Orthotopic transplantation of primary pancreatic tumour (PPT) cells into immunocompetent (wildtype C57BL/6) and immunodeficient (NOD/SCID IL2Rγnull (NSG)) mice were performed to further investigate metastatic patterns and the influence of the immune system.

Analyses of tumour, disseminated and metastatic cells via immunofluorescence staining and FACS were performed.

**Results and discussions**
We observed recombined (R) and non-recombined (NR) metastases in all our experimental settings (endogenous; implanted - wildtype and immunodeficient - mice). Interestingly the ratio of R to NR metastases changed according to the organ (e.g. 10:2 in lungs; 1:10 in lymphnodes). Surprisingly, the amount of NR metastases (non-EMT) in total were significantly higher than recombined (75% NR). On a side note we found that the recombined (EMT) tumour cells were much more likely to become circulating tumour cells (CCTs). A 5- to 50-fold increase of the recombined cell percentage was observed in the blood compared to the PPT.

**Conclusion**
The data suggest that there might be an EMT but also a non-EMT route to form metastases. Those routes seem to coexist and are to some point organ-specific. Also, there was no evidence for the amount of CCTs to predict the metastases pattern.

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**PO-205**

**CHARACTERISATION OF THE INTEGRIN ALPHA V-DEPENDENT ADHESOME IN MDA-MB-435S MELANOMA CELLS**

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**Introduction**
Integrins are heterodimeric glycoproteins that bind cells to extracellular matrix proteins. Upon integrin clustering, multimolecular integrin adhesion complexes (IACs) form that facilitate the linkage between integrins and the actin cytoskeleton and permit bidirectional signalling. The αV integrin is expressed in most tumour cells where it regulates an array of cellular functions and plays a role in anti-tumour drug resistance. The aim of this study was to assess αV-dependent changes in IAC composition in MDA-MB-435S melanoma cells in order to better understand the increased sensitivity to paclitaxel and vincristine upon integrin αV knockdown.

**Material and methods**
Integrin αV-specific shRNA was cloned into pSUPER.puro, transfected into MDA-MB-435S cells using Lipofectamine, and cell clones were selected using puromycin. The sensitivity of cells to antitumor drugs was determined using an MTT assay. Cell migration was monitored using a Transwell assay. IACs were isolated following crosslinking and their molecular composition analysed using mass spectrometry (MS)-based proteomics.

**Results and discussions**
In two MDA-MB-435S-derived cell clones with decreased expression of integrin αV, expressing 15% (2αV) or 5% (3αV) of the control cells amount, increased sensitivity to paclitaxel and vincristine, decreased sensitivity to cisplatin, and decreased migration were observed in line with previous results obtained following transient transfection with integrin αV siRNA. In cell clones 2αV and 3αV, which were smaller than control cells and lacked stress fibres, the number of focal adhesions was shown to be significantly lower as observed by interference reflection microscopy and immunofluorescence detection of phospho-paxillin, phospho-FAK and phospho-Src. MS analysis of isolated IACs from control MDA-MB-435S, 2αV and 3αV cells identified 282 proteins, including 36 out of 60 consensus adhesome proteins. As expected, in clones 2αV and 3αV, integrins αV, β3 and β5 were detected at much lower levels compared with control cells. In addition, lower levels of talin-1 and -2, vinculin, alpha-actinin-4, tensin-3, filamin-A and -B, liprin β1 and pleckrin were detected.

**Conclusion**
These data will enable follow-up analyses of the mechanisms of signalling by integrins αVβ3/β5 and therefore represent a valuable resource to improve our understanding of the mechanisms involved in adhesion control of cell sensitivity to antitumor drugs and metastatic potential.

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**PO-206**

**CALPONIN 3 (CNN3) PROMOTES EPITHELIAL TO MESENCHYMA TRANSITION AND DRUG RESISTANCE OF COLON CANCER CELLS**

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**Introduction**
Calponin 3 (CNN3) is one of the three isoforms in the Calponin family of actin-binding proteins. It is expressed in smooth muscle cells and non-smooth muscle cells and is required for cytoskeletal rearrangement and wound healing. Epithelial to Mesenchymal Transition (EMT) is an important step in the development of epithelial cancers during which the cancer cell exploits the preexisting wound healing program. Therefore, it is expected that CNN3 plays a role in EMT. The few available data on the role of CNN3 in cancer shows that CNN3 is expressed in mammary cells in response to ErbB2-overexpression and in Duke’s stage C of colon cancer, but there is no comprehensive analysis of its role in cancer which is the aim of this study.

**Material and methods**
Total of 21 cell lines were included in this study (8 from breast cancer, 12 from colon cancer, and a cervical cancer cell (HeLa) as a positive control for EMT). CNN3 expression was examined by Western blotting, followed by siRNA silencing in the metastatic cell line SW620. The influence of CNN3 silencing on EMT was detected by EMT markers expression by Western blot and collagen invasion assay. Cell viability was performed after exposure to 5 Fluorouracil (5FU) using Sulforhodamine B (SRB) assay. Furthermore, CNN3 expression was examined by immunohistochemistry in 56 formalin-fixed paraffin-embedded (FFPE) colon cancer tissue samples.

**Results and discussions**
CNN3 showed positive expression in 6/8 breast lines and 7/12 colon lines. The primary line SW480 was negative while its metastasis from the same patient (SW620) was positive, suggesting that CNN3 was associated with metastasis. The CNN3 silencing was >98% efficient in the metastatic cell line SW620. Silenced cells were less invasive compared to the control and showed...
loss of mesenchymal markers and acquisition of epithelial markers. CNN3 partially restored the colon cancer cell sensitivity to 5FU. CNN3 was expressed in 20/56 (39%) of FFPE colon cancer by immunohistochemistry and it was not related to p53 stabilisation consistent with our cell line findings.

