Title: Genomic and evolutionary landscapes of the normal Fallopian tube.

Background: High-grade serous ovarian carcinoma (HGSOC) is the deadliest gynaecological malignancy. It affects mostly women over the age of 40 with a peak incidence at the age of 70.[1] While scientific and medical advances have improved the outcomes of many other cancer types, the survival rates for HGSOC have been stagnant for the past four decades. This is partly due to the fact that most patients present at an advanced stage with multiple distant metastases. Furthermore, it is only in the last decade or so that we have begun to really understand the underlying pathogenesis of this disease. Specifically, similar to other types of epithelial tumours presenting in the ovary, the majority of HGSOC cases do not actually originate at this site. Instead, it is thought that the cell of origin resides in the fallopian tube (FT).[2] In particular, serous tubal intraepithelial carcinoma (STIC), a non-invasive lesion typically found in the distal portion of the FT, has been suggested as the precursor for HGSOC.[2]

On a molecular level, HGSOC is characterised by a high prevalence of *TP53* mutations, which are seen in virtually all cases, and marked chromosomal instability with ~ 80% of all tumours showing whole genome duplication (WGD) and highly rearranged genomes. HGSOC is also one of the defining tumours in carriers of *BRCA1/2* germline variants, associated with a lifetime risk of ~50%.

A recent analysis of over 2,500 cancer genomes showed that HGSOC has a long latency with many WGD and other driver events dating back more than 20 years prior to diagnosis (Fig 1).[3] The long latency is also supported by epidemiological data showing that oral contraceptive pill (OCP) and parity have a protective effect, linking the mostly post-menopausal disease presentation to the events that occurred during pre-menopausal years. Moreover, our recent work on normal endometrial epithelium in women aged 19 to 81 years, showed that in many cases, cancer driver mutations occur before the age of 40 and sometimes even before adolescence (Fig 2).[4] Finally, studies using p53 immunohistochemistry on normal, pre- and neoplastic FT epithelium suggested that aberrant *TP53* expression can be seen in normal FT epithelium, indicative of early driver mutations and microscopic clonal expansions (Fig 3).[5]

A latency of decades for such an aggressive cancer offers an exciting possibility that we might be able to detect the earliest mutant clones in the FTs before they progress to an advanced incurable malignancy.

Aims and objectives: The specific questions we aim to answer in this project are:
1. What is the mutation burden in normal FT epithelium and how does this change with age and contraception/pregnancy history?
2. Do carriers of *BRCA1/2* germline mutations exhibit different signatures and burden of mutations compared to wild-type individuals?
3. Are the earliest driver mutations evident in normal FT epithelium?
4. What are the clonal dynamics of normal FT epithelium, and what are the implications for normal stem cell biology in this organ?

Methods: We have recently developed a low-DNA input method that allows us to generate high quality whole genome and targeted sequencing data from 100-2000 laser-capture microdissected cells. Our analyses in other tissues, including the endometrium,[4] have already shed light on the somatic evolution occurring in apparently normal tissues as we age. Here, I

propose to perform targeted and whole genome sequencing of normal FT epithelium samples from 10 women (5 with *BRCA1/2* and 5 without *BRCA1/2*) to answer the above questions.

Expected results: The work will provide an unprecedented view on the precancerous evolution the FT epithelium in normal conditions and under BRCA deficiency. It will shed light on the earliest stages of HGSOC evolution and help establish whether genomic biomarkers exist that distinguish normal and malignant evolution. My recent work on normal endometrium has led to a series of oral and poster presentations at international conferences and a first author publication in *Nature*. I predict similar results from the work proposed here and will acknowledge BAGP in all presentations and publications.

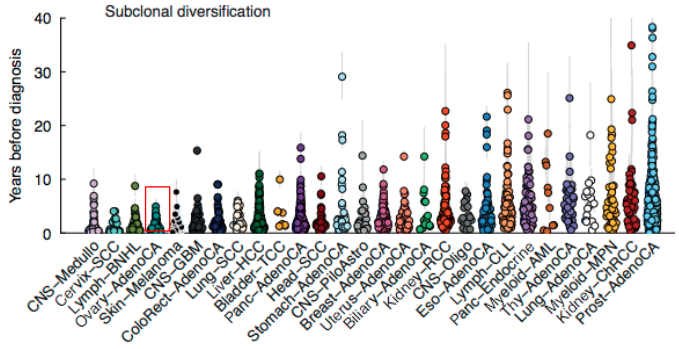

Figure 1 | Absolute timing driver mutations in cancer. Adapted from Gerstung et al., *Nature*, 2020. Here, high-grade serous ovarian carcinoma (HGSOC) is referred to as 'Ovary-Adenocarcinoma'.

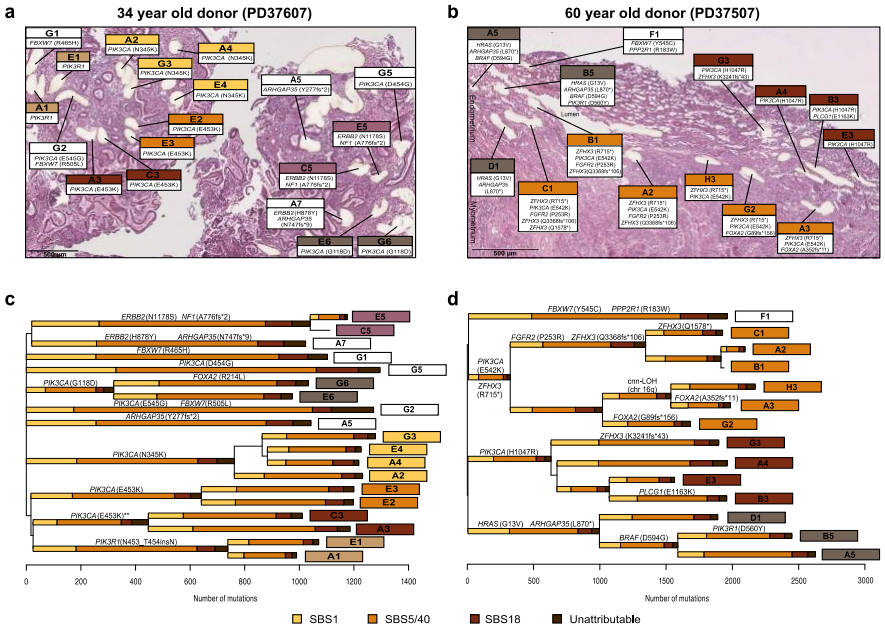

Figure 2 | Histology images and reconstructed phylogenetic trees of normal endometrial glands for two selected individuals: 34-year-old (a,b) and 60-year-old (c,d). a,b H&E images of individual endometrial glands after laser-capture microdissection (20x magnification). **c,d** Phylogenetic trees were reconstructed using single base substitutions; the length of each branch is proportional to the number of variants; a stacked barplot of attributed single base substitution (SBS) mutational signatures that contributed to each branch is then superimposed onto every branch. Endometrial glands sharing >100 variants were considered to belong to the same clade. Labels for glands that did not belong to any clades, are in white. SBS signatures are colour-coded (SBS1, SBS5 and SBS18); substitutions that could not be attributed to reference signatures labelled 'Unattributable'. Adapted from Moore et al, *Nature*, 2020.

References

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