regardless of PTEN status, whereas mTOR pathway upregulation is observed mainly in PTEN-competent CRC cells. **Conclusion** The presence of stromal cells (fibroblasts/endothelium) profoundly influences CRC response to PI3K/mTOR-targeting agents. Understanding the mechanisms underlying microenvironmental interactions (tumour, stroma, soluble factors) may be of fundamental importance to overcome therapeutic resistance and develop more effective therapies for patients affected by cancer.

**Results and discussions** CRC cell lines harbouring both \(BRAF^{V600E}\) and PTEN-loss expressed the highest levels of IL-8 and a ROC curve-based prediction algorithm based on these two mutations had 68% accuracy in predicting IL-8 production (\(p=0.002\)); on the other hand, VEGF levels inversely correlated with KRAS mutational status. IL-8 is tightly and significantly associated with the MEK/ERK-related with KRAS mutational status. IL-8 is tightly and significantly associated with the MEK/ERK

**Conclusion** These results showed that, in preclinical models of CRC, IL-8 expression is regulated by a \(BRAF/MEK/ERK/STAT3\) axis, and differential modulation of IL-8 after pharmacological treatments is dependent on \(BRAF\) mutational status. Such evidence allows better understanding of the molecular mechanisms of chemokine regulation, which can, in turn, contribute to the development of new targeted therapies in CRC.

**Poster Presentation: Tumour Biology**

**PO-294**

**BRAFV600E/PTEN-LOSS STATUS IS ASSOCIATED WITH INTERLEUKIN (IL)–8 EXPRESSION IN PRECLINICAL MODELS OF COLORECTAL CANCER (CRC)**

**Introduction** Mutational status in CRC is a strong predictor for overall survival; unfortunately, tumour microenvironmental (TME) and tumor-stroma interactions (TSI) also increase cancer cells’ drug resistance, leading to an urgent need to better understand the molecular mechanisms of acquired tumor-resistance, which remains crucial to determine overall patient benefit.

**Material and methods** Production of IL-8 and vascular endothelial growth factor (VEGF) was determined by ELISA under standardised culture conditions. Modulation of cytokine production after exposure to selective inhibitors of the MAPK and PI3K pathways was assessed, by both ELISA assay and real time-PCR. BRAF, MEK1, ERK1 and ERK2 expression were modulated using siRNAs specifically targeting these genes.

**Results and discussions** CRC cell lines harbouring both \(BRAF^{V600E}\) and PTEN-loss expressed the highest levels of IL-8 and a ROC curve-based prediction algorithm based on these two mutations had 68% accuracy in predicting IL-8 production (\(p=0.002\)); on the other hand, VEGF levels inversely correlated with KRAS mutational status. IL-8 is tightly and transcriptionally controlled by activation of the MEK/ERK pathway, as the MEK inhibitor trametinib downregulated ERK transcriptionally controlled by activation of the MEK/ERK

**Conclusion** These results showed that, in preclinical models of CRC, IL-8 expression is regulated by a \(BRAF/MEK/ERK/STAT3\) axis, and differential modulation of IL-8 after pharmacological treatments is dependent on \(BRAF\) mutational status. Such evidence allows better understanding of the molecular mechanisms of chemokine regulation, which can, in turn, contribute to the development of new targeted therapies in CRC.

**PO-296**

**IDENTIFICATION OF NOVEL PLAYERS OF TUMOUR-MACROPHAGE CROSSTALK IN LUNG CANCER**

**Introduction** Tumor-macrophage (Mφ) crosstalk has been extensively studied and both cytokine networks and cell-cell interactions at the tumour site directly impact therapeutic outcome. Tumour modelling brings up tools that can be explored to uncover targets for therapeutic intervention. We developed a model system based on the co-culture of lung tumour spheroids, fibroblasts and Mφ, which can emulate aspects of tumour microenvironment dynamics. In co-culture, Mφ infiltrate the tumour mass and display tumour promoting features, e.g. secretion of M2-like cytokines, among which CCL24 showed the highest upregulation. In a tumoral context, CCL24 was shown to be upregulated in primary tumours and liver metastasis of colorectal cancer and contributes to hepatocellular carcinoma malignancy. Therefore, this study aims to investigate this chemokine for its possible role in NSCLC invasion and metastasis.

