screening. DCIS is usually treated by surgery combined with radiotherapy, which can have a large impact on the life of patients. However, there is little to no evidence that treatment of low and intermediate grade DCIS reduces mortality, while women diagnosed with DCIS do perceive their risk of dying the same as patients with invasive disease. To reduce the negative perception and the overtreatment of DCIS, but assure proper treatment for high risk DCIS, it is critical to understand the progression of DCIS and which genetic factors initiate DCIS formation.

Material and methods To understand the initiation and progression of DCIS, existing non-germline mouse models are used for establishing genetic DCIS models. To generate these, multiple genes were selected from previous work and literature which are suspected to play a role in DCIS initiation. Firstly, *PIK3CA(H1047R)* and *Myc* were incorporated in lentiviral vectors and injected in the mammary gland of immunocompetent mice, while *Trp53* was conditionally knocked out by introducing a Cre lentiviral vector in the mammary gland of FVB $Trp53^{F/F}$ mice. In addition, these genetic aberrations were investigated in combinations. To further characterise human DCIS we derived fresh patient DCIS material to create *in vitro* tumouroid cultures, which we transplanted into NSG mice. Furthermore human DCIS cell lines will be stained using multiple fluorescent markers simultaneously to create a coloured 'cell library'. These will be injected intraductally and followed by intravital imaging to assess the dynamics of progression to an invasive breast cancer.

Results and discussions We have successfully shown that it is possible to propagate breast cancer cell lines and tumouroid lines, as well as DCIS cell lines *in vivo* using intraductal injections. Modifications of these lines are possible using lentiviral vectors, also allowing for intravital imaging. Besides this, the genetic models are initiated and primary DCIS tumouroids have been cultured *in vitro* successfully.

Conclusion Generating these models will provide a better understanding of the biological processes underlying DCIS initiation and progression. This knowledge can then be used to predict DCIS evolution and distinguishing patients with high risk DCIS from low risk DCIS, which will aid in better care.

PO-332 GENOMIC LANDSCAPES, NEOANTIGEN PROFILES AND BIOLOGICAL IMPACT OF MLH1 INACTIVATION IN CANCER CELLS

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Introduction Alterations in DNA repair pathways are thought to fuel tumour progression. Mismatch Repair (MMR) deficient cancers show peculiar biological features such as an indolent progression and a resolute therapeutic response to checkpoint inhibitors. The genomic and biological bases of the peculiar clinical features are poorly understood. Further progress in this area is limited by the paucity of models to study the impact of MMR genes inactivation at the genomic and biological levels. To address this issue we developed a bioinformatic workflow to monitor the neoantigen repertoire induced by inactivation of the *Mlh1* gene (a key player of the MMR machinery), in murine cell lines.

Material and methods We inactivated *Mlh1* throughout the CRISPR-Cas9 technology in CT26 (colon cancer), PDAC (pancreatic cancer) and TSA (breast cancer) murine cell lines. We performed whole exome sequencing (WES) at different time points and then we quantified the amount of mutations (SNVs and indels). We generated a pipeline that characterises the neoantigen repertoire, starting from annotated alterations and the HLA of specific murine strain. In parallel, we inoculated MMR-proficient and -deficient cells in immuno-compromised and -competent mice and monitored their growth.

Results and discussions In all pre-clinical models analysed we found a massive increment in the number of non-synonymous alterations (up to 100% increase respect to basal population) after *Mlh1* inactivation. Notably, analysis of MMR deficient mouse cells at different time points showed a renewal of mutational profile and consequently an accumulation of predicted neoantigens. We further characterised the SNVs and frameshifts acquired by *Mlh1*-knockout cells. In agreement with data in human tumours, the fraction of predicted neoantigens derived from frameshifts was higher than the SNV-derived neoantigens. When injected in immuno-compromised mice the *Mlh1*-knockout cells and their wild type counterpart showed comparable growth. On the contrary, MMR-deficient cells but not their control counterpart grew poorly in immuno-competent mice and responded promptly to treatment with checkpoint inhibitors.

Conclusion We find that *Mlh1* gene inactivation drives dynamic neoantigen profiles, which can be monitored with an *ad hoc* bioinformatic pipeline. These analyses provide mechanistic support to understand why MMR deficient cells engage the immune system of the host, foster immune surveillance and tumour control.

PO-333 SOMATIC ENGINEERING OF MAMMARY GLAND EPITHELIAL CELLS USING CRISPR/CAS9 FOR RAPID TESTING OF BREAST CANCER SUSCEPTIBILITY GENES IN MOUSE MODELS

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Introduction Breast cancer is one of the most commonly diagnosed malignancies in women. In Western countries it is the second leading cause of cancer death among women. Five to ten percent of all breast cancers have a hereditary component, and approximately twenty five percent of all hereditary breast and ovarian cases are attributable to mutations in the breast cancer 1 or breast cancer 2 (*BRCA1* or *BRCA2*) genes. *In vivo* studies represent a highly comprehensive and relevant setting for studying the complexity of this human disease. Genetically engineered germline mouse models of breast cancer paved the way for improved basic and translational research, but their generation is slow and expensive.

Material and methods To overcome these limitations, our lab previously developed CRISPR/Cas9-mediated somatic genome editing approaches to test the role of candidate factors in the adult mammary tissue. In particular, a conditional Cas9-expressing mouse model was used for intraductal injection of