Prospective observational study of the role of the microbiome in BCG responsiveness prediction (SILENT-EMPIRE): a study protocol

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ABSTRACT

Introduction The human microbiota, the community of micro-organisms in different cavities, has been increasingly linked with inflammatory and neoplastic diseases. While investigation into the gut microbiome has been robust, the urinary microbiome has only recently been described. Investigation into the relationship between bladder cancer (BC) and the bladder and the intestinal microbiome may elucidate a pathophysiological relationship between the two. The bladder or the intestinal microbiome or the interplay between both may also act as a non-invasive biomarker for tumour behaviour. While these associations have not yet been fully investigated, urologists have been manipulating the bladder microbiome for treatment of BC for more than 40 years, treating high grade non-muscle invasive BC (NMIBC) with intravesical BCG immunotherapy. Neither the association between the microbiome sampled directly from bladder tissue and the response to BCG-therapy nor the association between response to BCG-therapy with the faecal microbiome has been studied until now. A prognostic tool prior to initiation of BCG-therapy is still needed.

Methods and analysis In patients with NMIBC bladder samples will be collected during surgery (bladder microbiome assessment), faecal samples (microbiome assessment), instrumented urine and blood samples (biobank) will also be taken. We will analyse the microbial community by 16S rDNA gene amplicon sequencing. The difference in alpha diversity (diversity of species within each sample) and beta diversity (change in species diversity) between BCG-candidates will be assessed. Subgroup analysis will be performed which will lead to the development of a clinical prediction model estimating risk of BCG-response.

Ethics and dissemination The study has been approved by the Cantonal Ethics Committee Zurich (2021-01783) and it is being conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Study results will be disseminated through peer-reviewed journals and national and international scientific conferences.

Trial registration number NCT05204199.

Strengths and limitations of this study

This is the largest prospective observational study evaluating the faecal and bladder tissue microbiome in non-muscle invasive bladder cancer (NMIBC) patients receiving BCG-immune therapy.

By sampling at different predefined time points, our longitudinal study design will allow us to identify the temporal sequence in changes of the microbiome.

Additionally, bladder tissue, instrumented urine and blood will be sampled from patients without a malignancy and patients with low-risk NMIBC to establish a biobank for future microbiome associated studies.

Due to the study’s observational nature, any association may not be causal and will need evaluation in randomised controlled trials.

Unpredictable shortage of BCG can interfere with the study schedule.

INTRODUCTION

Background and rationale

Bladder cancer (BC) affects yearly approximately 430 000 persons worldwide. At the time of diagnosis, approximately 75% of all detected cancers will be confined to the urothelium or lamina propria, they are non-muscle invasive BC (NMIBC). Unfortunately, 20% of NMIBC will progress to invasive cancers and 50% of the patients with invasive disease will develop metastases. Despite systemic therapy, metastatic BC shows a high mortality rate with a median survival of 12–15 months and a 5% 5-year survival rate. Their incidence starts to increase at age 50 and then almost doubles from the age group 65–69 years, to the age group of ≥85 years. The exact aetiology of BC development remains unclear. The urothelium is exposed to the outside environment, not unlike the skin or the lung epithelium, making it susceptible to damage...
from environmental toxins. The most strongly attributable risk factor for BC is cigarette smoking, which causes approximately 50% of cases annually across both sexes. Chronic inflammation, caused by urinary tract infections or urothelial irritants, is also regarded as significant risk factor, though the exact mechanism is unknown.

The human microbiota, the community of microorganisms in different cavities, has been increasingly linked with inflammatory and neoplastic diseases. In the gut microbiota of patients with prostate cancer, the level of Bacteroides massiliensis was found to be elevated and those of Faecalibacterium prausnitzii and Eubacterium rectale were reduced, compared with levels in healthy controls. Bacteroides species possess β-glucuronidase genes that remove sugars when the glycated substrate from the liver reaches the large intestine. Increased circulating levels of sugar-free xenobiotics or mutagens are considered to cause prostate cancer. In a study conducted by Salgia et al investigating composition and diversity of the gut microbiome and treatment response in patients receiving immunotherapy for renal cell carcinoma, a higher microbial diversity was found to be associated with better treatment outcomes and treatment response was shown to be characterised by changes in microbial species over the course of treatment.

