The microbiota controls the microorganisms that live in close contact with the host, with mutual benefit for both counterparts. The contribution of the gut microbiota to the emergence of castration-resistant prostate cancer (CRPC) has not yet been addressed. We found that androgen deprivation in mice and humans promotes the expansion of defined commensal microbiota that contributes to the onset of castration resistance in mice. Specifically, the intestinal microbial community in mice and patients with CRPC was enriched for species capable of converting androgen precursors into active androgens. Ablation of the gut microbiota by antibiotic therapy delayed the emergence of castration resistance even in immunodeficient mice. Fecal microbiota transplantation (FMT) from CRPC mice and patients rendered mice harboring prostate cancer resistant to castration. In contrast, tumor growth was controlled by FMT from hormone-sensitive prostate cancer patients and Prevotella stercorae administration. These results reveal that the commensal gut microbiota contributes to endocrine resistance in CRPC by providing an alternative source of androgens.

Androgen deprivation therapy (ADT) remains the mainstay of treatment for patients with advanced prostate cancer. However, after an initial favorable response, patients often develop resistance to ADT with resulting tumor progression. Castration-resistant prostate cancer (CRPC) generally carries an unfavorable prognosis and new treatment strategies are needed (1). Although there is an urgent need for effective treatments for CRPC patients, therapies that delay the onset of CRPC in hormone-sensitive prostate cancer (HSPC) patients treated with ADT may also offer an alternative therapeutic strategy. The microbiota comprises the microorganisms that live in close contact with the host, usually with mutual benefit to one another (2). This relationship is described as symbiotic and is fundamental for the fitness of the host (2). Perturbations of this equilibrium can occur under pathological conditions, including cancer (3). Microbiota can directly affect tumor initiation through releasing of toxins (4–6) or can influence tumor cells through bacterial metabolites (7). In addition to this, the microbiota can contribute to tumor development through the promotion of inflammation (8) and shaping the tumor immune response (9, 10). Increasing evidence shows that the microbiota is important for the antitumor activity of both chemotherapy (11, 12) and immune checkpoint inhibitors (13–16), and modulation of the microbiota might enhance favorable treatment response in cancer patients. Previous findings in mouse models and human prostate tumor samples report the existence of a prostate microbiome that can support prostate tumor growth by promoting chronic inflammation (17, 18). In contrast, only a limited number of correlative studies have investigated the role of the gut microbiota in prostate cancer initiation and progression (19–21). Given the role played by the gut microbiota in cancer, an intriguing hypothesis is that the intestinal microbiota of patients suffering from prostate cancer could also participate in the host’s hormone metabolism (22), thus affecting prostate cancer growth.

To address the impact of the intestinal microbiota on CRPC progression, we used two mouse models: the TRAMP-C1 alloraft and the Pten⁻/⁻ prostate conditional mouse models. In both models, surgical castration (CTX) is followed by a castration-sensitive phase (CS; in Pten⁻/⁻ mice 4 weeks after CTX (i.e., 12 weeks of age); in TRAMP-C1 alloraft mice 6 days after CTX) in which the tumor size shrinks because of androgen ablation, and a subsequent castration-resistant phase (CR; in Pten⁻/⁻ mice 12 weeks after CTX (i.e., ≥20 weeks of age); in TRAMP-C1 alloraft mice ≥10 days after CTX) in which the tumor becomes resistant to androgen ablation and starts to grow again (23). To deplete the intestinal microbiota, we treated these tumor-bearing mice with a cocktail of broad-spectrum antibiotics (24) (ABX) that reduced the fecal CFU counts by about 10 orders of magnitude (fig. S1, A and B). Microbiota ablation resulted in delayed tumor growth and improved survival in the TRAMP-C1 CTX context, but not in sham-operated animals (Fig. 1, A and B), with no impact on animal weight (fig. SIC). Microbiota ablation significantly reduced Ki67-positive prostate cancer cells in tumors of castration-resistant mice without altering the percentage of apoptotic cells as detected by cleaved caspase 3 (cC3) positivity (Fig. 1C and fig. S1, D and E). Also in the Pten⁻/⁻ CTX model, microbiota ablation led to a reduction in prostate tumor volume as detected by mag- netic resonance imaging (MRI) measurements of tumor volume over a time course, with no effect on sham-operated animals (Fig. 1, D and E). Histopathological evaluation of the anterior prostate (AP) lobe showed a clear reduction in tumor aggressiveness (percentage of glands with invasive area) in mice treated with ABX (fig. S1, F and G). Loss of intestinal microbiota led to a reduction of Ki67-positive cells in castration-resistant mice but did not affect cell proliferation in sham-operated animals (fig. S1H). Of note, ABX treatment of prostate epithelial tumor cells cultured in two
dimensions did not impair tumor cell growth (fig. S1F). These data were validated in vivo in two additional CS mouse models of human prostate cancer: the LNCaP xenograft model and the LuCaP35 patient-derived xenograft (PDX) model. In both cases, ABX resulted in delayed onset of CRPC that improved survival (Fig. 1, F to I). Similar results were acquired in CTX mice treated with enzalutamide, a second-generation androgen receptor (AR) antagonist (25) (fig. S2, A and B). Overall, these data show that elimination of the intestinal microbiota affects prostate tumorigenesis in different mouse models, selectively in castrated mice.

