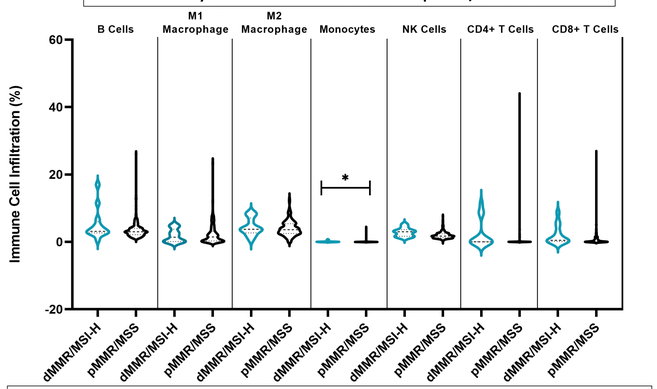

Figure 5. Immune cell tumor infiltration in dMMR/MSI-H and pMMR/MSS UTUC. Immune cell gene expression determined by Quantiseq analysis in dMMR/MSI-H (blue) and pMMR/MSS (black) UTUC. Immune cells queried include B cells, M1 Macrophage, M2 Macrophage, Monocytes, NK cells, CD4+ T cells and CD8+ T Cells. * = P-value < 0.05 determined by non-parametric Wilcoxon Rank Sum test.

Conclusions/Study Highlights

- In the largest analysis to date, we found a 3.9% prevalence of dMMR/MSI-H rate in UTUC. All dMMR/MSI-H tumors displayed TMB-H.
- PD-L1 positivity was comparable between dMMR/MSI-H and pMMR/MSS tumors.
- The pattern of immune cell infiltration in patients with UTUC shows more prevalent M1 macrophage in metastases as compared to primary/local tumors and more prevalent monocyte in pMMR/MSS as compared to dMMR/MSI-H tumors .
- dMMR/MSI-H tumors had a significantly higher rate of mutations in genes involved in chromatin remodeling and DDR biological pathways.
- These results could inform design of targeted and combination therapy trials in UTUC.

References

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