**Conclusion** The results suggested involvement of CNN3 in EMT and hence metastasis and also in resistance to standard chemotherapy in colon cancer. These data deserve further exploration in vivo and in clinical studies to validate the potential clinical applications of CNN3 in cancer treatment and prognosis.

**PO-208** **EMERGING ROLES OF HECT TYPE E3 UBIQUITIN LIGASE SMURF2 IN CANCER**

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**Introduction** Despite significant advances made in the treatment of specific cancer types, eradicating the disease, especially in its most dangerous metastatic forms, has yet to be achieved. The lack of an effective treatment together with the high mortality rates of the patients emphasise the urgency to explore novel therapeutic targets and paradigms, which can subsequently be incorporated in cancer treatment.

Recently, we discovered Smurf2 – an E3 ubiquitin ligase, chromatin modifier and signal transduction regulator, as a key cellular factor operating in cells to prevent cell transformation and carcinogenesis. We have also reported that Smurf2 acts as an important regulator of DNA damage response (DDR), gene expression, and genomic integrity maintenance spanning through and interlocking these components. Blank M et al., *Nature Med* 2012. These findings prompted us to further investigate and characterise molecular processes operating under Smurf2 control in mammalian cells.

**Material and methods** A gamut of different approaches ranging from genetics and biochemistry (including mass spectrometry analysis) through molecular cell biology to animal pathophysiology and human sample analyses (conducted on a variety of human normal and cancer cells and tissues) were used in our study.

**Results and discussions** Our research efforts identified a novel mechanism by which Smurf2 regulates genome integrity maintenance – through stability regulation of DNA topoisomerase IIα (Topo IIα) – one of the key cellular enzymes in chromatin organisation, dynamics and unaltered chromosome inheritance. We discovered that Smurf2 stabilises Topo IIα and prevents the formation of pathological DNA bridges; as well as modulates cell sensitivity to Topo IIα-targeting drug etoposide (Emamueli et al. *Cancer Res* 2017).

Our second line of investigation led to elucidation of Smurf2 as a bona fide negative regulator of nuclear A-type lamins, in particular of lamin A and its disease-associated form progerin (Borroni et al. *Ageing Cell* 2018). Both lamin A and progerin have been connected to genome integrity maintenance, DDR and gene expression, and linked to cancer. This association suggests that the targeting of A-lamins in cancer might be a promising direction to eradicate tumour cells, but more fundamental work is needed.

**Conclusion** Altogether, our findings support the notion that Smurf2 is intrinsically involved in the regulation of pivotal molecular and cellular processes operating in mammalian cells and leading to cancer.

**PO-207** **THE ROLE OF RSU-1 IN GLIOMA CELLS METASTASIS**

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**Introduction** Ras Suppressor-1 (RSU-1) was recently found to be associated with Focal Adhesion (FA) proteins. The main objective of this research work was the *in vitro* characterisation of a panel of glioma cancer cells in terms of aggressiveness, and the investigation of RSU-1 role on invasion of glioma cancer cells.

**Material and methods** A panel of four human neuroblastoma cell lines was used (H4, SW1088, A172, U87-MG). Invasion assay with matrigel-coated transwell and soft agar growth assay were performed in order to characterise the aggressiveness of glioma cell lines. The RSU-1 expression for glioma cells was tested by real time PCR and immunoblotting. Finally, glioma cells were transfected with siRNA against RSU-1 in order to find out the role of RSU-1 in glioma cells.

**Results and discussions** In order to assess the invasive potential of the four glioma cell lines, a transwell invasion assay was performed and A172 and U87-MG cells found to be more invasive than H4 and SW1088 cells. To determine the aggressiveness of the studied cell lines, soft agar assay was also performed. The number of colonies for the U87-MG and A172 was significantly larger than the H4 and SW1088, with the latter cell lines only forming a few small colonies. We then sought to find out whether RSU-1 gene is differentially expressed in the four cell lines and whether its expression is correlated with invasiveness. It was found that the more aggressive A172 and U87-MG cell lines, overexpress RSU-1 compared to the less aggressive H4 and SW1088 cell lines.

Subsequently, two cell lines were selected to be used for further experiments, H4 and A172 which are the least aggressive and more aggressive cells, respectively. Our results show that upon RSU-1 silencing, the invasion of A172 cells was significantly decreased whereas invasion of H4 cells was increased with respective changes observed in the expression of Matrix Metalloproteinase 13 (MMP13), a fundamental protease in cancer cell metastasis.

**Conclusion** Results confirmed that the A172 and U87-MG glioma cells are more aggressive than H4 and SW1088. Also, RSU-1 was found to be overexpressed in most aggressive cells in comparison to less aggressive cell lines. More aggressive A172 cells lacking RSU-1 showed decreased invasion while H4 cells showed increased invasion. Collectively, RSU-1 found to be critical for glioma cell invasion and further investigation of the implicated molecular mechanism is underway.

**PO-209** **BIOLOGICAL CHARACTERISATION OF SPECIFIC KRAS MUTATIONS: FROM BASIC BIOLOGY TO RESPONSE TO TREATMENT**

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**Introduction** KRAS is one of the most frequently mutated oncogenes in cancer and KRAS mutations are commonly associated