We next hypothesized that CTX altered mouse intestinal microbiota. To address this point, we performed 16S ribosomal RNA (rDNA) sequencing on fecal DNA from sham-operated and castrated Pten<sup>pc−/−</sup> mice. We identified a compositional difference in the two cohorts (fig. S3A), with enrichment of specific microbiota species in both CR and CS mice (fig. 1J and fig. S3B). Specifically, two species, Ruminococcus gnavus and Bacteroides acidificiens, were particularly enriched in the fecal microbiota of CR Pten<sup>pc−/−</sup> mice (fig. 1K). R. gnavus was also found enriched in fecal samples of castrated LNCaP mice, including those treated with enzalutamide (fig. S3, C to E). Intriguingly, bipolar androgen therapy in the TRAMP-C1 mouse model promoted tumor inhibition and decreased the abundance of Ruminococcaceae; this shows that changes in circulating host androgens affect gut microbiota composition (fig. S3, F to H).

Given the reported dependence of the immune response on the composition of the gut microbiota (9–15), we analyzed the systemic and tumor-infiltrating immune cell populations in castrated mice but found that ABX treatment induced only minimal variations in circulating level of cytokines (fig. S4A), percentage of tumor-infiltrating immune subsets (fig. S4B), and percentages of immune cell subsets in other organs (fig. S4, C to G). In confirmation, ABX treatment was also effective in TRAMP-C1 allograft mice treated with an anti-Ly6G depleting antibody targeting tumor-infiltrating myeloid cells (fig. S5A) and in NOD-SCID mice lacking T, B, and NK cells (fig. S5B). Overall, these data show that the intestinal microbiota is altered in CRPC and sustains tumor growth without regulating systemic and local immune responses.

To better dissect the functional role of the intestinal microbiota in sustaining CRPC growth, we performed fecal microbiota transplantation (FMT) experiments in TRAMP-C1 allograft recipients with feces from mice that had become CR from previous treatment (CR FMT) or from wild-type mice (HD FMT) and followed the evolution of the tumor volume upon castration. To avoid competition with the endogenous microbiota, we pretreated recipient mice with ABX for 7 days before CTX and FMT (24) (fig. S6A). FMT successfully engrafted in the hosts (fig. S6, B and C). Whereas CR FMT resulted in the rapid emergence of CRPC, HD FMT controlled tumor growth to levels comparable to ABX treatment (fig. 2A). Notably, CR FMT also had a significant impact on survival (fig. 2B). CR FMT induced an increase in tumor cell proliferation as measured by Ki67 staining, whereas HD FMT was associated with decreased Ki67 staining (fig. 2C and fig. S6D). Both treatments did not alter the percentage of apoptotic (cC3-positive) tumor cells (fig. S6E). These data were also validated in CTX Pten<sup>pc−/−</sup> mice treated with either CR or HD FMT (fig. 2, D to G, and fig. S6F). Histopathological evaluation of AP lobes showed a significant reduction in the frequency of adenocarcinoma and invasive cancer in HD FMT–treated CTX Pten<sup>pc−/−</sup> mice as compared to those receiving CR FMT (fig. 2H).

