The NFκB pathway plays an important role in inflammation and bone remodelling. The sesquiterpene lactone Parthenolide (PTN) is a potent NFκB inhibitor, and previous studies showed that PTN reduces osteolysis associated with breast cancer (Idris et al., 2009). Here, we took advantage of an in vivo supracalvarial injection, ex vivo mouse calvarial organ and in vitro co-culture models to assess the cell-autonomous effects of PTN on cancer cell-induced osteolysis. MicroCT analysis of calvarial bone showed that human MDA-231, mouse 4T1 and rat MLL cancer cells caused osteolysis when co-cultured with mouse calvaria, and these effects were significantly reduced by PTN (1 µM; MDA-231, 56%; 4T1, 62%; MLL, 36%, increase in BV/TV, p<0.05). Treatment of osteoblasts with PTN (1 µM) increased alkaline phosphatase levels (62% increase, p<0.01), enhanced bone nodule formation (42% increase, p<0.01) and reduced the ability of MDA-231 and 4T1 conditioned medium to enhance osteoblast support for osteoclastogenesis (MDA-231, 38% and 4T1, 24%; reduction, p<0.05) and induce mRNA expression of RANKL (85% reduction, p<0.01) and OPG (76% reduction, p<0.01). Moreover, PTN also inhibited RANKL (IC50; 1.6 µM), MDA-231 (IC50; 1.1 µM), 4T1 (IC50; 1.2 µM) and MLL (IC50; 1.4 µM) induced osteoclast formation in a dose dependent manner. Finally, intraperitoneal administration of PTN (1 mg/kg/day) in adult immune-competent mice prior to supracalvarial injection of MDA-231 conditioned medium caused a significant reduction of osteolysis (37% increase in BV/TV, p<0.05). This effect was found to be strongly associated with inhibition of NFκB-mediated pro-inflammatory actions of the bone- and tumour-derived factors RANKL, TGFβ, IL-8 and CXCL1 that alter the balance of osteoblasts and osteoclasts in bone metastatic microenvironment. Collectively, our findings suggest that, due to the combined anti-resorptive and osteoanabolic effects, PTN, or similar sesquiterpene lactones currently in clinical trials for advanced solid tumors, has potential as a promising therapeutic agent for the treatment of osteolytic bone disease.

Disclosure: The authors declared no competing interests.

CABS OC1.2
Breast Cancer Cells Compete for the Space in the Bone Metastatic Niche
Gloria Allocca, Hannah K. Brown, Ning Wang, Colby L. Eaton, Ingunn Holen
Medical School, University of Sheffield, Sheffield, UK

During dissemination to the skeleton, breast cancer cells are proposed to localise in a putative metastatic niche, situated in close proximity to the endosteal bone surface. To assess whether tumour cells compete for the space in this niche, we mapped the number and location of tumour cells in the tibia 5/12 days following injection of human breast cancer cells in immunocompromised mice. Female 12-week old BALB/c nude mice were injected i.v. with 1x10^5 MDA-MB-231-IV breast cancer cells labelled with a lipophilic dye (Vybrant-DiD/ Vybrant-CM-Dil). The number and location of tumour cells was mapped in three different regions of the tibia by multiphoton microscopy. Using Velocity 3D Image Analysis software we measured the distance between tumour cells and the nearest bone surface, and to other tumour cells. Competition studies were performed by partially occupying the niche by injecting 1x10^5 DiD labelled MDA-MB-231-IV cells and repeating the injection seven days later with cells labelled with CM-Dil, allowing separate identification of both cell batches in the niche. The tumour cells preferentially homed to the trabecular area of the bones rather than to the growth plate (p<0.005). In animals receiving two batches of tumour cells, the number of cells homing to bone from the second batch was significantly lower compared with the cells from the first injection (p<0.005). Moreover, the preferential homing pattern changed, with tumour cells evenly located in different regions of the bone. Tumour cells were located significantly closer to the bone surface than to other tumour cells (p<0.05 and p<0.01), regardless of whether the niche was ‘empty’ or partially occupied. Our results show that the preferential pattern of tumour cell homing is modified when the niche is partially occupied, suggesting a degree of competition for space in the bone metastatic niche. (In vivo work covered by UK Home Office license PPL 40/3462.)

