Introduction The neddylation, a process of Nedd8 modification, is important for the complete activity of cullin-scaffold RING-finger E3 ligases (CRLs) which promote proteins’ ubiquitination and degradation. The inhibition of neddylation is reported to interfere with the neddylation of cullins for subsequently inactivating CRLs, leading to cell cycle arrest and apoptosis of tumours. This study investigated the effects of neddylation status on angiogenesis and tumour growth using in vitro and in vivo models.

Material and methods Human umbilical vein endothelial cells (HUVECs) were used to examine the angiogenic effects of neddylation, including assays in viability, trans-migration, nitric oxide (NO) production, and angiogenic Matrigel tube formation. Further, protein profiles in neddylation, apoptosis, and angiogenesis-associated signalling pathways were verified in HUVECs by Western blotting. Additionally, dominant negative constructs of cullins were applied to investigate each cullin’s neddylation on VEGF-activated VEGFR2 degradation and signalling. Moreover, Matrigel-plug and xenograft tumour models were utilised to study tumour angiogenesis and growth.

Results and discussions Neddylation inhibition delayed vascular endothelial growth factor (VEGF)-activated VEGF receptor 2 (VEGFR2) degradation for sustaining VEGF phosphorylations and its down-stream MAPks/ACT-eNOS signalling, which accelerated the abnormal increment of NO, a biphasic pro- and anti-angiogenic factor, in HUVECs. Furthermore, neddylation inhibition exhibited biphasic effects on HUVECs’ viability, migration, and angiogenic tube formation. L-NAM, the NO inhibitor, notably blocked the proangiogenic activities and partly restored the anti-angiogenic and apoptotic activities of neddylation inhibition in HUVECs. Furthermore, we evidenced that the neddylation status of cullin 1 rather than other cullins was critically important in VEGF-activated VEGFR2 degradation and phosphorylations. Moreover, neddylation inhibitor displayed biphasic effects on angiogenic Matrigel-plug assay and on tumour angiogenesis and growth in xenograft tumour models.

Conclusion Neddylation inhibitor could block the neddylation of cullin 1 through delaying and sustaining VEGF-activated VEGFR2 degradation and signalling, which accelerated the abnormal amount of NO production on mediating the biphasic effects of endothelial angiogenesis and tumour growth. Therefore, exploring the neddylation status in angiogenesis may result in a new perspective for targeting tumours and angiogenesis-dependent diseases.

PO-315 INTERTUMOR HETEROGENEITY IN VASCUlARTY AND RESPONSE TO BEVACIZUMAB TREATMENT IN ARTIFICIAL MELANOMA BRAIN METASTASES
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Introduction Patients diagnosed with melanoma brain metastases have few treatment options and poor prognosis, and antiangiogenic agents targeting the vascular endothelial growth factor A (VEGF-A) could represent a potential treatment strategy. The purpose of this preclinical investigation was to study the vascularisation pattern and the effect of the VEGF-A targeting agent bevacizumab in artificial brain metastases established from four human melanoma cell lines with different angiogenic and invasive properties.

Material and methods A-07, D-12, R-18, and U-25 cells transfected with GFP were injected intracerebrally in nude mice treated with bevacizumab (10 mg/ml) or vehicle. Treatment was initiated one day before tumour cell injection, and continued twice a week until the mice became moribund. Moribund mice were killed and autopsied, and the brain was evaluated by fluorescence imaging or by histological examination.

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Expression and secretion of factors involved in angiogenesis and invasion was assessed by quantitative PCR and ELISA. 

**Results and discussions** The melanoma cells showed a preference for growth in the meninges and ventricles after intracerebral injection, and intertumor heterogeneity in the aggressiveness of meningial tumors reflected differences in angiogenic activity and expression of VEGF-A and interleukin 8 (IL-8). In contrast, growth and invasion of the brain parenchyma relied primarily on vascular co-option. The response to bevacizumab treatment depended on the angiogenic signature of the tumor cells and on the intracranial growth site. Bevacizumab treatment resulted in delayed meningial tumor growth and prolonged survival in cell lines showing high VEGF-A expression and high angiogenic activity in the meninges, whereas no difference in survival was observed between bevacizumab-treated and vehicle-treated mice in cell lines showing low VEGF-A expression and lower angiogenic activity in the meninges. 