While investigation into the gut microbiome has been robust, the urinary microbiome has only recently been described. Results from these recent investigations support the new dogma that the bladder possesses its own indigenous microbiome.

There is a growing number of studies investigating the microbiome in benign urological conditions, but only a few studies to date have explored the role of the urinary microbiome in urological malignancies. Xu et al showed that Streptococcus abundance was significantly elevated in urine specimen from urothelial carcinoma patients compared with healthy individuals. In another urinary microbiome study analysing mid-stream urine, Acinetobacter and Anaerococcus were found in higher abundances in patients with BC compared with the non-cancer patients.

Known virulence factors of Acinetobacter baumannii (which include the outer membrane protein OmpA, phospholipases, membrane polysaccharide components, penicillin-binding proteins and outer membrane vesicles) facilitate escape from the host immune response. Anaerococcus was reported to induce inflammation and remodelling of extracellular matrix (ECM). It is plausible that the interplay of ECM, microbiome and inflammation plays a key role in BC onset, progression and relapse.

Overall, patients with BC were found to have an increase in bacterial richness. These findings are in line with the results of Zeng et al showing that patients with low alpha diversity (variation of microbes in a single sample) had significantly prolonged recurrence-free survival than those with high alpha diversity.

Urologists have been manipulating the bladder microbiome for treatment of BC for more than 40 years, treating high-grade NMIBC with intravesical BCG (Bacillus Calmette and Guérin) immunotherapy, a live attenuated strain of the bacterium Mycobacterium bovis. It is believed that BCG activates both the innate and the acquired immunity of the bladder and thereby exerts its effect on tumour cells. A course of 6 weekly treatments is the most common protocol for induction therapy. While the dose, frequency and duration of treatment (maintenance therapy) have been subject to debate, maximising effectiveness while minimising side effects (as urinary frequency, urgency, dysuria, haematuria and rarer (eg, BCG sepsis)) has remained a challenge. Despite BCG instillation therapy, rates of recurrence and progression (BCG non-responder or BCG failure) is about 40% and a relevant clinical problem. Patients with BCG-unresponsive NMIBC need alternative bladder-sparing options. To this day, there is no established alternative intravesical standard therapy after BCG failure. Radical cystectomy and trimodal therapy for a selected patient collective remain the only standard treatment to prevent disease progression.

Current trials in BCG-unresponsive disease are underway, evaluating the application of immune checkpoint inhibitors against PD-L1 or PD-1 administered intravenously or intravesically in combination with BCG. Urinary, clinical and serum-based biomarkers for predicting response to BCG have been investigated. Of these, only clinicopathological features (eg, smoker vs non-smoker) and urinary cytokine profiles have been significantly associated with response rate, although none are integrated into widespread clinical practice.

A predictive tool prior to initiation of BCG therapy is still needed. This would avoid unnecessary exposure to BCG therapy for patients with no expected therapeutic benefit, spare them the risk of BCG side effects and it would open the possibility to adapt recruiting criteria for the above-mentioned BCG-unresponsive trials to speed up the clinical implementation of alternative bladder sparing therapies. And finally, it would help to alleviate the recurring problem of BCG shortage.

In a recently published congress paper, Sweis et al assessed the composition of the urine microbiome in NMIBC patients and evaluated associations with response to BCG therapy. They could show that global analysis of distances by operational taxonomic units indicated a significant difference between patients with and without recurrence.

Neither the association between the microbiome sampled directly from bladder tissue and the response to BCG therapy nor the association between response to BCG therapy with the faecal microbiome has been studied until now. A prediction model based on a combination of both microbial signatures has also never been presented.
Participants are allowed to retire at any point from the study but this will not affect medical treatment. Participants are allowed to withdraw from the study procedure and will be provided with enough time for questions. Next, written informed consent must be obtained.

The study will launch as a single-centre study at the University Hospital Zurich (USZ). After a start-up phase, we will expand the study to other Swiss centres.