Disclosure: The authors declared no competing interests.
CABS OC1.3
ERRalpha Regulates Prostate Cancer Cell Colonisation in Bone
Mathilde Bouchet1,2, Anais Fradet1,2, Carine Dellaux3,4, Geoffrey Vargas1,2, Lamia Bouazza1,2, Francesco Pantano1,2, Martine Croset1,2, Xavier Leroy4,7, Jennifer Rieusset2,8, Akelia Bellahcene9, Vincent Castronovo5, Philippe Clezardin1,2, Martine Dutergue-Coquillaud3,4, Edith Bonnelle1,2
1INSERM, Lyon, France, 2University Of Lyon, Lyon, France, 3CNRS, Lille, France, 4University Of Lille, Lille, France, 5University Of Liege, Liege, Belgium, 6UMR, Lyon, France, 7Centre Hospitaller Lille, Lille, France

Eighty percent of patients dying from prostate carcinoma (PCa) have developed bone metastases that are incurable. Because we found the orphan nuclear receptor ERα (oestrogen receptor related receptor alpha) expressed in bone metastases from PCa patients, we modulated its expression in PC3 cells. We showed that PC3 cells over-expressing wild-type ERα (PC3-ERRa) stimulate rapidly osteolytic bone lesions in SCID mice (n=10) ((1.20±0.34* for osteolysis and 18.5±5.4** for skeletal tumor burden (TB/STV)%)) compared with that observed with PC3-CT cells (0.49±0.22 (osteolysis) and 2±0.73 (TB/STV)) (Ethical approval DR2014-32). Surprisingly bone destruction was combined with new bone formation, as 70% of the metastatic limbs bearing PC3-ERRa inhibits spheres formation, which was associated with the stimulation of pro-osteoclastic factors mRNA of Cox2, Runx2 and Cathepsin K by PC3-ERRa. Moreover, we found that in vivo this was combined with the up-regulation of ET1, Wnt5a and Wnt5a that may explain the occurrence of bone formation in vivo. Interestingly, tumoural microenvironment was also affected by PC3-ERRa cells, as mouse periosin (POSTN), was over-expressed by the cancer-associated-fibroblasts in vitro. Moreover, we found that PC3-ERRa inhibits spheres formation, which was associated with a decrease of Nanog and Oct4 expression in vivo. Finally, we showed that elevated expression of ERα mRNA in PCa (cohort of 60 patients) (Ethical approval CSTMT-042) is associated with high level of ET1, Cox2, POSTN and Wnt5a. In conclusion, our data provided for the first time evidence that ERα can promote both osteolysis and osteosclerosis in animal models of PCa bone metastases. They also suggest its implication in the stromal niche via the POSTN/Wnt signalling and in the inhibition of the self-renewal capacity and pluripotency of tumour cells.

Disclosure: The authors declared no competing interests. This work was funded by the French National Cancer Institute (INCa); and Association for Prostate Tumor Research (ARTP).

CABS OC1.4
The Dark Side of Bone Anabolics? Intermittent PTH Modifies the Microenvironment to Increase Skeletal Breast Cancer Metastasis In Vivo
Penelope D Ottewell1, Hannah K Brown1, Kerstin Wiegand1, Ning Wang1, Gloria Allocca1, Peter I Croucher2, Colby L Eaton1, Ingunn Holen1
1University of Sheffield, Sheffield, UK, 2Garvan Institute of Medical Research, Sydney, Australia