In the FMT setting, we also did not find major differences in the relative abundance of tumor-infiltrating immune cell populations (fig. S6, G to J). Because the metagenomic analysis of the CR microbiota had revealed specific enrichment in R. gnavus and B. acidificiens, we tested the functional impact of these two species in promoting CRPC growth by performing colonization experiments. TRAMP-C1 allograft mice were treated with ABX for 7 days prior to CTX to eliminate the competing endogenous microbiota. After CTX, mice were either left untreated or administered R. gnavus or B. acidificiens orally every other day. Administration of R. gnavus increased tumor growth relative to untreated animals (fig. 2I). Overall, these data show that the murine microbiota and R. gnavus are able to sustain tumor growth, whereas HD FMT delays the onset of CRPC.

Because the intestinal microbiota is known to affect the host’s metabolome (26), we performed untargeted metabolomic analyses of sera from Pten<sup>pc−/−</sup> CTX mice treated with or without ABX. This revealed different metabolomic profiles in the two cohorts (fig. S7A). Interestingly, we detected a significant reduction in circulating dehydroepiandrosterone (DHEA) and testosterone in microbiota-depleted animals, even if the abundance of the upstream metabolite pregnenolone was not altered (fig. S7B). In line with these findings, the expression levels of AR target genes were significantly reduced in animals devoid of intestinal microbiota (fig. S7C). Targeted metabolomic analysis in Pten<sup>pc−/−</sup> mice confirmed that both DHEA and testosterone were decreased in CTX mice treated with ABX, whereas in intact mice ABX treatment did not alter androgen levels (fig. 3, A and B). Moreover, CR FMT and R. gnavus administration in TRAMP-C1 mice resulted in increased circulating DHEA and testosterone levels when compared to HD FMT treatment (fig. 3, C and D).

We next assessed whether the bacteria found enriched in the gut of CR mice were capable of synthesizing androgenic steroids. A bacterial strain isolated from the human microbiota, Clostridium scindens, can convert glucocorticoids into androgens (27, 28). We therefore hypothesized that R. gnavus and B. acidificiens may have a similar metabolic capability. To further explore this, we cultured these two strains in the presence of a panel of metabolites of the androgen biosynthesis pathway: cholesterol (the progenitor metabolite of the androgen synthesis pathway), pregnenolone, hydroxypregnenolone, cortisol, and aldosterone (fig. S7D). We analyzed the ability of bacterial species to convert in vitro these compounds into other intermediates of the pathway by using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (fig. S7E). Interestingly, we found that only R. gnavus and B. acidificiens converted pregnenolone and hydroxypregnenolone, but not other metabolites, into downstream metabolites of the pathway, including DHEA and testosterone (fig. 3, E and F, and fig. S7F). As negative controls, we tested seven bacterial strains indigenous to the gut microbiota (Enterococcus faecalis, Enterobacter cloacae, Klebsiella pneumoniae 27, Proteus mirabilis, Serretia marcescens, Staphylococcus haemolyticus, Staphylococcus epidermidis, Ruminococcus gnavus, and Enterobacter aerogenes) with testosterone and pregnenolone (fig. S7G).

### Table 1. Patients’ characteristics.

<table>
<thead>
<tr>
<th>Clinical data (at time of swab)</th>
<th>HSPC (n = 19)</th>
<th>CRPC (n = 55)</th>
</tr>
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<tbody>
<tr>
<td>Age (years; mean ± SD)</td>
<td>69.84 ± 11.45</td>
<td>69.03 ± 11.51</td>
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<td>Prostate-specific antigen (PSA) (µg/liter; mean ± SD)</td>
<td>14.03 ± 28.51</td>
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<tr>
<td>Neutrophil/lymphocyte ratio (NLR) (mean ± SD)</td>
<td>2.7 ± 1.05</td>
<td>3.78 ± 2.66</td>
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<tr>
<td>Gleason score at diagnosis (mean ± SD)</td>
<td>7.43 ± 2.12</td>
<td>8.06 ± 1.63</td>
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<tr>
<td>Overall survival (months; mean ± SD)</td>
<td>9.7 ± 3.12</td>
<td>8.8 ± 2.98</td>
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<tr>
<td>Enzalutamide/abiraterone treatment</td>
<td>4/19</td>
<td>22/55</td>
</tr>
<tr>
<td>Docetaxel/cabazitaxel treatment</td>
<td>0/19</td>
<td>5/55</td>
</tr>
<tr>
<td>Metastasis</td>
<td>12/19</td>
<td>51/55</td>
</tr>
</tbody>
</table>
Depletion of the intestinal microbiota in castrated but not in sham-operated mice affects CRPC growth. C57BL6/N mice were challenged subcutaneously with $2.5 \times 10^5$ TRAMP-C1 cells. When tumors became palpable, mice were either castrated (CTX) or sham-operated (sham). After surgery, mice were either administered normal drinking water or antibiotic cocktail (ABX: neomycin 1 g/liter, ampicillin 1 g/liter, vancomycin 0.5 g/liter, and metronidazole 2 mg per mouse every other day).

