

## Sex specific alterations in the urinary and intratissutal microbiome in therapy naïve urothelial bladder cancer patients

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Despite being actively investigated for several other malignancies, the impact of the microbiome on tumorigenesis, response to therapy and patient outcomes has not been thoroughly assessed in urothelial bladder cancer (BCa). Noteworthy, the relationship between urinary and bladder tissue microbiomes has not yet been investigated. Herein, we aimed to assess: i) the sex-specific microbiome differences in the urine and bladder tissue; ii) the extent to which paired urine samples mirror the bladder tissue-associated microbiome.

A total of 166 biological samples were analysed: morning, mid-stream voided urines from 49 therapy-naïve patients (36 males, 13 females) undergoing radical cystectomy for MIBC and from 59 age-matched healthy controls (34 males, 25 females), plus bladder tissue specimens (paired BCa/non-BCa tissues) of 29 patients (21 males, 8 females). Exclusion criteria included history of recurrent urinary tract infections, positive urine dipstick test at the time of sample collection, recent antibiotic therapy, history of intravesical or neoadjuvant treatment for BCa. Microbiome was analyzed by amplicon-based approach. Sequences with a high-quality score were analyzed using the QIIME (v1.9.1). Intra- and inter-diversity between samples and identification of taxonomic biomarkers by using the linear discriminant analysis (LDA) effect size (LEfSe) were considered significant at p-value  $\leq 0.05$ .

Comparing neoplastic vs. non-neoplastic paired tissues, alpha-diversity did not show distinct clustering of the samples. At the taxonomic level, the genus *Burkholderia* was enriched in the neoplastic specimens in both genders. When we compared urines of patients vs. controls, the male and female urinary microbiome was dominated by members of the three major bacterial phyla Proteobacteria, Firmicutes and Bacteroidetes. At lower taxonomic levels, the bacterial taxa differently enriched in BCa vs. controls were gender-specific, with 18 and 36 bacterial taxa differentially represented in BCa and controls in men and women, respectively. Comparing paired urinary and tissue-associated microbiome, we defined the “common BCa microbiome”, representing 34 and 16 bacterial families (>80% of total relative abundance) shared in the urines and tissues of male and female patients, respectively.

We provide novel characterization of the gender-specific microbiome in the urines and paired bladder tissues of BCa patients. A sex-specific “common BCa microbiome” was detailed, highlighting potentially actionable bacterial taxa.