Intermittent administration of parathyroid hormone (PTH) increases bone volume through positive effects on osteoblasts. However, it is not known whether expansion of osteoblastic cell populations also modifies breast tumour growth in bone. We have investigated the effects of PTH pre-treatment on bone and subsequent skeletal colonisation and growth of breast cancer cells in vivo. Twelve-week old female BALB/c nude mice (n=3-8/group) received PBS (control) or rhPTH 1-34 at 40/80ug/kg/day for 5 days +/- intracardiac injection of DiD-labelled MDA-MB-231-tld-tomato-luc2 cells on day 5. Animal cohorts were sacrificed day 5, 7, 10, 15 (bone studies) or week 1, 2 or 9 (tumour studies). Bone was assessed by µCT, histology, bone homormophometry and measurement of serum bone turnover markers. Tumour growth was monitored by in vivo imaging and bone homing investigated using two-photon microscopy. PTH treated animals had significantly increased numbers of osteoblasts compared with control (27.79 (40ug/kgPTH) and 24.07 (80ug/kgPTH) vs. 12.42(PBS) on day 5; p<0.01) and elevated serum P1NP levels (92.93:40ug/kgPTH and 89.31:80ug/kgPTH) vs. 30.87(PBS) day 7; p<0.01), whereas trabecular bone volume, osteoclast numbers and serum TRAP levels were unaffected. These effects were no longer detectable by day 10. Animals receiving tumour cell injections on day 5 of PTH treatment did not have higher number of colonising tumour cells in tibia/femur (90.6 in 80ug/kg PTH vs. 83.8 in PBS, p>0.05), and the number of tumours detected in the long bones was comparable (1.86:40ug/kgPTH) and 1.58:80ug/kgPTH) vs. 1.29(PBS), p>0.05). However, PTH caused increased tumour growth in skeletal sites not normally affected in this model, with higher number of tumours in sites outside the hind limbs (5.71:40ug/kgPTH and 5.25:80ug/kgPTH vs. 2.57(PBS); p<0.0001 and p<0.001). These results demonstrate that pre-treatment with PTH modifies the microenvironment, leading to increased breast tumour growth in a range of skeletal sites. (Covered by UK Home Office licence PPL40/3462.)

Disclosure: The authors declared no competing interests. This work was supported by a Cancer Research UK program grant “Defining the bone metastasis niche”.

CABS OC1.5
The Pain Mediator NGF is Induced by Multiple Myeloma in vivo, and Relieved by Therapeutic Activation of Adiponectin Signalling
Sam Olechnowicz, Megan Weivoda, Szi Kay Leung, Seint Lwin, James Edwards, Claire Edwards
University of Oxford, Oxford, UK

Multiple myeloma (MM) is a plasma cell neoplasm which causes osteolytic bone disease, and at diagnosis the most common symptom is bone pain. Adiponectin (Adpn) is a...
myeloma-suppressive adipokine which negatively correlates with the major pain mediator Nerve Growth Factor (NGF) in osteoarthritis. We sought to determine whether NGF was increased in MM and the mechanism of NGF regulation within the MM-bone microenvironment. Mice were inoculated with 5TG1-MM cells and tumour burden was associated with a significant increase in serum NGF (p<0.01). Immunohistochemistry revealed NGF expression in stromal cells within the growth plate, while CGRP+ nerves were detectable in the nearby periosteum. 5TGM1-MM or RPMI-8226 human MM cells did not express NGF, while cells found within bone (2T3 osteoblasts, ST2 bone marrow stromal cells (BMSCs), HS5 BMSCs and ATDC5 chondrocyte cells) expressed high NGF. Coculture of MM cells with BMSCs or osteoblasts significantly increased NGF expression in BMSCs or osteoblasts (p<0.05). MM-derived cytokines such as TNFα up-regulated NGF in 2T3 cells, ATDC5 chondrocytes and HS5 BMSCs (p<0.05). Adiponectin inhibited an LPS-induced increase in NGF in 2T3 osteoblasts and blockade of Adiponectin signalling towards Adiponectin receptors AdipoR1 or AdipoR2 resulted in a significant 2-fold increase in NGF, which was further increased upon combination with TNFα. The Adiponectin L-4F was compared with bortezomib and melphalan in vivo in 5TGM1-MM-bearing mice. All treatments gave a similar reduction in tumour burden (L-4F; 36%, bortezomib; 34%, melphalan; 36%, p<0.01), yet only L-4F induced a significant 2-fold reduction in serum NGF induction (p<0.01). Our results demonstrate that NGF is increased in MM in vivo, likely due to MM-induced up-regulation of stromal NGF. NGF is likely to be a cause of MM-induced bone pain, therefore Adiponectin-targeted therapies may provide an improvement over traditional approaches by reducing tumour burden while also acting to inhibit bone pain.