- **Fig. 1.** Depletion of the intestinal microbiota in castrated but not in sham-operated mice affects CRPC growth.

- **A** Tumor volume (sham, $n = 14$; sham ABX, $n = 4$; CTX, $n = 20$; CTX ABX, $n = 10$). (A) Tumor volume (sham, $n = 14$; sham ABX, $n = 4$; CTX, $n = 20$; CTX ABX, $n = 10$).
- **B** Survival curve. (B) Survival curve. (C) Percentage of Ki67-positive cells (sham, $n = 4$; sham ABX, $n = 3$; CTX, $n = 4$; CTX ABX, $n = 5$).
- **D** Experimental scheme and representative MRIs of sham, sham ABX, CTX, or CTX ABX mouse at 13 and 20 weeks of age. (D) Waterfall plot representing proportional change in tumor volume (%). (E) Waterfall plot representing proportional change in tumor volume (%).
- **F** Tumor volume [mm$^3$] (sham, $n = 14$; sham ABX, $n = 4$; CTX, $n = 20$; CTX ABX, $n = 10$).
- **G** Tumor volume [mm$^3$] (sham, $n = 14$; sham ABX, $n = 4$; CTX, $n = 20$; CTX ABX, $n = 10$).
- **H** Percent survival (sham, $n = 14$; sham ABX, $n = 4$; CTX, $n = 20$; CTX ABX, $n = 10$).
- **I** Percent survival (sham, $n = 14$; sham ABX, $n = 4$; CTX, $n = 20$; CTX ABX, $n = 10$).
- **J** Normalized OTU count relative to CR/CS.
- **K** Normalized OTU count relative to CR/CS.

The composition of the CS and CR microbiota was analyzed through 16S rDNA sequencing of the feces of sham (n = 6) and CR (n = 5) Pten$^{pc/-}$ mice. NRG mice [non-obese diabetic (NOD)–Rag1(null)–γ chain(null) mice] were challenged with LNCaP or LuCaP35, castrated or sham-operated when the tumor was palpable, and either left untreated or treated with ABX. (F and H) Tumor volume (F) and survival curve (H) of LNCaP allograft (sham, $n = 4$; sham ABX, $n = 3$; CTX, $n = 5$; CTX ABX, $n = 7$). (G and I) Tumor volume (G) and survival curve (I) of LuCaP35 allograft (sham, $n = 5$; CTX, $n = 6$; CTX ABX, $n = 6$). The composition of the CS and CR microbiota was analyzed through 16S rDNA sequencing of the feces of sham (n = 6) and CR (n = 5) Pten$^{pc/-}$ mice. (J) Waterfall plot representing the abundance of the significantly different OTUs identified at species level as a ratio CR/CS. (K) Normalized OTU counts relative to R. gnavus and B. acidifaciens (sham, $n = 6$; CR, $n = 5$). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 [two-way analysis of variance (ANOVA) and Sidak’s multiple-comparison test in (A), (F), and (G); Mantel-Cox test in (B), (H), and (I); unpaired two-sided Student’s t test in (C), (E), and (K)]. NS, not significant. Data in (A), (C), (E), (F), (G), and (K) are means ± SEM.
Fig. 2. Fecal microbiota transplantation from CRPC mice supports tumor growth in castrated recipient mice. TRAMP-C1 allograft mice were treated with ABX for 7 days before CTX, then either left untreated (n = 9), treated with ABX (n = 9), or received FMT from HD (n = 8) or CR (n = 9) mouse donors. (A to C) Tumor volume (A), survival curve (B), and immunohistochemical quantification of Ki67 staining (C) (CTX, n = 4; CTX ABX, n = 6; CTX FMT CR, n = 4; CTX FMT HD, n = 4). Ptenpc-/- mice were treated with ABX for 7 days before CTX, then either left untreated, treated with ABX, or received FMT from CR or HD donors. (D) AP tumor volume at 20 weeks of age (CTX, n = 24; CTX ABX, n = 19; CTX FMT CR, n = 11; CTX FMT HD, n = 10). (E) Representative hematoxylin and eosin (H&E), Ki67, and cC3 immunohistochemical staining in AP lobes of Ptenpc-/- mice at 20 weeks of age. Scale bar, 45 μm. (F and G) Quantification of Ki67-positive cells (CTX, n = 11; CTX ABX, n = 9; CTX FMT CR, n = 7; CTX FMT HD, n = 6) (F) and cC3-positive cells (CTX, n = 12; CTX ABX, n = 9; CTX FMT CR, n = 5; CTX FMT HD, n = 6) (G). (H) Histopathological score of anteroposterior lobes of CTX (n = 6), CTX ABX (n = 5), CTX FMT CR (n = 5), and CTX FMT HD (n = 5) Ptenpc-/- mice at 20 weeks of age. PIN, prostatic intraepithelial neoplasia. (I) Tumor volume of TRAMP-C1 allograft mice treated with ABX for 7 days before CTX, then either left untreated (n = 8) or treated with R. gnavus (n = 5) via oral gavage. CTX FMT HD was used as internal control. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 [two-way ANOVA and Sidak’s multiple-comparison test in (A); Mantel-Cox test in (B); unpaired two-sided Student’s t test in (C), (H), and (I); Mann-Whitney test in (D); one-way ANOVA and Tukey’s multiple-comparison test in (F) and (G)]. Data in (A), (C), (D), (F), (G), (H), and (I) are means ± SEM.
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**A**

