expressed by glioma cells modulates tumour growth and efficacy of GB conventional treatments, i.e. chemotherapy (temozolomide, TMZ) and radiotherapy (X-rays).

**Material and methods** Stable inhibition of HAF expression was established in human glioblastoma cells by RNA interference (U251shHAF). Orthotopic GB models were developed in mice (8/group) for U251shHAF and U251Sc cells (scrambled-shRNA infected cells) as control. Tumour development was assessed with 7T MRI (T2w sequence). At the end of the experiments, an immunohistology study was performed to characterise the vascularisation (PECAM), glial (GFAP) and inflammatory (CD68) reactions. In vitro, the radio- and chemosensitivity of U251shHAF were studied by clonogenic assay and cell cycle analysis following X-rays irradiation (X-RAD 225Cx) or TMZ exposition. Annexin-V binding and propidium iodure uptake followed by flow cytometry was used to quantify apoptotic and necrotic cells.

**Results and discussions** The stable inhibition of HAF expression in U251 cells leads to around 70% of its extinction in either normoxia or hypoxia (1% O2). Accordingly, the expression of VEGFA and CAIX, both known as HIF-1 and HIF-2 dependent genes, was decreased in U251shHAF cultured in hypoxia (1% O2) compared to U251Sc cells. Loss of function of HAF leads to a significant growth delay of U251shHAF tumours of 3 weeks compared to U251Sc tumours, although both tumours display similar vascularisation, glial and inflammation reactions.

In other hand, HAF silencing in glioma cells does not modify their sensitivity to X-rays or TMZ as suggested by the similar results obtained for both U251shHAF and U251Sc cells, through clonogenic assay, cell cycle and apoptosis analyses.

**Conclusion** Our results suggest that HAF might be of poor prognosis for GB since its inhibition in glioma cells reduces tumour growth without alleviating glioma cell chemoresistance and radioresistance.

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**CHARACTERISATION OF THE IMMUNE MICROENVIRONMENT OF COLORECTAL CANCER USING A NOVEL HIGH-PLEX PROTEIN ANALYSIS TECHNOLOGY**

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**Introduction** Spatial characterisation of the immune microenvironment within tumours enables a better understanding of immunology and oncology. However, it has proven difficult to perform such studies in a highly multiplexed manner using limited samples. To address this unmet need, we have developed an imaging and tissue-sampling platform designed to simultaneously analyse up to hundreds of tumour and immune proteins in a single FFPE tissue section with spatial resolution. This novel technology, called Digital Spatial Profiling (DSP) was applied to the characterisation of the therapeutic response of colorectal cancer patients to immunotherapy.

**Material and methods** FFPE colorectal tumour specimens were subjected to DSP to determine the spatial expression of 30 +immune related proteins. Following antigen retrieval, sections were stained with a cocktail of 30+DNA barcoded antibodies in combination with fluorescently labelled antibodies which were used to define the immune-enriched regions of the tumour. Using the fluorescent signal as a guide, regions of interest (ROI) were delineated followed by UV excitation of the defined ROI’s, which releases the antibody-bound DNA barcodes for downstream quantitation on the NanoString nCounter technology.

**Results and discussions** Comparing colorectal tumours characterised by Microsatellite stable (MSS), DSP was able to differentiate immune hot and cold tumours despite MSS status. Since there is a subset of patients with MSS colorectal cancer that still responds to immunotherapy, this suggests DSP could ultimately be used to identify unique spatial biology and immune characteristics that might further expand beyond MSS and MSI status to help predict patients’ response to therapy.

**Conclusion** Using this novel approach, we demonstrate multiplexed protein analysis of defined micro-regions within a tumour enabling systematic interrogation of the immune microenvironment within the tumour. We demonstrate the ability of this technology to reveal immune biology that can point to novel biomarkers or therapeutic targets.