Disclosure: The authors declared no competing interests. This work was supported by the NIH/NCI R01 CA137116 and Leukaemia and Lymphoma Research (UK).

**CABS OC1.6**

*The Anti-Diabetic Drug Metformin Reduces Tumour Burden and Osteolytic Bone Disease in Multiple Myeloma In Vivo*

Siobhan Webb1, Rosie Butler1, Amanda Bacon1, Ann Snailth1, Sarah Gooding1, Jessica Whittburn1, Claire Edwards1,2


Multiple myeloma (MM) is a fatal haematological malignancy characterised by accumulation of malignant plasma cells in the bone marrow (BM), and severe lytic bone disease. Metformin is widely prescribed in diabetes, and is associated with improved outcomes in diabetic patients with MM, suggesting a potential anti-myeloma effect of metformin. The aim of the current study was to investigate the effect of metformin within the myeloma-bone microenvironment in vitro and in vivo. C57Bl/KaLwRij mice were inoculated with 5TG1-MM cells and treated with metformin either from time of tumour inoculation (continuous) or from time of established tumour (delayed). MM-bearing mice treated with metformin exhibited a decrease in myeloma-specific serum IgG2bk concentrations as compared to control (Control; 4.29 mg/ml ± 0.3 mg/ml, metformin-continuous; 1.51 mg/ml±0.6 mg/ml, p<0.001, metformin-delayed; 0.7 mg/ml ± 0.7 mg/ml, p<0.001). MicroCT analysis demonstrated a significant decrease in osteolytic lesion number in MM-bearing mice treated with metformin (Control; 26±3.6, metformin-continuous; 11.4±0.7, p<0.001, metformin-delayed; 9±1.5, p<0.01). Metformin induced a dose-dependent decrease in MM cell viability. MM cell lines exhibited a differential sensitivity to metformin; RPMI 8226 cells had highest basal metabolic activity and sensitivity to metformin. Metformin treatment of MM cells activated AMPK, decreased IGF-1 gene expression and induced apoptosis, detected by an increase in cleaved caspase-3 and PARP. Metformin had no effect on BM stromal cell (BMSC) viability. Direct contact of MM cells with BMSCs decreased the anti-MM effect of metformin. BMSC-conditioned media (CM) had a protective effect against the anti-MM effects of metformin at 24h that was lost by 72h. In contrast, BMSC CM protected against the anti-MM effects of the proteasome inhibitor bortezomib at all time points. Our studies demonstrate a strong anti-tumour effect of metformin in the MM-bone microenvironment, suggesting that metformin may be effective for the treatment of MM and the associated bone disease.

Disclosure: The authors declared no competing interests. This work was supported by NIH/NCI R01 CA137116.

**CABS OC3.1**

*Targeting of Epithelial-to-Mesenchymal Transition by a Novel Small Molecule Inhibitor Attenuates Prostate and Breast Cancer Invasiveness and Bone Metastases*

Jan Kroon1, Onno van Hooij2, Henry Cheung1, Cindy van Rijt-van de Westerlo2, Kees Jansen2, Eugenio Zoni1, Jeroen Buijs1, Maaike van der Mark1, Henk Viëtor1,3, Gabi van der Pluijm1

1. Leiden University Medical Center, Dept. Urology, Leiden, The Netherlands, 2. Radboud University Medical Center, Dept. Urology, Nijmegen, The Netherlands, 3. Oncodrone BV, Nijmegen, The Netherlands, 4. SPECS, Zoetermeer, The Netherlands