![Graph A](image)

**B**

![Graph B](image)

**C**

![Graph C](image)

**D**

![Graph D](image)

**E**

![Graph E](image)

**F**

![Graph F](image)

**G**

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**H**

![Graph H](image)

**I**

![Graph I](image)

**J**

![Graph J](image)

**K**

![Graph K](image)
Escherichia coli). We then tested the ability of conditioned media (CM) of *R. gnavus* incubated with pregnenolone to activate the AR signaling on TRAMP-C1 cells cultured in full androgen deprivation (FAD) conditions (Fig. 3G). The CM of *R. gnavus* incubated with culture broth alone was used as a control. Expression of AR target genes highlighted the ability of *R. gnavus* CM, cultured in the presence of pregnenolone, to stimulate the transcription of AR target genes relative to control CM (Fig. 3H).

We next hypothesized that in CTX mice, circulating pregnenolone could reach the gut through the enterohepatic circulation, where it is metabolized to DHEA and testosterone by the gut microbiota (Fig. 3I). In CTX mice and CTX patients, cholesterol can be metabolized into pregnenolone by the adrenal glands (17). Expression of AR activity sustains androgen-dependent prostate cancer growth in vitro and in vivo, and that this metabolic activity sustains androgen-dependent prostate tumor growth.

To validate the translational potential of our findings, we analyzed the gut microbiota of two patient cohorts with HSPC (n = 19) and metastatic CRPC (n = 55) (Table 1, fig. S10A, A and B, and table S1). We performed shotgun whole-genome metagenome (WGM) sequencing of rectal swabs, leading to >16 million short DNA sequence reads per sample. These analyses highlighted a specific microbial signature in CRPC patients, with 33 species specifically enriched in CRPC and 10 species enriched in HSPC microbiota (Fig. 4A). We did not observe overall differences in the diversity indices of the two cohorts (fig. S10C). Interestingly, in the CRPC cohort, we observed an enrichment of members of the *Ruminococcus* and *Bacteroides* genera as compared to HSPC patients, mirroring the analysis performed in the murine model (fig. S10, D and E). Among the species enriched in the hCRPC microbiota, we found five species belonging to the *Bacteroides* genus and two species belonging to the *Ruminococcus* genus, whereas in the hHSPC microbiota we found four commensal species belonging to the *Prevotella* genus (Fig. 4, A and B). The commensal bacteria that were most significantly associated with a poor clinical outcome (defined on patient survival from the day of the swab, for 14 months), independent of disease stage, were *Ruminococcus* sp. DSM_100440, *Ruminococcus* sp. OM05_10BH, *Streptococcus vestibularis*, and *Clostridiales bacterium VE202_14* (collectively RRSC) (fig. S11A, A to D), whereas a more favorable clinical outcome was associated with *Prevotella* spp. BCRC_S1118, Marseille_P4119, and 885 (collectively PPP) (fig. S11, E to G). Moreover, patients with the simultaneous presence of the RRSC species (termed an “unfavorable” fingerprint) had poorer survival relative to the patients where the four species were not simultaneously present (fig. S11H). In contrast, patients with the concomitant presence of the PPP species (termed a “favorable” fingerprint) were associated with improved survival (fig. S11I). Only a minority of the surviving patients at follow-up (HSPC 9.21% and CRPC alive 24.32%) had the unfavorable fingerprint, with the favorable microbiota fingerprint associating with a greater number of surviving patients after 14 months of follow-up (Fig. 4C and fig. S11J). In the CRPC patient cohort, *Ruminococcus* sp. DSM_100440 and *C. bacterium* VE202_14 were associated with a poor clinical outcome (P < 0.01), whereas *Prevotella* sp. 885 was linked to a favorable prognosis (P < 0.1). Next, we stratified mCRPC patients according to different ADT regimens (abiraterone versus enzalutamide) and we found that patients treated with enzalutamide but not abiraterone presented an expansion of the *Ruminococcaceae* family (fig. S12, A and B). KEGG pathway analysis performed on the gut microbiota of HSPC and CRPC patients showed that the steroid hormone biosynthesis pathway was enriched in the microbiota of patients with CRPC (Fig. 4D).