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**MIRNAS IN TUMOR-DERIVED MICROVESICLES PROMOTE ANGIOGENESIS AND TUMOUR CELL GROWTH**

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**Introduction** Intercellular communication between cells and their microenvironment is important for tumour growth. Tumor-derived microvesicles (MV) have recently received great deal of attention because of their ability to induce an aggressive phenotype, immune escape, angiogenesis and drug resistance through the horizontal transfer of cellular macromolecules between cancer cells. We evaluated the role of miRNAs in tumor-derived MVs on angiogenesis and tumour growth in addition to searching for its target genes.

**Material and methods** MVs were isolated by ultracentrifugation in A549, H460 and BEAS2B cell lines. Candidate miRNAs in MVs were selected by miRNA array. RNA sequencing with validation by Western blot was done following the treatment of MVs in HUVEC cells.

**Results and discussions** A549 cell-released MVs increased the tube formation in HUVEC cells more than those of H460 or BEAS2B cells. Upon miRNA array, 11 miRNAs in A549 cell-released MVs were up-regulated and 2 miRNAs were down-regulated by more than 2-fold compared to those in MVs of H460 and BEAS2B. Among them, miR-619–5p the most
significantly increased angiogenesis in endothelial cells and growth rate of cancer cells. We found that miR-619–5p directly targeted RCAN1.4 which has the capability to inhibit endothelial cell proliferation and angiogenesis and ectopic expression of miR-619–5p markedly decreased RCAN1.4 expression.

Conclusion Our findings provide the first evidence that tumor-derived MVs can promote angiogenesis in endothelial cells and aggressive phenotype in cancer cells through the transfer of miRNAs including miR-619–5p. Clinical validation and exploring the way of therapeutic intervention should be followed.

**PO-311 CHARACTERISATION OF COLORECTAL TUMOUR ENDOTHELIAL CELLS**

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**Introduction** Endothelial Cells (ECs) have a substantial influence on the tumour microenvironment. This aberrant tumour micro-environment mediates cancer progression. Although the tumour vasculature is understood to be unique, few studies have directly isolated and compared tumour-associated and normal endothelial cells from the same tissue/organ. With the advent of the tumour vasculature ‘normalisation’ hypothesis, and cancer immunotherapy, understanding the specific characteristics of tumour endothelial cells, and their respective influence on tumours, may be a crucial step in developing effective and comprehensive, anti-tumour therapies.

**Material and methods** We applied a fully optimised 8 colour flow cytometry marker panel to characterise and compare endothelial cells from colorectal cancer biopsies, and normal-adjacent, non-involved colonic tissue. ECs were gated as live, CD31+ and CD45- cells. The markers CD144 (VE-cadherin), CD34 (ICAM-1), CD102 (ICAM-2), CD34 (Hematopoietic progenitor cell antigen), and CD105 (Endoglin) were assessed for differences in expression. Tumours were enzymatically digested and analysed on the same day. Matched samples from n=10 patients were collected.

**Results and discussions** Tumour-associated endothelial cells (TEC) express significantly less CD144, CD34, CD102 and CD34 when compared to normal ECs (NEG). Conversely, TEC demonstrate higher expression of CD105. No consistent trend was observed for CD31 expression. Using this unique approach, we were able to uncover previously unidentified subpopulations within NEC, that are significantly altered in TEC. Additionally, using various gating strategies – we analysed specific subpopulations of cells that appear to be significantly increased or reduced between colorectal tumours and normal colon.

**Conclusion** Taken together these results accurately depict a tumour endothelium that is functionally inept, and permit new insights into the origin, and adaptation, of tumour endothelial cells, which is still debated. The remarkable consistency between patients in CD54/CD34/CD105 expression may argue for a tightly regulated tumour endothelium. Additionally, this optimised endothelial marker panel provides a useful tool for further studies in the Tumour Biology and Angiogenesis field.

**PO-312 MOLECULAR IMAGING OF INTEGRIN αvβ3 UPRREGULATION IN A MOUSE MODEL OF BRAIN METASTASIS**

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**Introduction** Angiogenesis is an important marker of tumour stage and aggressiveness. Many anti-angiogenic therapies exist, although clinical success remains poor partly due to difficulties in patient selection. Molecular magnetic resonance imaging (MRI) using targeted microparticles of iron oxide (MPIO) has been used to image angiogenic vessels in solid tumours by targeting integrin αvβ3 with the peptide RGD. The aim of this study was to determine whether RGD conjugated MPIO can be used to detect angiogenic vessels in a mouse model of brain metastasis.