To understand the differences and impact of the microbiota on cancer therapy response and to establish a local biobank for future microbiome projects focusing on identifying survival outcome predictors and investigating the pathophysiological and metabolic properties of the altered microbiome in NMIBC patients and their impact on BCG therapy response or failure in accordance to the Human Microbiome Project and the integrative Human Microbiome Project.

Our second aim is to collect additional samples (blood, instrumented urine, bladder tissue, faeces) to establish a local biobank for future microbiome projects focusing on identifying survival outcome predictors and investigating the pathophysiological and metabolic properties of the altered microbiome in NMIBC patients and their impact on BCG therapy response or failure in accordance to the Human Microbiome Project and the integrative Human Microbiome Project.

METHODS AND ANALYSIS
Recruitment and consent

Overall patient recruitment will be performed at the USZ. The project leader and coworkers of the ‘Klinik für Urologie’ will recruit Patients without a urological or gastrointestinal malignancy undergoing non-oncological bladder surgery or transurethral resection of the prostate (TUR-P) (group A); low-risk NMIBC patients (group B) and NMIBC patients scheduled for BCG therapy (group C).

Study information will be provided during the first consultation; therefore, patients will have sufficient time for consideration. At the standardised consultation hour prior to surgery, patients will be informed again about the study procedure and will be provided with enough time for questions. Next, written informed consent will be obtained.

All patients must sign and date the most current Ethics Committee approved written informed consent before any project-specific assessments or procedures are performed. Participants will not gain any benefit by participating, it may be however greatly beneficial for future patients. This information will clearly be stated in the patient information. Participation is voluntary and it will not affect medical treatment. Participants are allowed to retire at any point from the study.

Eligibility criteria

General inclusion criteria

Subjects, who will fulfil all the following inclusion criteria, may be included into this project:

- Signed informed consent.
- Ability to understand and follow study procedures and understand informed consent.
- Age 18–90 years.

General exclusion criteria

If a subject fulfils any of the following exclusion criteria he/she may not be included:

- Antibiotic treatment within the last month.
- Immuno/chemotherapy within the past 6 months.
- Immunosuppressive therapy.
- Major medical, neoplastic (with the exception of skin cancer), surgical or psychiatric condition requiring ongoing management. Minor, well-controlled conditions, such as medically controlled arterial hypertension or occupational asthma, may be present.
- Additional major diagnosis known to affect the gut or bladder microbiota (eg, liver cirrhosis, systemic sclerosis, inflammatory bowel disease, inflammatory bowel syndrome, coeliac disease, neuropathic bladder).
- Major past intestinal surgery, especially in small intestine or colon. Cholecystectomy, appendectomy, past perianal surgery or past hernia repair may be present.
- Major gastrointestinal symptoms (diarrhoea, constipation, abdominal pain, vomiting, unexplained weight loss, rectal bleeding or blood in the stool).
- Bladder augmentation surgery.
- Indwelling urinary catheter.

Group-specific criteria

We will include patients fulfilling inclusion criteria for one of the following groups:

Group A: Patients without a urological or gastrointestinal malignancy undergoing non-oncological bladder surgery or TUR-P fulfilling the following criteria. At the time of study inclusion, no urological symptoms or relevant urological disease (unclear macrohaematuria, cystolithiasis, prostate cancer).

Group B: Low-risk NMIBC (primary, solitary, T1/a low grade ≤3 cm, no carcinoma in situ (CIS)).

Group C: NMIBC patients, BCG candidates, assessed as intermediate (between the category of low and high risk) or high risk (T1 or high grade or CIS or multiple, recurrent and large >3 cm) Ta/low grade tumours.