To assess whether the species enriched in the gut microbiota of the CRPC patients were capable of synthesizing androgens, we performed in vitro analysis of nine bacterial species enriched in the CRPC microbiota (*Dysgonomonas mossii*, *Ruminococcus* sp. DSM_100440, *S. vestibularis*, *Drancourtella massiliensis*, *Parasutterella excrementihominis*, *Sellimonas intestinalis*, *Lactobacillus paracasei*, *Campylobacter hominis*, *Asaerachobacter celatius*) and two species enriched in the HSPC microbiota (*Prevotella stercoralis*, *Actinomyces ihuae*). These bacteria were incubated with a panel of metabolites of the androgen biosynthesis pathway and the conversion to downstream metabolites was monitored, following the same experimental scheme used for the murine species (fig. S7E). Only *Ruminococcus* sp. DSM_100440, enriched in CRPC microbiota,
had the ability to transform pregnenolone and hydroxy pregnenolone into downstream androgenic steroids, including DHEA and testosterone (Fig. 4, E and F). *Ruminococcus* sp. DSM_100440 and/or OM05_10BH were more abundant in fecal samples of patients having a high serum testosterone levels (>10 ng/dl, 3/4, 75%) $\chi^2(1, n = 15) = 2.7841, P = 0.095205$ (fig. S12).

We next used the CM of *Ruminococcus* sp. DSM_100440 incubated with pregnenolone to treat two patient-derived organoids (PDOs), CP50 and CP50C, that are sensitive and insensitive (due to the presence of ARs) to androgen deprivation, respectively (30) (fig. S13A). *Ruminococcus* sp. DSM_100440 CM stimulated the transcription of AR target genes in CP50 but did not do so in CP50C (fig. S13, B and C). Intriguingly, abiraterone, a selective inhibitor of CYP17A1, inhibited the bacterial conversion of pregnenolone in DHEA and testosterone (fig. S13, D to F). RNA sequencing of *R. gnavus* treated with pregnenolone showed up-regulation of 22 genes $\log_{2}(F) > 3.5$, where FC is fold change relative to vehicle, some of which share high sequence homology with human CYP17 (fig. S13, G to I). These data support the existence of a bacterial enzyme that synthesizes androgenic steroids; however, further investigation is needed to identify the bacterial enzyme(s) responsible for the steroid biosynthesis. To examine whether human CRPC microbiota can promote tumor growth in vivo, we generated a mouse model where the gut microbiota of tumor-bearing mice were human-colonized by performing FMT experiments with feces from hHSPC or hCRPC patients. TRAMP-C1 mice pretreated with ABX were subjected or not to CTX and received FMT, and tumor growth was then monitored (Fig. 4G and fig. S14A). In line with the results obtained with murine FMT, hHSPC FMT limited tumor growth when compared to hCRPC FMT in CTX but not in sham-operated mice (Fig. 4G). In fact, hHSPC FMT reduced intratumoral expression of the AR target gene *Pckbp5* (fig. S14B). Notably, *Ruminococcus* sp. DSM_100440 could also reverse the efficacy of ABX in castrated TRAMP-C1 allograft mice when compared to *P. stercorea* (enriched in HSPC) (Fig. 4H). As observed in the other studied mouse models, administration of *Ruminococcus* sp. DSM_100440 increased the circulating levels of DHEA and testosterone in recipient mice (fig. S14, C and D). Overall, these data provide a microbial blueprint of hCRPC microbiota and identify microbial species that alone or in combination affect prostate cancer outcome.