Transformed epithelial cells can activate embryonic programmes of epithelial plasticity and switch from a sessile, epithelial phenotype to a motile, mesenchymal phenotype also referred to as epithelial-to-mesenchymal transition (EMT). EMT is associated with poor prognosis in patients with osteotropic cancers. E-cadherin (CDH1) is an essential homotypic cell adhesion molecule that is often down regulated during this process. EMT-like processes are increasingly linked to therapy resistance and metastasis-initiating cells, thus providing the rationale for the development of novel small-molecule inhibitors that a) block the acquisition of an invasive phenotype in osteotropic cancer cells via EMT or b) revert their invasive, mesenchymal phenotype into epithelial phenotype (MET) by upregulation of CDH1 expression. High throughput screening of ~43,000 LMW compounds, followed by compound design & optimisation in vitro led to the identification ten candidate therapeutic compounds. These compounds displayed significant inhibitory effects on cancer cell invasion (>80%) and induced E-cadherin (re)expression, most likely through the interference with the binding of transcriptional repressors to the CDH1
E-box elements. We identified a unique compound, OCD155, can effectively and dose-dependently block the acquisition of an invasive phenotype in osteotropic prostate and breast cancer cells (PC-3M-Pro4Luc2 and MDA-MB-231/Bluc). When tested in our in vivo models of prostate and breast cancer bone metastasis, treatment of mice with OCD155 strongly and dose-dependently inhibited skeletal metastasis (number of metastases, tumour burden) according to preventive and curative protocols. At the dosages tested, no adverse effects of OCD155 were observed (body weight, liver toxicity parameters). To the best of our knowledge, our studies are the first to demonstrate the efficacy of new small molecule EMT inhibitor in the treatment of experimental skeletal metastasis.

Disclosure: The authors declared no competing interests. This research was supported by the EU Framework 6 PRIMA and the International Innovation Program PRONET (Agentschap.nl).

CABS OC3.2

BMP7 Inhibits Prostate Cancer Metastases by Depletion of Metastasis-Initiating Cancer Stem Cells
Jeroen Buijs1, Lapeng Chen2, Lilian Bijl1, Lilian de Geus1, Ellen de Morré3, Jan Kroon1, Maaike van der Mark1, Eugenio Zoni1, Geertje van der Horst1, Wytse van Weerden3, Ewa Snaar-Jagalska2, Gabri van der Pluim1
1Dept. of Urology, Leiden University Medical Centre, Leiden, The Netherlands, 2Institute of Biology, Leiden University, Leiden, The Netherlands, 3Dept. of Urology, Erasmus Medical Centre, Rotterdam, The Netherlands

Prostate cancer is the most common cancer in men, and bone is the preferred site for metastasis. Current treatment options for metastasised prostate cancer are not curative since conventional therapies (hormone, chemo-, and radiation therapy) seem relatively ineffective in targeting prostate cancer cells with stem/progenitor-like characteristics (CSCs). It is becoming increasing clear that the generation of CSCs may be linked to the acquisition of an invasive phenotype via epithelial-to-mesenchymal transition (EMT). Previously we found that bone morphogenetic protein (BMP)-7 inhibited EMT and bone metastasis formation but the effect of BMP7 on prostate CSCs has remained largely elusive. In this study, we show that BMP7 is the most potent inducer (180x) of BMP reporter signalling of all tested BMPs (BMP2, BMP4, BMP6, BMP2/7, BMP4/7). In clonogenic assays, BMP7 (2 nM) reduced the formation of holoclones (colonies enriched for CSCs) of PC-3 and PC-3M-Pro4 prostate cancer cells. While TGFβ pretreatment increased, BMP7 pretreatment reduced migration, and inhibited proliferation at later time points. Interestingly, when PC-3M-Pro4 cells were FACS-sorted for high aldehyde dehydrogenase (ALDH) enzymatic activity, BMP7 was shown to differentially inhibit clonogenic capability of the CSC subpopulation (ALDHhi vs. ALDHlo).

In vivo, intravascular injection of red-fluorescent prostate cancer cells in zebrafish with a GFP+ vasculature -a model of dissemination and metastasis- led to rapid dissemination, extravasation and metastatic colonisation of distant sites. Strikingly, BMP7 pretreatment (2 nM) of PC-3M-Pro4/mCherry cells inhibited extravasation and reduced formation of distant metastases at 1 and 3 days post inoculation, respectively. Furthermore, BMP7 pretreatment increased the metastasis-free survival (62.5% vs 100% in vehicle) and reduced the number of distant metastases in a model of intracardiac injection of PC-3M-Pro4/Luciferase cells in nude mice allowing for real-time cell-tracking. In conclusion, we have shown that BMP7 targets the CSC subpopulation in human prostate cancer leading to impaired formation of distant metastasis.