Sample collection

We will extract microbial DNA from each faecal and TUR-B sample (obtained by endourological tumour excision). We will sample bladder tissue from participants without urological malignancies (group A, non-oncological bladder surgery and TUR-P candidates). Samples will be taken from the bladder neck. In patients with NMIBC (group B and C) we will take samples from within the bladder.
tumour and from adjacent normal tissue. In patients with one tumour/papillary lesion, samples from within the tumour confirmed by the pathologist (cancerous tissue) and one from the perimeter of the tumour 5 cm away from the lesion in projection to the bladder neck (non-cancerous tissue). In patients with multiple tumours, cancerous tissue will be sampled from the most obvious lesion (size and configuration), presence of tumour will be confirmed by a pathologist and non-cancerous tissue will be sampled 5 cm away in projection to the bladder neck. Collection of instrumented urine will be performed during baseline visit with a sterilised flexible cystoscope or, in patients without a prior cystoscopy, during the surgery via the resectoscope and prior to BCG instillation (group C) via one-off catheter. For faecal sampling, the patients will be instructed, and an information leaflet provided. The study schedule table shows an overview of the planned sample collection (table 1).

Blood (2×10 mL for plasma, 10 mL for serum) will be drawn prior to the respective procedures and when possible, combined with medically required blood draws. Analysis of the microbial community will be performed by 16S rDNA gene amplicon sequencing as outlined in the statistical section.

We will perform the described sample analysis at baseline with samples from BCG candidates. We will repeat faecal analysis after first induction course of BCG therapy and repeat it again for BCG failure patients with additional 16S rDNA gene amplicon sequencing of Re-TUR-B Samples.

**Statistical plan**

**Sample size determination**

The primary endpoint of this study is to evaluate the difference in microbiome composition between BCG responders and non-responders (BCG failures) from BCG candidates using 16S rDNA gene amplicon sequencing. BCG failures are differentiated in two subcategories:

1. Recurrence: defined as histopathologically proven detection of any tumour after the start of intravesical BCG therapy, regardless of grade or stage.
2. Progression: defined as an increase in stage to muscle-invasive disease.

Since the within-group variation in microbiome composition, as well as the effect size between the groups is uncertain and hard to predict, we have performed a power analysis as outlined by Kelly et al. We used micro-power R package to implement simulation of different effect sizes and calculate the PERMANOVA power corresponding to the number of subjects per group. We used 5, 15, 30 subjects per group, obtaining increased power with more subjects per group (see figure 1). For two groups comparison, we predict that if we include 30 patients per group we will obtain a 90% power that allows us to detect effect size ($\omega^2$) of 0.035, this is a conservative assumption of effect size which would be less than the effect observed in other microbiome studies. To enhance statistical power, we plan therefore to recruit 30 patients per group per year. The sample size will amount to 60 patients in total for the main study and to 60 additional patients (30 in group A and 30 in group B) to establish a local biobank for future follow-up microbiome projects including but not limited to identification of survival outcome predictors.

**Statistical methods**

For microbiome analysis (16S rRNA sequencing), the difference in alpha diversity (diversity of species with-in each sample) between BCG candidates will be assessed using Shannon index, which combines richness and diversity, measuring both the number of species and the
inequality between species abundances (significance will be determined using Mann-Whitney test or Kruskal-Wallis test for more than two group comparisons). The differences in microbiome composition between groups will be estimated by calculating several distance metrics, such as weighted UniFrac distance and weighted Jaccard distance. The between-group differences will then be analysed using multivariate analysis of variance with permutation (PERMANOVA), and analysis of group similarities. Qiime2 analysis pipeline will be used to check sequence quality and rare fraction to ensure enough sequencing depth across all samples.

Subgroup analysis will be performed as secondary analysis: We will analyse smoking status (smokers vs non-smokers) and sex (male vs female). Further analyses include alpha and beta (variation of microbial communities between samples) diversity differences between BCG responder and subgroups of non-responder (BCG recurrence group and progression group), analysis of microbiome changes in BCG non-responder patients and analysis of stool microbiome changes after the first BCG induction cycle.

Combination of these predictor metrics and the following variables: age, sex (male, female), stage (Ta, T1), CIS (yes, no, only), grade (high, low), smoking status (yes, no), will then be used to develop a clinical prediction model to estimate risk of BCG response (or non-response). After model development, we will perform bootstrapping for estimation of internal validity. Model development will be performed according to PROBAST (Prediction model Risk Of Bias ASsessment Tool) recommendations.43

Handling of missing data

Newly recruited individuals will replace drop-outs until the final number of ‘Project Participants’ per group will be reached. The primary analysis will not use individuals with missing data. However, secondary analyses (e.g., comparison of microbiome characteristics at baseline) might use data from these individuals if statistically appropriate.