The gut microbiota is a recognized player in host fitness (2), modulating numerous bioactive molecules in the intestine, blood, and various extraintestinal organs, and may affect many cancer types through different mechanisms (3, 8, 11–15). However, its role in prostate cancer has remained unexplored. Some studies have reported altered fecal microbiota in prostate cancer patients, but the mechanisms through which the microbiota affects tumor growth have not been directly addressed (19–21). By using different mouse models of prostate cancer including PDXs, we show that androgen deprivation (CTX, CTX+Enza) drives the expansion of a peculiar intestinal microbiota, and that the gut microbiota affects CRPC growth by contributing to the host’s androgen metabolism. Our findings show that these particular species can contribute to androgen metabolism, prostate cancer growth, endocrine treatment resistance, and disease outcome.

ADT is the standard first-line therapeutic strategy in patients with lethal prostate cancer, and numerous mechanisms of resistance to this treatment have been described, including increased AR expression, AR splicing (31), activation of aberrant cell signaling, recruitment of myeloid-derived suppressor cells (23) or plasma cells (32), and paracrine factors secreted by stromal cells as well as lineage plasticity (33–35). In patients treated with ADT, optimal castration is reached when patients’ testosterone plasma level is reduced below 50 ng/dl. However, clinical evidence demonstrates that patients having androgen levels below 32 ng/dl have better outcomes than patients with testosterone levels between 32 and 50 (36). Thus, subtle variations in plasma androgen levels can affect the prognosis of prostate cancer patients. Our findings, if further validated in the clinic, may provide novel opportunities for the therapy of prostate cancer patients. Indeed, we have shown that FMT with hormone-sensitive microbiota or administration of *P. stercorea* can decrease androgen levels in CTX mice and delay the onset of CRPC. FMT has become the first-line therapy against *Clostridium difficile* infection (37), with clinical trials also demonstrating its efficacy in ulcerative colitis (38, 39), and a number of ongoing clinical trials are studying this therapeutic strategy further. However, translation to transformative clinical trials in prostate cancer using FMT could be challenging, because HSPC patients become CRPC several years after starting ADT (40).

Moreover, we have identified in CRPC patients a fecal bacterial signature associated with decreased overall patient survival. If validated in prospective and larger clinical trials evaluating antibiotic and bacterial consortium transplantation strategies, this signature might be used as a minimally invasive biomarker to identify patients that could benefit from microbiota manipulation strategies.