Disclosure: The authors declared no competing interests. This study was supported by the Netherlands Organization for Scientific Research (NWO, VENI-Grant, 916.131.10).

CABS OC4.1

Vectorisation of Hypoxia Activated Prodrugs to Chondrosarcoma Proteoglycans: Evaluation and Characterisation of Antitumoural Activity
Voisiere Aurélien1, Weber Valérie1, Dauplat Marie-Mélanie3, Degouil Françoise1, Chezal Jean-Michel1, Rédini François2, Miot-Noirault Elisabeth1, Peyrolde Caroline1
1Inserm, Université d’Auvergne, Clermont-Ferrand, France, 2Inserm, Nantes Atlantique Université, Nantes, France, 3Centre Jean Perrin, Clermont-Ferrand, France

Chondrosarcoma, represents the second most frequent primary malignant bone tumour in adults after osteosarcoma. Because of its abundant chondrogenic extracellular matrix, its poor vascularisation and hypoxic microenvironment, chondrosarcoma is highly resistant to conventional chemo and radio-therapeutic treatments. Today, only effective treatment remains surgical resection. UMR990 Inserm/UdA laboratory develops a new innovative therapeutic targeting strategy which exploits the two characteristics of chondrosarcoma microenvironment: a chondrogenic extracellular matrix (ECM) and a hypoxic tissue. Based on the affinity of the quaternary ammonium (QA) moiety for proteoglycans, we developed a strategy that uses the quaternary ammonium function to selectively address hypoxia activated prodrugs to the ECM of chondrosarcoma, that exhibit a high fixed charged density (FCD). We propose thus, to vectorise, with QA as vectors to PGs of chondrosarcoma, cyclophosphamide derivative hypoxia activated prodrugs with nitroimidazole or nitrofurane cleavable entity. These compounds were studied in vitro and in vivo comparatively to their non-vectorised equivalents and to a vectorised but non cleavable equivalent. The in vitro results, on the HEMC-SS human chondrosarcoma cell line, show that QA derivatives of nitroimidazole prodruk exhibited the best hypoxia versus normoxia differential cytotoxic activity (4.5 times more apoptotic cells in hypoxia than in normoxia). In vivo, on an HEMC-SS xenograft SCID mice model, this molecule causes a significant tumour growth inhibition of 62.1% as compared to only 8% for its non-vectorised equivalent. Interestingly, haematological side effects were less pronounced for the QA-prodrug respectively to the non-vectorised molecule. This novel innovative therapeutic strategy offers a real hope for treatment of cartilage cancer, relatively rare pathology, but particularly redoubtable.

Disclosure: The authors declared no competing interests. This work was supported by Ligue Contre le Cancer Auvergne Région, and State-Region Planning Contract (CPER).
CABS OC4.2
Beta Haemoglobin (Hbb): a Novel Marker of Breast Cancer Progression
Nadia Rucci¹, Mattia Capulli³, Marco Ponzetti¹, Luca Ventura², Simona DelleMonache¹, Patrizia Sanità¹, Adriano Angelucci², Anna Teti¹
¹Department of Biotechnological and Applied Clinical Sciences, University of L’Aquila, L’Aquila, Italy, ²Department of Pathology, San Salvatore Hospital, L’Aquila, Italy