**Conclusion** The melanoma cell lines showed different response to bevacizumab treatment, and these differences reflected differences in intracranial vascularisation patterns and in expression of VEGF-A.

**PO-316 ISOQUERCETIN: A NOVEL AGENT TO INCREASES VASH1 AND SUPPRESS COLON CANCER**

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**Introduction** Angiogenesis represents an important factor supporting the growth and propagation of many tumors; however, current antiangiogenesis agents exhibit limited efficacy or elevated adverse effects. As natural plant-based products with numerous beneficial physiologic effects, flavonoids represent attractive alternatives as cancer therapeutics. To the best of our knowledge, this study represents the first demonstration of acute but not prolonged bevacizumab treatment in a mouse xenotransplant model of colorectal cancer.

**Material and methods** Balb-c nude mice were implanted with 1.5×10⁷ cells subcutaneously. Tumor volume was monitored daily. Following euthanasia, tumors were processed for histological analysis (histologic grade, microvessel count) and immunohistochemical determination of VASH1 expression. Statistical analysis of the data (ANOVA and polynomial regression) adopted a 5% significance level.

**Results and discussions** We identified that acute but not prophylactic administration of Q3G in a mouse xenotransplant tumor model Q3G increased VASH1 expression, decreased vascular proliferation, and inhibited tumor growth. Our studies suggest that Q3G therefore represents a vascular disrupting agent, inhibiting tumour growth by limiting tumour blood supply and neovascularization through the upregulation of the angiogenesis inhibitory factor, VASH1.

**Conclusion** Thus, Q3G targeting of VASH1 expression may serve as a novel antiangiogenesis strategy for treating colorectal cancer.

**PO-317 A NOVEL BISPECIFIC ANTIBODY TO HARNESS THE HERG1-b1 MACROMOLECULAR COMPLEX FOR CANCER THERAPY.**

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**Introduction** Among hindrances in cancer treatment, the lack of appropriate markers to be exploited for targeted therapy, and the need of new potential drugs are two big challenges. hERG1 potassium channels area novel class of oncological targets and one of the most intriguing aspects of their involvement in tumour establishment and progression is the interaction with adhesion molecules, such as integrins. It has been recently demonstrated that macromolecular complexes formed between hERG1 and b1 integrins selectively occurs in many types of cancer (Becchetti A et al., 2017). In this scenario, hERG1 could be exploited as a therapeutic target providing non cardiotoxic strategies aimed at blocking hERG1.

**Material and methods** A scDb, a bifunctional single-chain diabody, directed against hERG1/b1 complex, was developed via SOE-PCR methodology. Such antibody was tested on HCT116 cells in lateral motility and western blotting experiments. Moreover immunohistochemistry (IHC) was performed on metastatic colorectal cancer (mCRC) paraffin embedded samples using the scDb, an anti-hERG1 and an anti-b1 integrin.

**Results and discussions** Performing IHC on sequential sections of mCRC confirmed the specificity of the scDb for both hERG1 and b1 integrin. In vitro data provide evidences that the administering of the bispecific antibody has an impact on lateral motility. Moreover, signalling pathways are also affected by the antibody treatment, as AKT phosphorylation and HIF1α levels are decreased when the molecule is administered. Such findings might suggest a possible effect of the bispecific antibody on the VEGF-A signalling pathway, which are consistent with our previous hypothesis (Becchetti A et al., 2017) of a possible cross-talk leading to a deep impact on VEGF expression and, thus, on neoangiogenesis.

**Conclusion** scDb-hERG1/b1 could be used as a potential new treatment for cancer patients and as an early molecular diagnostic marker. In fact, the selective expression of hERG1/b1 complex in cancer cells and its role in angiogenesis and cancer progression suggests that a molecule selectively targeting the complex will be an invaluable tool for cancer treatment.