**Material and methods** Three lung cancer cell lines (NCI-H157, NCI-H1650, NCI-H1437), representing tumour stages with different invasion potential and EMT scores, and 3 sources of fibroblasts (cancer-associated – CAF, normal – NF, human dermal fibroblasts – hDF) were supplemented with increasing concentrations of CCL24. Gene expression of EMT markers, transwell invasion and EDU proliferation assays were performed.

**Results and discussions** In the model, a tumor-supportive secretory profile was observed, with identification of cytokines associated with M2-like Mφ polarisation (G-CSF, IL4, IL13, IL10). Hierarchical clustering analysis identified CXCL13, CCL22, CCL23 and CCL24 as cytokines specifically upregulated in triple cultures. CCL24 supplementation resulted in an increase in the invasion potential of lung cancer cell lines (20% to 40%), most evident in non-invasive cell line NCI-H1437. This was concomitant with an upregulation of EMT markers Slug and Snail (up to 5-fold) in NCI-H1437 cells. hDF showed a marked increase in the invasion potential (up to 2-fold), while for CAF and NF the increase was around 20%. No effects in proliferation were observed.

**Conclusion** This data suggests that CCL24 promotes the invasion but not the proliferation of lung cancer cell lines, which could be linked to an EMT process in non-invasive cell lines presenting an epithelial phenotype. We are currently investigating the downstream effectors, namely upregulation of MMPs and angiogenesis stimulating factors.
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PO-297 PLEXINA4 PLAYS A ROLE IN CANCER PROGRESSION AND IMMUNE CELL INFILTRATION
1,2,4AI Oliveira*, 1,2W Celus, 2,3BM Costa, 2,3M Mazzone. 1VIB KU Leuven, Center for Cancer Biology CCB, Leuven, Belgium; 2Lab of Tumor Inflammation and Angiogenesis, Department of Oncology- KU Leuven, Leuven, Belgium; 3Life and Health Sciences Research Institute ICSVS, School of Medicine- University of Minho, Braga, Portugal; 4ICS/3B/3-PT Government Associate Laboratory, School of Medicine- University of Minho, Braga, Portugal

Introduction Immune cells play a major role in tumour progression, metastasis and response to anti-cancer therapies. Indeed, escape from the immune system is a hallmark of cancer. Plexins are attractive/repressive guidance molecules in the central nervous system that are also expressed in some immune cells. Starting from our previous observations that Sema3A/Nrp1 underpin a pathway that is indispensable for the immunosuppressive phenotype of tumor-associated macrophages (TAMs), we investigated how the expression of the Sema3A co-receptor PlexinA4 in stromal cells influences tumour progression and immune cell infiltration.

Material and methods Lung, breast and brain tumour mouse models were used in both full PlexinA4 KO mice as well as in a PlexinA4 chimeric model. Immune cells infiltration and/or localization in the tumour were assessed by FACs and IHC. FACs-sorting was used to isolate tumour infiltrating immune-cells that were then evaluated by qRT-PCR.

Results and discussions Using sorted tumor-associated immune cells and their respective non-tumour associated counterparts, we found that PlexinA4 is upregulated in tumor-infiltrating immune cells, prompting us to explore the role of plexin signalling cascade in these cells. Using PlexinA4 full KO mice, we first found that PlexinA4 deficiency in the stroma reduces tumour growth. Additionally, we generated a chimeric mouse model, where only the immune system is affected by the depletion of PlexinA4, and we observed a reduction of tumour growth in various subcutaneous and orthotopic cancer models, such as lung, breast and brain tumours. This reduction in tumour growth was accompanied by an increased infiltration of Cytotoxic T cells (CTLs).

Conclusion Together, our results point to the possible use of PlexinA4 blocking antibodies as a new anti-tumour immunotherapy, alone or in combination with standard immune checkpoint inhibitors and chemotherapy regimes in refractory tumours.