**Material and methods** Mice (n=34) were injected intracerebrally with 4 T1 murine metastatic mammary carcinoma cells. Mice underwent MRI following intravenous injection of either RGD-MPIO or control scrambled RDG-MPIO, at days 7, 14, 21, 28 or 35 using a T2* weighted sequence to detect MPIO-induced hypointensities. Following imaging, brains were perfusion-fixed for histology.

**Results and discussions** In mice receiving RGD-MPIO, postcontrast hypointensities were evident at most time-points, and were significantly increased in tumour-bearing vs. contralateral striatum at day 35 (p<0.05). At days 7 and 14, a trend towards increased hypointensities was observed in RGD-MPIO injected mice compared to both the control hemisphere and mice injected with RDG-MPIO. No significant differences were found between the tumour-bearing and contralateral striatum in RDG-MPIO injected mice. Thus, hypointensities observed in mice injected with RGD-MPIO likely reflect specific binding to endothelial αvβ3.

At later stages, however, hypointensities were also observed in gadolinium-enhancing tumours in mice receiving RDG-MPIO and unconjugated MPIO. Histological analysis indicated that MPIO of both types were largely present in macrophages associated with tumour blood vessels, suggesting that perivascular macrophages may actively phagocytose MPIO once the BBB is no longer intact. Nevertheless, endothelium-specific binding was also evident histologically in mice imaged with RGD-MPIO, but not with RDG-MPIO.

**Conclusion** RGD-MPIO appear to detect integrin αvβ3 positive blood vessels in a mouse model of brain metastasis. However, molecularly targeted MRI using MPIO may be precluded once tumours reach later stages with overt BBB breakdown, since retention is no longer target-specific.

**PO-313 INHIBITION OF NEDDYLATION ACCELERATES VEGF-ACTIVATED VEGF2-NITRIC OXIDE SIGNALLING FOR RESULTING IN BIPHASIC EFFECTS ON ANGIOGENESIS AND TUMOUR GROWTH**

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10.1136/esmoopen-2018-EACR25.826

**Introduction** Androgen deprivation therapy, understanding the specific characteristics of tumour endothelial cells, and their respective influence on tumours, may be a crucial step in developing effective and comprehensive, anti-tumour therapies.

**Material and methods** We applied a fully optimised 8 colour flow cytometry marker panel to characterise and compare endothelial cells from colorectal cancer biopsies, and normal-adjacent, non-involved colonic tissue. ECs were gated as live, CD31+ and CD45- cells. The markers CD144 (VE-cadherin), CD34 (ICAM-1), CD102 (ICAM-2), CD34 (Hematopoietic progenitor cell antigen), and CD105 (Endoglin) were assessed for differences in expression. Tumours were enzymatically digested and analysed on the same day. Matched samples from n=10 patients were collected.

**Results and discussions** Tumour-associated endothelial cells (TEC) express significantly less CD144, CD34, CD102 and CD34 when compared to normal ECs (NEG). Conversely, TEC demonstrate higher expression of CD105. No consistent trend was observed for CD31 expression. Using this unique approach, we were able to uncover previously unidentified subpopulations within NEC, that are significantly altered in TEC. Additionally, using various gating strategies – we analysed specific subpopulations of cells that appear to be significantly increased or reduced between colorectal tumours and normal colon.

**Conclusion** Taken together these results accurately depict a tumour endothelium that is functionally inept, and permit new insights into the origin, and adaptation, of tumour endothelial cells, which is still debated. The remarkable consistency between patients in CD54/CD34/CD105 expression may argue for a tightly regulated tumour endothelium. Additionally, this optimised endothelial marker panel provides a useful tool for further studies in the Tumour Biology and Angiogenesis field.