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 targetted 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-221**

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

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10.1136/esmoopen-2018-EACR25.738

**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 expression 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 hallmarks 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 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.
cases, strongly enhanced tumour progression without affecting the overall tumour morphology. Most importantly, in both NFE2L2-SOX2PC and SOX2PC mice, we observed a highly immunogenic microenvironment, as reported for human patients, with high level of PD-L1 and extensive immune infiltrations of leukocytes mostly of myeloid lineage.

**Conclusion** Our data show that neoplastic squamous differentiation is strongly associated to the oncogenic activation of SOX2. Concomitant activation of the anti-oxidant pathway accelerates tumour progression, making NFE2L2-SOX2PC autochthonous model, with its characteristic immunophenotype (massive myeloid immune infiltrations) suitable for a diversity of intervention studies.

**Results and discussions** The sea otter genome contains 24 129 genes, of which 17 421 were mapped to the human genome. We identified several mutated genes with a known involvement in human lymphomas. We observed a stop-gain mutation in EP400, a paralog of human EP300. EP300 is a tumour suppressor which has been previously implicated in human Diffuse Large B-Cell and Follicular lymphomas. We also observed an indel in SPEN, a NOTCH pathway regulator, the deregulation of which is associated with human hematopoietic neoplasms.

**Conclusion** These observations suggest gene and pathway deregulation are a shared genetic commonality between sea otter and human lymphomas. Comparing the mutational spectrum in human, canine, and sea otter lymphoid cancers may elucidate aetiology and treatment options, and facilitate a One Health approach to neoplasia.

**Introduction** Lymphoid cancers are a biologically diverse group of neoplasms. Disease aetiology is multifactorial, implicating viral and infectious agents, immune dysfunction, and genetic factors. Lymphoid cancers affect domestic and laboratory animals; among members of Mustelidae, lymphoma has been reported in domestic ferrets, yet rarely in sea otters, with only 3 reported cases since 2002. However, since 2006, 3 sea otters at the Vancouver Aquarium (British Columbia, Canada) developed lymphoma. All 3 sea otters were rescued as young animals and have lived at the aquarium for the majority of their lives. A 21 year old female was diagnosed with chronic lymphocytic leukaemia, while 2 males, aged 12 and 16, were diagnosed with lymphoma. Both males received chemotherapy before being humanely euthanized due to declining quality of life. A high rate of blood cancers in this population motivated the molecular characterisation of the most recent lymphoma.

**Material and methods** Whole genome sequencing was performed on peripheral blood and a lymphoma needle biopsy. The tumour biopsy was sequenced on the Illumina HiSeq X and Oxford Nanopore MinION, generating 264.5 and 4.63 passed filtered Gbp, respectively. De novo assembly was performed on the Illumina DNA and RNA data using ABySS 1.3.4. Structural variants were called with trans-abyss 1.4.10. Somatic variants were called by Strelka (v1.0.15) using matched tumour-normal samples aligned against the *Enhydra lutris kenyoni* reference genome. Variants were annotated by snPEFF (v4.3) using an in-house custom gene annotation database. The mutations in the sea otter tumour were subsequently compared to human and canine lymphomas.

**Results and discussions** The sea otter genome contains 24 129 genes, of which 17 421 were mapped to the human genome. We identified several mutated genes with a known involvement in human lymphomas. We observed a stop-gain mutation in EP400, a paralog of human EP300. EP300 is a tumour suppressor which has been previously implicated in human Diffuse Large B-Cell and Follicular lymphomas. We also observed an indel in SPEN, a NOTCH pathway regulator, the deregulation of which is associated with human hematopoietic neoplasms.

**Conclusion** These observations suggest gene and pathway deregulation are a shared genetic commonality between sea otter and human lymphomas. Comparing the mutational spectrum in human, canine, and sea otter lymphoid cancers may elucidate aetiology and treatment options, and facilitate a One Health approach to neoplasia.