the expression of various Wnt-associated genes, such as Survivin (BIRC5) and CD44.

**Conclusion** This work suggests that ASCL2, functioning within the Wnt signalling pathway, may be a potential novel driver of breast tumourigenesis. Investigation using both in vitro methods and data mining, will continue to further elucidate the functional role of ASCL2 in breast cancer tumourigenesis.

**PO-462** FUNCTIONAL EX VIVO ASSAY REVEALS HOMOLOGOUS RECOMBINATION DEFICIENCY IN BREAST CANCER BEYOND BRCA GENE DEFECTS

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**Introduction** Tumours of germline BRCA1/2 mutated carriers show homologous recombination (HR) deficiency (HRD), resulting in impaired DNA double strand break (DSB) repair and high sensitivity to Poly-(ADP-Ribose)-Polymerase (PARP) inhibitors. Although this therapy is expected to be effective beyond germline BRCA1/2 mutated carriers, a robust validated test to detect HRD tumours is lacking. In the present study we therefore evaluated a functional HR assay exploiting the formation of RAD51 foci in proliferating cells after ex vivo irradiation of fresh breast cancers (BrC) tissue: the RECAP test.

**Material and methods** Fresh samples of 170 primary BrC were analysed using the RECAP test. The molecular explanation for the HRD phenotype was investigated by exploring BRCA deficiencies, mutational signatures, amount of tumour infiltrating lymphocytes (TILs) and microsatellite instability (MSI).

**Results and discussions** RECAP was completed successfully in 148 out of 170 samples (87%). 24 tumours showed HRD (16%), while 6 tumours were HR intermediate (HRi) (4%). HRD was explained by BRCA deficiencies (mutations, promoter hypermethylation, deletions) in 16 cases. Several non-BRCA deficient HRD tumours showed BRCA-related mutational signatures, suggesting that they are likely also bona fide HRD cases. HRD tumours showed an increased incidence of high TIL counts (p=0.023) compared to HR proficient (HRP) tumours and MSI was more frequently observed in the HRD group (2/20, 10%) than expected in unselected BrC (1%) (p=0.017).

**Conclusion** The RECAP test is a robust assay detecting both BRCA1/2 deficient and BRCA1/2 proficient HRD tumours. This test identifies approximately 30% more patients that may benefit from PARPi treatment than BRCA gene testing only.

**PO-466** MULTIPARAMETRIC ANALYSIS OF LUNG CANCER TISSUE SECTIONS USING IMAGING MASS CYTOMETRY

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**Introduction** A novel tool for multiparametric molecular pathology analysis of tissue sections is described. Imaging Mass Cytometry (IMC), a new mass spectrometer-based technology, provides visualisation and spatial distribution of up to 40 markers in a single tissue section with 1 µm resolution. IMC uses metal-tagged antibodies (mAbs), a pulsed UV laser for ablating regions of interest, and a stream of inert gas to transfer material generated by the laser to the mass cytometer.

**Material and methods** Tissue microarray (TMA) of human lung cancer was stained according to IMC protocol using a mix of 23 mAbs.

The samples were inserted into the ablation chamber of the Hyperion Imaging System. CyTOF® Software v6.7 and MCD Viewer 1.0 were used for data acquisition and rendering of pseudo three-colour images. Quantification of selected markers was done with Definiens Tissue Studio® (DTS) software. Standard HPA protocol was used for immunohistochemical (IHC) staining.

**Results and discussions** IMC analysis of human lung cancer TMA stained with a panel of 23 mAbs revealed that core morphology was consistent with anatopathological tumour classification. Immune cell phenotype and spatial distribution assessed by IHC and IMC were equivalent.

IMC generated data was used to quantitate PD-L1 marker expression. Percentages of cells expressing different levels of PD-L1 and average intensity of PD-L1 related signal were calculated for tumour and stroma regions of each core. In addition, PD-L1 corresponding signal intensity was calculated in cytoplasm and membrane compartments of marker-positive cells.

**Conclusion** IMC technology is a powerful tool for multiparametric histopathological analysis. It allows researchers to simultaneously detect up to 40 markers with single-cell detection resolution equivalent to conventional IHC. Using DTS software we demonstrate quantitation of multiple markers in lung tumour sections probed with a panel of 23 mAbs.

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**PO-464** ENDOGLIN-TARGETED THERAPY DEMONSTRATES STRONG PRECLINICAL ANTI-TUMOUR ACTIVITY IN EWING SARCOMA USING ANTIBODY-DRUG CONJUGATES

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**Introduction** Ewing sarcoma (ES) is a bone/soft tissue neoplasia putatively originated from mesenchymal stem cells (MSC). Among the proteins that define the ESC signature, endoglin (ENG, CD105) is considered one of the highly expressed molecules. This protein is a transmembrane co-receptor of the TGf-β family. The poor outcome experienced by ES patients with disseminated disease highlights the necessity of developing new therapeutic strategies. The generation of two novel