Participant timeline

The scheduled visits and assessments are described in table 1.

Data management system

For the present project, the electronic data capture software REDCap (Research Electronic Data Capture) (Vanderbilt University) will be used for data processing and management.

Patient and public involvement

Neither patients nor public were involved in the development of the study protocol.

ETHICS AND DISSEMINATION

Ethics

The study has been approved by the Cantonal Ethics Committee Zurich (2021-01783) and will be carried out in accordance with principles enunciated in the current version of the Declaration of Helsinki and Swiss regulatory authority’s requirements. Central ethics committee (CEC) will be informed about study stop/end in agreement with local requirements. Each substantial protocol amendment will be notified for approval to the CEC prior to implementation.

Dissemination plan

On completion of the study, it is our intent to present the results as oral communications and abstracts at national and international urological meetings and we will publish the results in peer-reviewed journals.

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Contributors UB, MS and CP wrote the study protocol. CP is the principal investigator for this study. UB is responsible for running the clinical trial and is the coordinating investigator. UB drafted the protocol in the journal format. YM developed the statistical plan and wrote the statistical section in the study protocol. SS, BMS, YM, JHR, PHS, MK, LJH and DE have contributed to the revision of the manuscript.

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REFERENCES

1. IARC Publications Website - GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v1.0. Available: https://publications.iarc.fr/Databases/iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012 [Accessed 5 Jul 2020].

2. Burger M, Catto JWF, Dalbagni G, et al. Epidemiology and risk factors of urotheal bladder cancer. *Eur Urol* 2013;63:234–41.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016;66:7–30.

4. Shariat SF, Sfakianos JP, Droller MJ, et al. Male bladder microbiome relates to lower urinary tract symptoms. *Eur Urol Focus*. 2019;15:376–82.

5. Freedman ND, Silverman DT, Hollenbeck AR, et al. Association between smoking and risk of bladder cancer among men and women. *JAMA* 2011;306:737–45.

6. Vermeulen SH, Hanum N, Grotenhuis AJ, et al. Gram-positive anaerobic cocci--commensals and opportunistic pathogens. *FEMS Microbiol Rev* 2013;37:520–3.

7. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2015;13:594–600.

8. Uchiyama K, Naito Y, Takagi T. Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. *Pharmacol Ther* 2019;199:164–72.

9. Lee KW, Song HY, Kim YH. The microbiome of the urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. *Br J Cancer* 2015;112:594–600.

10. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014;12:661–72.

11. Uchiyama K, Naito Y, Takagi T. Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. *Pharmacol Ther* 2019;199:164–72.

12. Lee KW, Song HY, Kim YH. The microbiome in urological diseases. *Investig Clin Urol* 2020;61:338.

13. Golombok DM, Ayangbesan A, O’Malley P, et al. The role of gut microbiome in the pathogenesis of prostate cancer: a prospective, pilot study. *Urolology* 2018;111:122–8.

14. Gill CIJR, Rowland IR. Diet and cancer: assessing the risk. *Br J Nutr* 2002;88 Suppl 1:s73–87.

15. Salgia NJ, Bergerot PG, Maiga MC, et al. Stool microbiome profiling of patients with metastatic renal cell carcinoma receiving anti-PD-1 immune checkpoint inhibitors. *Eur Urol* 2020;78:498–502.

16. Whiteside SA, Razvi H, Dave S, et al. The microbiome of the urinary tract—a role beyond infection. *Nat Rev Urol* 2015;12:81–90.

17. Thomas-White K, Brady M, Wolfe AJ, et al. The bladder is not sterile: history and current discoveries on the urinary microbiome. *Curr Bladder Dysfunct Rep* 2016;11:18–24.

18. Nelson DE, Van Der Pol B, Dong Q, et al. Characteristic male urine microbiomes associate with asymptomatic sexually transmitted infection. *PloS One* 2010;5:e14116.

