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 meningal 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 bevacinuzabab treatment depended on the angiogenic signature of the tumor cells and on the intracranial growth site. Bevacizumab treatment resulted in delayed meningal tumor growth and prolonged survival in cells lines showing high VEGF-A expression and high angiogenic activity in the meninges, whereas no difference in survival was observed between bevacinuzab-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 bevacinuzab treatment, and these differences reflected differences in intracranial vascularisation patterns and expression of VEGF-A.

**PO-316**  
ISOQUERCETIN: A NOVEL AGENT TO INCREASE 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 that the flavonoid isoquercetin (Q3G) functions as a novel inhibitor of angiogenesis in colorectal cancer by targeting vasohibin 1 (VASH1). Vasohibin-1 (VASH1) is an endogenous angiogenesis inhibitor. However, the clinical relevance of VASH1 in colon cancer and its regulation on cancer angiogenesis and cancer cell biological characteristics are still unknown. The aim of this study was to evaluate the flavonoid isoquercetin (Q3G) as a novel VASH1-targeted inhibitor of angiogenesis in colorectal cancer.

**Material and methods** Balb-c nude mice were implanted with human colon adenocarcinoma HT-29 cells. The tumor volume was monitored daily. Following euthanasia, tumors were subjected to 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 tumor growth by limiting tumor 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.

**PO-318**  
INTESTINE-SPECIFIC HOMEBOX GENE ISX INTEGRATES IL6 SIGNALING, TRYPTOPHAN CATABOLISM, AND IMMUNE SUPPRESSION

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**Introduction** The intestine-specific homeobox transcription factor intestine specific homeobox (ISX) is an IL6-inducible proto-oncogene implicated in the development of hepatocellular carcinoma, but its mechanistic contributions to this process are undefined.

**Material and methods** In this study, we provide evidence that ISX mediates a positive feedback loop integrating inflammation, tryptophan catabolism, and immune suppression.

**Results and discussions** We found that ISX-mediated IL6-induced expression of the tryptophan catabolic enzymes Indoleamine2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase in hepatocellular carcinoma cells, resulting in an ISX dependent increase in the tryptophan catabolite kynurenine and its receptor aryl hydrocarbon receptor (AHR). Activation of this kynurenine/AHR signaling axis acted through a positive feedback mechanism to increase ISX expression and enhance cellular proliferation and tumorigenic potential. RNAi-mediated attenuation of ISX or AHR reversed these effects. In an IDO1-dependent manner, ectopic expression of ISX induced expression of genes encoding the critical immune modulators CD86 (B7-2) and programmed death ligand-1 (PD-L1), through which ISX conferred a significant suppressive effect on the CD8+ T-cell response. In hepatocellular carcinoma specimens, expression of IDO1, kynurenine, AHR, and PD-L1 correlated negatively with survival.

**Conclusion** Overall, our results identified a feed-forward mechanism of immune suppression in hepatocellular carcinoma organized by ISX, which involves kynurenine-AHR signaling and PD-L1, offering insights into immune escape by hepatocellular carcinoma, which may improve its therapeutic management.

**PO-319 ROLE OF STANNIOCALCIN-1 ON MACROPHAGE DIFFERENTIATION AND CYTOKINE PRODUCTION**

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**Introduction** Inflammation is one of the cancer hallmarks that promotes tumour formation. The infiltration of immune cells is linked to the growth and metastasis of various types of solid tumours. Clinical and experimental data indicate that cancer tissues with high infiltration of tumour-associated macrophages (TAMs) are associated with poor prognosis and resistance to therapeutics. Therefore, an understanding on the factors responsible for macrophage-homing and differentiation of M1 and M2 TAMs is recognised as an important immunotherapeutic strategy. Stanniocalcin-1 (STC1) is a paracrine factor associated with inflammation and carcinogenesis. Recent evidences suggested the roles of STC1 on anti-inflammation and alteration of macrophage functions. However, the role of STC1 in macrophage differentiation is not clear. With the benefit of hindsight, we hypothesised that STC1 may play a role in differentiation and inflammatory functions of macrophages.

**Material and methods** Human Leukaemia cells (THP-1) were differentiated into macrophages by 5 nM phorbol 12-myristate 13-acetate (PMA) for 6 hour, followed by directed M1 or M2 polarisation, induced by additional treatment with IFNγ +LPS, or ‘IL-4+IL-13’ respectively for another 18 hour incubation. To generate STC1 gene knockdown on THP-1, ON-TARGET Human STC1 siRNA was used. Cells were treated with siRNA/Lipofectamine complex before differentiation-treatments.

**Results and discussions** Transcript levels of macrophage (M1 and M2) markers and STC1 were significantly upregulated by PMA. An intermitted removal of PMA significantly abolished the upregulation of STC1 and TNFα (M1-marker) but not M2-markers (e.g. CD163). This suggested that STC1 was not a determining factor on differentiation but might play a supporting function in the process, especially in M1 polarisation. Drug-induced M1 differentiation remarkably upregulated mRNA levels of M1-markers and STC1 as compared with M2 differentiation. Intriguingly, STC1-knockdown before differentiation and M1 polarisation caused significant inductions of M1-marker transcripts, but no significant effects on M2-polarised macrophages. This suggested STC1 induction acts to counteract M1 cytokine production.

**Conclusion** The present study demonstrated the involvement of STC1 in the process of macrophage differentiation, particularly in suppressing the expression levels of pro-inflammatory cytokines. The characterisation of STC1 in the functionality of macrophages can provide insight into its role in TAMs.

**Receptors and Signal Transduction**

**PO-320 ANTI-INFLAMMATORY EFFECTS OF GOSSYPOL ON HUMAN LYMPHOCYtic JURKAT CELLS VIA REGULATION OF MAPK SIGNALLING AND CELL CYCLE**

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**Introduction** Gossypol, a natural polyphenolic compound extracted from the cotton seed oil, have been reported to possess pharmacological properties via modulation of cell cycle and immune signalling pathway. However, whether gossypol has anti-inflammatory effects against phytohemagglutinin (PHA)-induced cytokine secretion in T lymphocytes through similar mechanism remains unclear.

**Material and methods** Human T-cell leukaemia Jurkat cells were employed and cultured in the presence or absence of gossypol with or without PHA stimulation. The cell cycle was analysed by flow cytometry. The cytotoxicity was measured by evaluating lactate dehydrogenase (LDH) activity using LDH detecting kit. The protein expressions in Jurkat cell were analysed by Western blot assay. Total RNA from Jurkat cells were extracted and the gene-expressions were measured by real-time polymerase chain reaction technique. IL-2 levels were determined by an ELISA kit.

**Results and discussions** Using the T lymphocytes Jurkat cell line, PHA exposure caused dramatically interleukin-2 (IL-2) mRNA expression as well as IL-2 secretion. All of these PHA-stimulated events were attenuated in a dose-dependent manner by pretreating with gossypol. However, gossypol did not show any in vitro cytotoxic effects at doses of 5–20 mM. As a