of cancer-related death in Western civilization. There is an urgent need to provide diagnostic tools able to reliably stratify aggressive from slow progressing tumours. As a result, over-diagnosis of PCa remains a prevalent issue and patients frequently suffer from severe side effects following therapeutic intervention. Recently, whole-exome sequencing has identified the MLL family member KMT2C amongst the most frequently mutated genes in human prostate cancer. However, the exact role of this histone lysine methyltransferase in tumorigenesis remains elusive. In this study we aim to investigate the contribution of the methyltransferase activity of Kmt2c to prostate tumorigenesis using a Pten-deficient mouse model of PCa.

**Material and methods** We have developed a mouse model with a double deletion of the tumour suppressor Pten and the methyltransferase domain of Kmt2c in the prostate epithelium (PtenKmt2cPE-/-). Mice were sacrificed at 19 weeks of age and prostate tissue was analysed according to size, morphology and selected markers involved in tumorigenesis in comparison to Pten-deficient control mice (PtenPE-/-). To establish human relevance, we investigated the correlation of KMT2C expression to biochemical recurrence (BCR) and exploited publicly available databases to gain insight from the mutational frequency spectrum of KMT2C in PCa.

**Results and discussions** Deletion of the Kmt2c methyltransferase activity (PtenKmt2cPE-/-) resulted in severely reduced life expectancy, increased tumour size and weight accompanied by a more aggressive histopathological morphology. PtenKmt2cPE-/- tumours showed increased proliferation rates and a deregulation of several histone marks. In a dataset of human PCa patients we found that low expression as well as truncating mutations of KMT2C are similarly associated with poor prognosis. Analysis of TMAs of human PCa patients showed decreased KMT2C expression in bad prognostic high Gleason grade PCa tumour samples.

**Conclusion** Our findings indicate that loss of Kmt2c methyltransferase activity accelerates PCa tumorigenesis in the PtenKmt2cPE-/- mouse model as well as in human patient samples. We therefore propose Kmt2c as a tumour suppressor in prostate cancer.

**Animal Models of Cancer**

**PO-218 CYTOKINE-TRANSGENIC NOG MICE ENGRAFTED WITH HUMAN PERIPHERAL BLOOD CELLS SUPPORT NATURAL KILLER CELL EXPANSION**

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**Introduction** Given the central role natural killer (NK) cells exert in immune responses and the great therapeutic potential they hold due to their ability to mediate antibody-dependent cellular cytotoxicity (ADCC), it is necessary to optimise available preclinical models for ongoing immuno-oncology efforts. Currently, most preclinical in vivo studies of ADCC-dependent efficacy rely on syngeneic models, which mandate the generation and use of surrogate therapies to overcome differences between mouse and human immune systems. Human NK cell-dependent cancer therapies could profit from a humanised immune system (HIS) model that supports human NK cells. However, so far HIS models have largely failed to support human NK cell-engraftment, rendering them inadequate for ADCC studies. Recent work with human cytokine-transgenic hIL-2 NOG and hIL-15 NOG mice showed that the development of human NK cells from engrafted human hematopoietic stem cells (HSCs) is favoured. If hIL-2 NOG and/or hIL-15 NOG mice might also be suited to support NK cells after engraftment of peripheral blood mononuclear cells (PBMCs) remains to be investigated. NK cell maintenance after engraftment of PBMCs would make a cost-effective and efficient model for in vivo studies of human NK cells and therapeutic human antibodies.

**Material and methods** To test this hypothesis, CIEA NOG mouse® (NOG), hIL-2 NOG, and hIL-15 NOG mice were engrafted with PBMCs from a single donor and at one of three increasing cell doses. The onset of graft vs host disease and peripheral blood human immune cell subsets were monitored for several weeks.

**Results and discussions** Results showed that hIL-15 NOG mice had a slightly diminished survival compared to conventional NOG. Nevertheless, hIL-15 NOG survived up to 7 weeks after PBMC engraftment without any signs of graft vs host disease. Although hIL2-NOG mice showed the best engraftment rate for NK cells and other immune cell subpopulations, the overall survival was severely decreased post engraftment. Comparing NK cell-engraftment in hIL-15 NOG and conventional NOG mice, we observed a tenfold increase of NK cell numbers in hIL-15 NOG mice independent of engrafted PBMC numbers.

**Conclusion** Results suggest that hIL-15 NOG mice engrafted with PBMCs make a highly suitable HIS model for studying NK cell activity.

**NEW CELL GENETIC TRACING AND SINGLE-CELL PATHWAYS INVOLVED IN CANCER METASTASIS**

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**Introduction** Traditionally, metastasis has been seen as the final and often fatal step in the progression of solid malignancies. This vision of tumour progression has been recently challenged, as genetic analyses of circulating tumour cells and functional studies in animal models, have suggested that the dissemination of tumour cells can be a very early event, even at a premalignant stage. Whether this implies that the colonisation of the distant tissue happens at such an early stage is still unclear. In accordance with an early metastatic implantation model, there is evidence in cancer of early parallel evolution of primary and metastatic tumours as well as tissue specific evolutionary branches among different metastases from the same tumour. Transcriptional analysis of cells with different metastatic potential in mouse tissues have been used to identify the genes and pathways involved in metastasis specificity. Moreover, intratumour heterogeneity has been observed in multiple cancers and has been postulated as a critical aspect
for tumour metastasis and treatment resistance. In this context, the use of new molecular and sequencing strategies like cell lineage tracing systems and single-cell sequencing, to genetically modified mouse models, could provide new opportunities to unravel the molecular mechanisms behind metastatic potential.

