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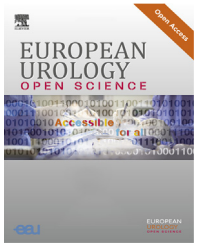

European Association of Urology

Opinion: Open Science

Defining the Mycobiome in Bladder Cancer

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The fact that bladder cancer and the microbiome are interwoven is no secret. Bacillus Calmette-Guérin, an attenuated formulation of *Mycobacterium bovis*, has been a standard treatment for superficial bladder cancer for decades [1]. Other microbes have also been examined in detail in this context, including *Lactobacillus casei*. In small, randomized trials reported in the 1990s, oral preparations of *L. casei* prevented superficial recurrence of bladder cancer [2]. However, a detailed understanding of the landscape of microbes in bladder cancer has lagged behind therapeutic applications. Undertakings such as the National Institutes of Health–sponsored Human Microbiome Project have used cutting-edge genomic analysis techniques to characterize microbial composition in the gut across broad populations [3]. Since then, multiple studies have identified a distinct gut microbial composition in cancer patients, with many of these studies suggesting links between composition and therapeutic efficacy [4]. In particular, multiple recent studies have shown compelling associations between greater gut bacterial diversity and response to immune checkpoint inhibitors (ICIs) in a spectrum of diseases, including melanoma and renal cell carcinoma (RCC); these studies also point to specific bacterial species associated with response [5,6].

While the bacterial composition of the gut in cancer patients is increasingly well documented, what remains a relative "black box" is the composition of the fungal component of the microbiome, the mycobiome. In their article in *European Urology Open Science,* Bukavina and colleagues [7] offer initial insights into the bladder cancer mycobiome. In a comparison of 29 patients with localized bladder cancer to 32 control patients, the authors observed substantially greater fungal diversity in patients with bladder cancer. Whereas *Saccharomycetales* spp. constituted the dominant fungal organism among control patients, these species represented just under half of the fungi identified in bladder cancer patients. Prominent fungi in the latter group included *Hypocreales*, *Tremellales*, and *Sporidiobiolaies* spp.

The authors offer some plausible hypotheses for these observations, pointing to a study reporting decreases in *Saccharomycetes* in colon cancer patients [8] and evidence that *Hypocreales* spp. may produce toxins that inhibit proteosomal activity [9]. The authors also provide observations for a subset of patients receiving neoadjuvant chemotherapy, although this can only serve as a hypothesis-generating analysis given that only seven patients were assessed. Still, a trend towards greater diversity was seen for complete responders (ypT0; n = 4) in comparison to nonresponders (>ypT0; n = 3).

Saccharomycetes/Saccharomycetales spp. were more abundant in nonresponders, while *Hypoecreales* spp. were more abundant in responders. Clearly, this work will require validation in larger samples.

The work by Bukavina and colleagues [7] can be juxtaposed against data our group presented on the mycobiome in RCC. In an analysis of 24 patients with metastatic RCC, we identified *Saccharomyces* spp. as the dominant fungal organism, with a median relative abundance of 87% [6], which appears to mirror the control patient cohort (as opposed to the cancer patient cohort) for the bladder cancer study [7]. The majority of patients in our cohort had received VEGF-directed therapy or ICIs. Among other findings, we identified an association between *Malassezia* spp. and nonresponse to VEGF-directed agents. Notably, mycobiome enrichment of *Malassezia* spp. has been found in pancreatic cancer [10].

Of course, differences between RCC and bladder cancer experiences are to be expected. On top of obvious differences between patient populations (eg, advanced vs localized disease), patients received entirely distinct systemic

DOI of original article: https://doi.org/10.1016/j.euros.2022.06.005

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https://doi.org/10.1016/j.euros.2022.11.023
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therapy regimens. Whereas our patients received either targeted therapy or ICIs, Bukavina and colleagues describe a population of patients receiving cytotoxic therapy. Another significant difference lies in the study methodology: we analyzed stool collected by patients with RCC at home, whereas bladder cancer patients in the study by Bukavina et al had stool manually collected from the distal colon at the time of cystectomy. Presumably, our method could allow for overgrowth of aerobic organisms and depletion of anaerobes. Ultimately, future explorations of the mycobiome should work to harmonize methods for stool collection and analysis to facilitate interstudy comparability.

As the authors concede, much larger studies are needed to fully interrogate the role of the mycobiome in bladder cancer. Any ongoing randomized study in these disease types can be used as a platform for validating the predictive capabilities of (1) mycobiome diversity and (2) specific fungal elements. The ideal finding would be identification of specific species that can be tailored to enhance an antitumor response. It has already been shown that fecal microbiome transplantation from donors who have responded to ICIs can convert ICI nonresponders to responders in the melanoma setting [5]. Our group has taken this one step further. In a cohort of patients with metastatic RCC, we showed that CBM588, an oral, live bacterial product containing a specific strain of *Clostridium butyricum,* could enhance response to ICI therapy in a small randomized trial [6]. Therefore, a logical hypothesis is that a fungal microorganism with immunomodulatory properties could ultimately be used to treat bladder cancer.

Conflicts of interest: Benjamin D. Mercier and Daniela V. Castro have nothing to disclose. Sumanta K. Pal reports consulting agreements with Genentech, Aveo, Eisai, Roche, Pfizer, Novartis, Exelixis, Ipsen, BMS, and Astellas.

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