be one of the underlying mechanisms of differential sensitivity to cisplatin.

**PO-339** INTRATUMOUR HETEROGENEITY IN A PANCREATIC CANCER MOUSE MODEL

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**Introduction** Intratumour heterogeneity has been observed in multiple cancers and has been postulated as a critical aspect for tumour metastasis and treatment resistance. Therefore, a further characterisation of its role in cancer progression and metastasis has become essential. Pancreatic cancer in humans has a dismal prognosis with only 8% of patients surviving more than 5 years after diagnosis. Mouse models of pancreatic cancer, based on the expression of an oncogenic version of Kras in the pancreas, have been widely used to study the molecular pathways involved in pancreatic cancer progression, nevertheless there is still controversy about their utility to study the genetic complexity observed in the human tumours.

**Material and methods** We have performed multi-sampling exome and genome sequencing in primary and metastatic lesions, as well as in primary cell cultures generated in an oncogenic Kras-mediated mouse model of pancreatic cancer.

**Results and discussions** We have observed that murine tumours recapitulate the genetic complexity observed in the human tumours like a monofocal origin of the aggressive disease, multi-clonal intratumour heterogeneity with clone convergent evolution, independent waves of metastatic colonisation and the presence of chromotripsis.

**Conclusion** Our results show that the oncogenic Kras-based mouse model is a very good tool for the study of the dynamics of intratumour heterogeneity and that it also reflects faithfully the genomic processes observed in human pancreatic tumours. Consequently, we propose that a deeper genomic study of these murine tumours could provide a better understanding of the role of intratumour heterogeneity in tumour progression and metastasis.

**PO-340** TUMOUR HETEROGENEITY IN PATIENT-DERIVED GILOBLASTOMA ORGANOIDS

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**Introduction** Glioblastoma (GBM) is the most malignant primary brain tumour with a median survival of 15 months despite intensive multimodal treatment including surgery, chemoradiation and adjuvant chemotherapy. A major obstacle in the improvement of patient survival is intratumoral heterogeneity, responsible for differences in treatment sensitivity and a spatial and temporally dynamic process that drives tumour resistance mechanisms. New clinically relevant predictive models are needed that encompass this heterogeneity to discover improved tumor-tailored treatments.

Cancer organoids are 3D cancer stem-cell cultures that can self-maintain a hierarchy of pluripotent cancer stem cells and differentiated tumour cells. At present, cancer organoid models for GBM are still understudied. Our goal is to demonstrate whether GBM organoids maintain genetic and phenotypic heterogeneity with respect to the patient biopsy and if standard treatment is predictive for resistance mechanisms. If so, GBM organoids may be used as co-clinical avatars to predict treatment response and guide adaptations to the treatment plan.

**Material and methods** In this project, fresh GBM biopsies from 30 patients will be used to establish GBM organoids. Organoids are formed by suspending tumour cells in Matrigel plugs and cultured in stem cell medium. At set time points, GBM organoids have been harvested and embedded using paraffin- and cryopreservation. Sections are stained for hypoxia, proliferation, apoptosis and glioma stem cell markers. GBM organoids will be exposed to current standard treatment options (i.e. radiotherapy and temozolomide) and phenotypic changes will be determined. RNA expression analysis will be used to assess tumor-driving signal transduction pathways before and after treatment.

**Results and discussions** GBM organoids have been derived from five patients with different clinical subtypes and have been maintained in culture for several months. Our results show that GBM organoids develop a hypoxic core and an outer rim that has abundant proliferation. Data on the hypoxia, proliferation and presence of glioma stem cells will be presented before and after treatment. Future studies will include next-generation sequencing of glioma driver genes during tumour evolution in culture before and after treatment.

**Conclusion** Our results demonstrate the feasibility of maintaining primary GBM organoids in culture for phenotypic and genotypic analysis. Future studies will demonstrate the genetic stability of the GBM organoids and their response to treatment.

**PO-341** THE ROLE OF CANCER/TESTIS ANTIGENS FROM MAGE-A FAMILY AND NY-ESO-1 IN DUCTAL CARCINOMA IN SITU (DCIS)

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**Introduction** Cancer/testis antigens (CTA) are a large family of tumor-associated antigens expressed in human tumours of different histological origin, but not in normal tissues except for testis and placenta. Several immunohistochemical studies confirmed the association of CT antigen expression and ER
negativity in breast tumours and demonstrated their frequent expression in tumours with higher nuclear grade. The expression of cancer/testis antigens in ductal carcinoma in situ is not studied so extensively as in invasive breast cancer.

**Material and methods** This retrospective study included archived paraffin-embedded specimens from 83 patients diagnosed with DCIS in the period between 2007 and 2014. Antigens multi-MAGE-A, MAGE-A1, MAGE-A10 and NY-ESO-1 and were demonstrated by immunostaining. TILs were determined on all sections together with the histopathological variables of DCIS.

**Results and discussions** All tested antigens showed association (positive or negative) with histopathological parameters. Expression of MAGE-A1 was significantly associated with cytoplasmic staining (p=0.007). Simultaneously cytoplasmic and nuclear staining was in statistically significant positive correlation with local recurrence (p=0.005) and central necrosis (p=0.016) and in negative correlation with expression of ER receptors (p=0.003) and PR receptors (p=0.009). Antigen MAGE-A10 was significantly associated with tumor-infiltrating lymphocytes (p=0.05). The additional analysis of TILs showed statistically significant positive correlation with grade (p=0.023), and central necrosis (p<0.001), and negative with tumour size (p=0.623), ER receptors (p=0.003) and PR receptors (p=0.027).

**Conclusion** Cancer/testis antigens from MAGE family (MAGE-A1, multi-MAGE-A and MAGE-A10) and NY-ESO-1 correlate with histopathological predictive variables of DCIS. The expression of antigen MAGE-A10 could have an important role in treatment of patients with negative histopathological predictive variables, but further analysis is required. Simultaneous cytoplasmic and nuclear protein expression of MAGE-A family and NY-ESO-1 cancer-testis antigens represent an independent marker for local recurrence. Cancer/testis antigens are not perfect indicators of invasiveness for DCIS, but in combination with other histopathological predictive variables they can be used as a guidance for better treatment of this patients. But, this is the small study and further larger studies are necessary to confirm our findings.