**Material and methods** In order to construct a new fluorescent-based lineage tracing system, we have performed a systematic evaluation of the fluorescent characteristics of different proteins and have tried several orientation and locations of loxP sites in order to provide a random and proportional repertoire of fluorescent labelling after CRE recombination. Additionally, we have set up droplet-based microfluidic technology to perform single-cell sequencing on murine tumour primary samples.

**Results and discussions** Here we have constructed a new allele able to produce up to 15 different colour combinations that can be uniquely identified by confocal microscopy and FACS. Additionally, we have set up the infrastructure and protocol to perform single-cell RNA-Seq and targeted sequencing on tumour primary samples.

**Conclusion** We have generated very promising new tools that could open new opportunities to study the molecular mechanisms behind metastatic potential in mouse and human tumours.

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**PO-220** DEFINING THE ROLE OF HEPARANASE IN BREAST CANCER PROGRESSION USING THE PYMT-MMTV MOUSE MODEL

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**Introduction** Heparanase (HPSE) is a beta-d-endoglucuronidase and the only mammalian enzyme that cleaves heparan sulphate (HS), a major structural and regulatory component of the extracellular matrix (ECM) and the vascular basement membrane (BM). The expression of HPSE is tightly controlled and under physiological conditions is limited to immune cells, endothelial cells, placental trophoblasts and keratinocytes. However, during pathological conditions such as cancer, HPSE expression is dysregulated and high expression often correlates with poor patient survival. The cleavage of HS by HPSE expressing tumours degrades the ECM/BM and releases HS bound growth factors and cytokines, promoting cellular signalling in a positive feed-back mechanism. This in turn leads to enhanced primary tumour growth, metastasis, angiogenesis and inflammation. HPSE is therefore a critical promoter of the hallmark of cancer and has generated significant interest as an anti-cancer drug target. However, despite decades of research, the precise mechanistic role of HPSE in the tumour microenvironment remains poorly defined. Breast cancer is the most prevalent malignancy in women worldwide. Clinical data reveals that the expression of HPSE in mammary tumours results in poor patient survival.

**Material and methods** We recently generated a HPSE-deficient C57Bl/6 mouse strain (C57Bl/6xHPSE−/−) that were crossed with spontaneous mammary tumour developing PyMT-MMTV mice to generate the PyMT-MMTVxHPSE−/− mice, providing us with a valuable in vivo model to characterise the role of HPSE in early mammary tumour development, tumour progression and metastasis.

**Results and discussions** Our data indicate that although HPSE promoted tumour angiogenesis, overall tumour development and metastasis between PyMT-MMTV and PyMT-MMTVxHPSE−/− mice remained comparable. By examination of the tumour microenvironment, we also demonstrate that the tumour stroma positively contributes to mammary tumour HPSE activity, promoting tumour angiogenesis. In contrast, HPSE expressed in the tumour-bearing host appeared to play no significant role in tumour growth and metastasis. These data suggest that in the PyMT-MMTV model and indeed, in certain cancer settings such as breast cancer, HPSE may not play as significant a role to what has been proposed over the last two decades.

**Conclusion** These findings may have important implications for the ongoing development and application of HPSE inhibitors in the treatment of cancer.

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**PO-221** MOUSE MODELS OF LUNG SQUAMOUS CELL CARCINOMA FOR PRECLINICAL INTERVENTION STUDIES

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10.1136/esmoopen-2018-EACR25.739

**Introduction** Lung squamous cell carcinoma (LSCC) is a lethal disease accounting for 30% of all lung cancer cases. Currently, there are no effective treatments: patients are treated with a combination of surgery, chemotherapy and radiotherapy. So far numerous high-throughput screenings have been carried out in order to identify the representative mutations of this tumour. Their contribution is precious but remains descriptive.

**Material and methods** Mice were engineered by using Flp-Recombinase mediated cassette exchange technology in ES cells. Mutations were activated by Intratracheal injection of adenoviruses carrying Cre recombinase under different promoters.

Genome profiling and histological analysis were performed on lung isolated from mice that showed signs of distress due to tumour development.

**Results and discussions** We published that mice carrying SOX2 over-expression combined with PTEN and CDKN2AB loss (hereafter SOX2PC) developed central and peripheral LSCC according to the targeted cell-of-origin, fully mimicking the human counterpart.

To examine gene contribution, we uncoupled the inactivation of tumour suppressor genes and found that mice defective either of PTEN or CDKN2AB in combination with SOX2 over-expression, only showed epithelial hyperplasia, indicating that the combination is necessary for the tumour to progress.

To our knowledge, no studies have been published that mice carrying SOX2PC developed central and peripheral LSCC according to the targeted cell-of-origin, fully mimicking the human counterpart.

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