19. Nickel JC, Stephens A, Landis JR, et al. Search for microorganisms in men with urologic chronic pelvic pain syndrome: a culture-independent analysis in the MAPP research network. *J Urol* 2015;194:127–35.

20. Lewis DA, Brown R, Williams J, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol* 2013;3.

21. Xu W, Yang L, Lee P, et al. Mini-Review: perspective of the microbiome in the pathogenesis of urothelial carcinoma. *Am J Exp Urol* 2014;2:57–61.

22. Wu P, Zhang G, Zhao J. Profiling the urinary microbiota in male patients with bladder cancer in China. *Front Cell Infect Microbiol* 2018;8:1–10.

23. Popovic B V, Itum M, Chow C. The human urinary microbiome associated with bladder cancer. *Sci Rep* 2018;8.

24. Bajic P, Wolfe AJ, Gupta GN. The urinary microbiome: implications in bladder cancer pathogenesis and therapeutics. *Urology* 2019;126:10–15.

25. Han J, Gu X, Li Y, et al. Mechanisms of BCG in the treatment of bladder cancer-current understanding and the prospect. *Biomed Pharmacother* 2020;129:110393.

26. Herr HW, Morales A. History of Bacillus Calmette-Guerin and bladder cancer: an immunotherapy success story. *J Urol* 2008;179:53–6.

27. Alhunaidi O, Zlotta AR. The use of intravesical BCG in urothelial carcinoma of the bladder. *Ecmancermedicscience* 2019;13:905.

28. Bajic P, Wolfe AJ, Gupta GN. The urinary microbiome: implications in bladder cancer pathogenesis and therapeutics. *Urology* 2019;126:10–15.

29. Han J, Gu X, Li Y, et al. Mechanisms of BCG in the treatment of bladder cancer-current understanding and the prospect. *Biomed Pharmacother* 2020;129:110393.

30. Babjuk M, Burger M, Compérat EM, et al. European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) - 2019 Update. *Eur Urol* 2019;76:639–57.

31. Kulkarni GS, Hermanns T, Wei Y, et al. Propensity score analysis of radical cystectomy versus Bladder-Sparing Trımodal therapy in the setting of a multidisciplinary bladder cancer clinic. *J Clin Oncol* 2017;35:2299–305.

32. Volkmann CS, Oo HZ, Genitsch V, et al. Eortc nomograms and risk groups for predicting recurrence, progression, and disease-specific and overall survival in non-muscle-invasive stage Ta-T1 urothelial bladder cancer patients treated with 1–3 years of maintenance Bacillus Calmette-Guérin. *Eur Urol* 2016;69:60–9.

33. Golla V, Lenis AT, Faiena I, et al. The interplay of extracellular matrix and microbiome in urotheal bladder cancer. *Nat Rev Urol* 2019;16:137–90.

34. Van Der Pol B, Dong Q, et al. Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PLoS One* 2012;7:e36298.

35. Bajic P, Van Kunken ME, Burge BK, et al. Male bladder microbiome relates to lower urinary tract symptoms. *Eur Urol Focus* 2020;6:376–82.

36. DiTommaso A, Gentilini E, Ciardiello F. The human urinary microbiome: susceptibility and immune responsiveness. *Bladder Cancer* 2020;6:26–39.

37. McCon nell MJ, Acts L, Pachón J. Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol Rev* 2013;37:130–55.

38. Mihotich EC, Frick-Vaughn SW, Carson DJ. Microaerobic cocci--commensals and opportunistic pathogens. *FEMS Microbiol Rev* 2013;37:520–3.

39. Alfano M, Canducci F, Nebuloni M, et al. The interplay of extracellular matrix and microbiome in urotheal bladder cancer. *Nat Rev Urol* 2016;13:77–90.

40. Zeng J, Zhang G, Chen C, et al. Alterations in Urobacteria in patients with bladder cancer and implications for clinical outcome: a single-institution study. *Front Cell Infect Microbiol* 2020;10:555508.

41. Droller MJ, Sfakianos JP, Shariat SF, et al. Integrative HMP (iHMP) Research Network Consortium. The male bladder microbiome relates to lower urinary tract symptoms. *Eur Urol Focus*. 2019;15:376–82.