an NGS panel, identifying challenges in case classification and possible solutions.

**Methodology** We performed the FoundationOne CDx NGS panel on 60 EC and assigned molecular subtype: POLE mutated (POLEmut), mismatch repair deficient (MMRd), p53 abnormal (p53abn) or no specific molecular subtype (NSMP).

**Result(s)** In 55 patients the molecular classification was successful. A pathogenic POLE mutation was detected in 9 cases (POLEmut). 20 were MMRd (12 MSI-high, 8 MSI-indeterminate based on the NGS panel MSI classifier) and a known or likely MMR gene mutation was found in 7 of these. Of the remaining 26 cases, 17 carried a TP53 mutation (p53abn) and the remaining 9 were considered to be NSMP. The tumor mutation burden (TMB) was significantly different (p<0.001) in the molecular subtypes (A) and high TMB (>55 mut/MB) was 100% specific for POLEmut EC (p<0.001). High TMB was specific for known pathogenic POLE mutations and was not elevated in cases with solely non-pathogenic POLE mutations (B).

In non-POLEmut cases, TMB was higher in MSI-high and MSI-indeterminate than microsatellite stable (MSS) cases (C), and a TMB of >7 mut/MB was 100% specific for MMRd EC. There was one MSS EC with a TMB of 18 mut/MB but it showed loss of MLH1 and PMS2 proteins on immunostaining and was classified as MMRd.

**Conclusion** An NGS panel can be used in the molecular classification of EC when there is sufficient tumor cellularity. TMB can be used as an adjunct in molecular subtype diagnosis for tumors that are difficult to classify. Additionally, TMB can potentially serve as a diagnostic adjunct in cases with POLE mutation of unknown significance.

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**INTRODUCTION/BACKGROUND**

Endometrial cancer has a dominant place among gynaecological cancers and is the fourth most common malignancy in women. Accumulating reports have associated gynaecological precancer and cancer with dysbiotic microenvironments. Our aim was to identify a microbial signature in endometrial cancer and explore its role in disease pathogenesis.

**Methodology** Eligibility criteria included patients undergoing total abdominal/laparoscopic hysterectomy for endometrial cancer or benign indications. Microbiome swabs were collected along the female genital tract (FGT) (vagina, external cervical os, endometrium, fallopian tubes and ovaries) and rectum. The V1-V2 hypervariable regions of 16S rRNA genes were sequenced (Illumina MiSeq platform), data were analysed with Mothur software package and OTU taxonomies were determined. Benign and malignant endometrial organoids were cultured and treated with increasing concentrations (10%, 20%, 30%) of *L. crispatus* -conditioned media. Proliferation was assessed by the CellTiter-Glo™ 3D cell viability assay and cytokine/chemokine secretion (IL-1β, IL-1ra, IL-2, IL-6, IL-8, IL-10, G-CSF, GM-CSF, IFN-γ, TNF-α, CCL4/MIP1beta, CCL5/RANTES) by the Magnetic Multiplex Cytokine Array (R&D systems).

**Result(s)** Sixty-one women were recruited; 37 had endometrial cancer and 24 were benign controls. We confirmed the presence of a genuine, low- abundance microbiome above background contamination in the endometrium, fallopian tubes and ovaries in a subset of benign and endometrial cancer patients, which was one- four orders of magnitude lower than the heavily colonised vagina, cervix and rectum. In 75% (12/16) of benign patients, we found that the most abundant species of the lower genital tract could also be recovered from the whole length of the upper genital tract, while the microbial continuum was less cohesive in endometrial malignancy. We also demonstrated that *Lactobacillus* depletion and high microbial diversity along the genital tract are characteristic in endometrial cancer patients with concurrent enrichment of *Porphyromonas*, *Prevotella*, *Peptoniphilus* and *Anaerococcus*. Different histotypes and grades of endometrial cancer were not marked by microbial differences. *L.crispatus*, a FGT commensal that is depleted in endometrial cancer, was shown to reduce viability of endometrial cancer organoids at high concentrations and impact on cytokine secretion by benign and malignant endometrial organoids.

**Conclusion** Endometrial cancer displays a distinct microbial signature. *L.crispatus* may exert an anti-proliferative effect in endometrial cancer and interfere with inflammatory pathways.

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**INTERIM ANALYSIS OF 10-YEAR DATA REGARDING TREATMENT AND PROGNOSIS OF UTERINE CARCINOSARCOMA CASES ACROSS THE THAMES VALLEY CANCER ALLIANCE NETWORK**

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