PO-298 PEGYLATED RECOMBINANT HUMAN HYALURONIDASE PH20 (PEGPH20) INCREASES TUMOUR UPTAKE AND EFFICACY OF CETUXIMAB IN A HUMAN PANCREATIC CANCER XENOGRAFT MODEL
J Souratha, R Ospood, J Cowell, A Fatallah, C Thompson, M Printz, D Maneval, D Kang*. Halozyme Therapeutics, Research, San Diego, USA

Introduction Hyaluronan (HA) accumulation in the ECM of many solid tumours correlates with tumour progression and poor prognosis. In preclinical models, enzymatic degradation of ECM HA with PEGPH20 is associated with remodelling of the tumour stroma, reduction of tumour interstitial fluid pressure, and expansion of tumour blood vessels, resulting in facilitated delivery of anti-cancer agents. Cetuximab (CET), a chimeric monoclonal antibody, targets human EGFR preventing tyrosine kinase-mediated phosphorylation and subsequent signal transduction. Studies were performed to quantitatively assess tumour CET content, plasma PK, and efficacy of CET ±PEGPH20 in human xenograft tumour bearing mice.

Material and methods Nude mice bearing EGFR-positive peritumoral human pancreatic BxPC-3 tumours overexpressing human HA synthase 3 (HAS3) were used to evaluate the effects of PEGPH20 in combination with CET. To evaluate PK, mice were dosed IV with 37.5 μg/kg PEGPH20 and IP with 0.03 mg/kg CET. Plasma samples were collected and analysed for CET concentration by ECL immunoassay. To evaluate tumour uptake, CET was radiolabeled with 89Zr and administered IV ±PEGPH20, and PET scans were acquired over 24 hours. To evaluate anti-tumour efficacy, 37.5 μg/kg PEGPH20 was delivered IV, 0.03 mg/kg CET was administered IP biweekly, and tumour growth was measured over time.

Results and discussions Significantly higher 89Zr uptake into BxPC-3/HAS3 tumours was observed by PET imaging in PEGPH20-treated mice, compared to vehicle alone. PEGPH20 administration immediately prior to 89Zr-CET resulted in significantly increased tumour maximum standardised uptake value (SUV) compared to CET alone (9.4 vs 6.5 meanSUVmax; p=0.039). Similarly, PEGPH20 administration 24 hours prior to 89Zr-CET showed increased tumour SUV that was nearly significant compared to CET alone (8.8 vs 6.5 meanSUVmax; p=0.051). PK analysis indicated decreased plasma CET exposure when combined with PEGPH20. Whether PEGPH20 was administered immediately prior to CET or administered 24 hours prior to CET, plasma CET levels at 24 hour after CET dosing were approximately half the concentration of that obtained when CET was administered alone. Administration of PEGPH20 alone inhibited tumour growth by 46% compared to vehicle. The addition of PEGPH20 increased CET tumour growth inhibition (TGI) from 67% to 82% (p<0.05).

Conclusion Tumour imaging and PK studies in nude mice demonstrated increased tumour uptake and reduced systemic exposure of CET, which corresponded with enhanced TGI when CET was combined with PEGPH20.

PO-299 ABBRENT TNDCS EXPRESSION IN COLON STROMAL FIBROBLASTS PROMOTES COLORECTAL CANCER CARCINOGENESIS
K.L. Cheng1, C.C. Chen1, K.C. Yang1.

1National Taiwan University, Graduate Institute and Department of Pharmacology, Taipei, Taiwan

Introduction Colorectal cancer (CRC) consists of a complex mixture of malignant tumour cells and nonmalignant stromal cells that form the tumour microenvironment. Activated stromal fibroblasts, or cancer-associated fibroblasts (CAFs), characterised by increased proliferative activity, extracellular matrix protein (ECM) production and α-smooth muscle actin (α-SMA) expression, are known to promote CRC tumorigenesis and progression. We have recently identified a stromal fibroblast-enriched protein thioredoxin domain-containing protein 5

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