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ACKNOWLEDGMENTS

We thank the members of Alimonti lab, IOR/IRB institute for instrument and core animal facility (Bellinzona), and all the collaborators for their help, meticulous analysis of the data, and critical input; the Clinical Microbiology Unit, in particular S. Favero; and D. Westaby for contribution to clinical specimen collection. Funding: Supported by ECR consortia (868136) and Swiss Cancer League (KFS4267-08-2017) grant, Dr. Josef Steiner Foundation, Swiss Card-Onc-Grant of Alfred and Annemarie von Sick grant, Helmut Horton, SNSF (310030_176045), PCUK (RIA15-ST2-012), PCF challenge award (20OHAL04), Lega Toscana Conto il Cancro, and Fondazione Leonardo. Also supported by a Prostate Cancer Foundation Young Investigator 2019 award (A.C.), the Prostate Cancer Foundation through a PCFY award and Prostate Cancer Foundation Young Investigator 2019 award (P.R.). University of Florida Health Cancer Center Funds and University of Florida Department of Medicine Gatorade Fund (C.J.), and Prostate Cancer UK, the Movember Foundation through the London Movember Centre of Excellence (GEO13_2-002), the John Black Charitable Foundation and Prostate Cancer Foundation (8BCHAL06), Cancer Research UK (Centre Programme grant), Experimental Cancer Medicine Centre grant funding from Cancer Research UK and the Department of Health, and Biomedical Research Centre funding to the Royal Marsden (J.d.B.). J.d.B. is a National Institute for Health Research (NIHR) Senior Investigator. The views expressed in this article are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. We thank all foundations for their support. Author contributions: A.C. and A.A. conceived the project; A.A., N.P., E.Z., and A.C. designed the experiments and interpreted the results; N.P., E.Z., A.C., G.A., B.C., A.R.E., D.B., and E.P. performed the in vivo and in vitro experiments; M.T. performed bioinformatic analyses with support from M.B.s and J.-P.T.; A.R. and M.B.o performed bacteria RNA sequencing and analysis; M.T., N.P., and A.A. interpreted shotgun metagenomic sequencing analyses; J.T. performed metabolomic analyses; M.M. performed the RT-qPCR experiment; E.Z., N.P., A.C., and B.C. performed FACS analyses; S.M.o performed immunohistochemical experiments; A.R.e performed gland scoring; R.P.M., P.R., F.F., J.H., M.F., E.D.A., A.B., and S.M.e. selected and provided human samples and helped with patients’ data; A.N., L.B., and J.W. performed in vitro PDO experiments; M.G. and M.B.o performed treatments in the MRI facility; A.P., and A.E. performed MRI analysis and analyzed the results; J.G., R.Z.G., and C.J. performed and analyzed 16S rRNA sequencing; G.M.L. provided the microbiology facility at the EOC microbiology unit; J.-P.T., F.G., and M.R. contributed to data discussion and interpretation; S.G. and J.d.B. supervised human experiments and helped in clinical data interpretation; E.Z., N.P., A.C., and A.A. wrote the paper. Competing interests: J.T. is employed by the company Theareo Srl, which is involved in the development and marketing of metabolomics-based diagnostic tests. S.G. is a member of the advisory board, steering committee, or speakers’ bureau of Orion, Janssen, Amgen, Merck Sharp & Dohme, Menarini/Silicon Biosystems, Orion, Pfizer, Qiagen, Sanofi Aventis, Sierra Oncology, Taiho, Teva, and Vertex Pharmaceuticals. J.d.B. is an employee of the ICR, which has received funding or other support for his research work from AZ, Astellas, Bayer, Cellicentric, Daiichi, Eisai, Genentech/Roche, Genmab, GSK, Harpoon, Janssen, Merck Serono, Merck Sharp & Dohme, Menarini/Silicon Biosystems, Orion, Pfizer, Qiagen, Sanofi Aventis, Sierra Oncology, Taiho, Teva, and Vertex, and which has a commercial interest in abiraterone. PARP inhibition in DNA repair-defective cancers, and PI3K/AKT pathway inhibitors (no personal income). J.d.B. is an inventor, with no financial interest, on patent 8,822,438 submitted by Janssen that covers the use of abiraterone acetate with corticosteroids. N.P., E.Z., A.C., M.T., and A.A. are inventors on patent application with filing number 102021000229174 submitted by the Institute of Oncology Research (IOR) that covers the use of antibiotics, FMT, and probiotics in prostate cancer therapy. Data and materials availability: All data are available in the main text or the supplementary materials. 16S sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under bioproject ID PRJNA474258. Whole-genome metagenomics data from human fecal swab have been deposited in NCBI SRA under bioproject ID PRJNA496465. Bacterial RNA-seq data have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE180863.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.abf8403
Materials and Methods
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View/request a protocol for this paper from Bio-protocol.

23 November 2020; resubmitted 9 June 2021
Accepted 8 September 2021
10.1126/science.abf8403
Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis


*Science*, 374 (6564),

**Microbes hijack prostate cancer therapy**
Androgens such as testosterone and dihydrotestosterone are essential for male reproduction and sexual function. Androgens can also influence the growth of prostate tumor cells, and androgen deprivation therapy (ADT) either by surgical means (castration) or pharmacological approaches (hormone suppression), is the cornerstone of current prostate cancer treatments. Pernigoni *et al.* found that when the body was deprived of androgens during ADT, the gut microbiome could produce androgens from androgen precursors (see the Perspective by McCulloch and Trinchieri). Gut commensal microbiota in ADT-treated patients or castrated mice produced androgens that were absorbed into the systemic circulation. These microbe-derived androgens appeared to favor the growth of prostate cancer and helped to facilitate development into a castration- or endocrine therapy–resistant state.—PNK

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