Bone is the preferentially site of distant colonisation for breast carcinoma (BrCa). BrCa patients with metastases restricted to bone (BO) show a longer overall survival compared with BrCa patients developing bone and visceral metastases (BV). In a gene expression analysis, we found few genes whose expression was significantly different between the two groups of bone metastases. Among them, haemoglobin beta (HBB) was one of the most upregulated in BV vs. BO. Based on these data, we evaluated HBB expression in primary human BrCa, finding HBB in 34 out of 57 samples. Moreover, the percentage of HBB positive cancer cells was significantly higher in the invasive lesions than in the in situ counterpart (6-fold, P<0.05). Higher expression of HBB was also observed in ductal infiltrating carcinoma vs. the lobular invasive histotype (2.2-fold, P<0.05). Interestingly, a positive correlation (P<0.05) was observed between HBB and the Ki67 score. We next compared HBB expression between poorly aggressive (MDA-MB-231) BrCa cells, finding a higher expression in the latter. HBB overexpression in MDA-MB-231 (MDA-HBB) and MCF7 (MCF7-HBB) cells increased migration and invasion ability compared with control (MDA and MCF7-empty), along with an increased expression of MMP9. Moreover, MDA-HBB and MCF7-HBB conditioned media induced tube formation (1.5-fold increase P<0.05). Consistently, the in vivo growth rate of orthotopically implanted MDA-HBB was higher compared with MDA-empty. Endpoint tumour weight was increased too (1.9 fold, P=0.002), while histology revealed less fibrosis in MDA-HBB and MCF7-HBB-derived tumours (0.4 fold, P<0.001) along with increased angiogenesis. Finally, local recurrence and visceral metastases were observed only in MDA-HBB implanted mice (incidence, 60%). Similar results were observed in MCF7-HBB orthotopically injected mice. Altogether, our findings demonstrate a positive correlation between HBB expression and BrCa aggressiveness, paving the way for the use of HBB as a BrCa progression marker.

Disclosure: The authors declared no competing interests.

This work was supported by the “Associazione Italiana per la Ricerca sul Cancro” (AIRC) (grant numbers 11950).

CABS OC4.3
FZD5 mediates the anti-proliferative, but not the pro-apoptotic effects of WNT5A on prostate cancer cells
Stefanie Thiele¹, Andy Göbel¹, Sandra Hippauf¹, Tilman D. Rachner¹, Franz Jakob², Martina Rauner¹, Lorenz C. Hofbauer¹,³
¹Technical University, Dresden, Dresden, Germany, ²University of Würzburg, Würzburg, Germany, ³Center for Regenerative Therapies Dresden, Dresden, Germany

Wnt proteins and their cognate receptors play a significant role in malignant diseases, in particular in PCa. Previously, we could show that WNT5A inhibits PCa cell proliferation and induces apoptosis in vitro, leading to reduced PCa growth in vivo. However, the involved receptors remain unknown. Here, we determined which receptors mediate the WNT5A-induced effects on PCa cells. The expression profile of 12 different Wnt receptors was analysed in three human (PC3, C42B, MDA-PCa-2b) and two mouse (RM1, TRAMP-C2) PCa cell lines. Frizzled (FZD) 10 and FZD9 showed the lowest expression levels in the PCa cell lines, while FZD1; FZD6, and Ryk were highly expressed. To determine which receptors mediate the anti-proliferative and pro-apoptotic effects of WNT5A in PCa, we knocked down FZD5 and receptor tyrosin-like orphan receptor (ROR) 2 with specific siRNA in PC3 cells 24 h before the induction of WNT5A overexpression. After knock-down of ROR2, WNT5A was still able to suppress proliferation by 31%. However, FZD5 knock-down completely reversed the suppressive effect of WNT5A on proliferation. The knock-down of FZD5 and ROR2 itself did not change PCa cell proliferation. Interestingly, the increase of apoptosis after WNT5A overexpression could not been reversed by neither knock-down of FZD5 or ROR2, suggesting another receptor involved in this process. Of note, knock-down of FZD5 even further increased apoptosis after WNT5A overexpression. A cDNA array containing samples from 9 healthy and 39 patients with prostate cancer was evaluated for WNT5A, FZD5 and ROR2 expression. WNT5A, FZD5, and ROR2 mRNA expression was significantly higher in the prostate cancer samples compared with healthy controls (p<0.001). However, only FZD5 expression correlated highly positively with WNT5A expression (r²=0.8801, p<0.001). These data suggest that FZD5, but not ROR2, mediates the anti-proliferative effects of WNT5A on prostate cancer cells.

Disclosure: The authors declared no competing interests.