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CUKC is jointly held by Cardiff University and its Chinese partners; Capital Medical University, Peking University and Yiling Group once a year. The conference aims to provide an opportunity for researchers and scholars in the area of cancer treatment and research to communicate, exchange, and share their knowledge so as to promote development and advances in this area. CUKC supports and strengthens the collaboration between China and the UK who recognise the need and benefit of Fighting Cancer Together.

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FBO2A01
THE ROLE OF CALCIUM SIGNALLING IN DEVELOPMENT OF PANCREATITIS AND PANCREATIC CANCER

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Acute pancreatitis is mostly initiated by combinations of fatty acids and alcohol, or by bile acids, causing massive release of Ca^{2+} stored inside the pancreatic acinar cells followed by excessive entry of Ca^{2+} from outside the cells. The loss of Ca^{2+} from the stores and the overloading of the cytosol with Ca^{2+} cause trypsin activation and inhibition of mitochondrial function, ultimately resulting in necrosis. Repeated attacks of acute pancreatitis lead to chronic pancreatitis, characterized by loss of acinar cells. There is also proliferation of the peri-acinar stellate cells due to increased Ca^{2+} signalling in these cells, partly driven by increased availability of the pro-inflammatory peptide bradykinin. The increased number of active stellate cells increases the production of a cancer-promoting matrix. The pathological Ca^{2+} signals in both acinar and stellate cells have been characterized and inhibitors of these signals, which may have therapeutic value, have been identified.

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FBO2A02
EXPRESSION OF CD26/DPPIV PROMOTES GROWTH AND METASTASIS OF PANCREATIC CARCINOMA

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Background/Aim: Pancreatic carcinoma (PC) is a malignant tumour with highly aggressive behaviour and poor prognosis. Currently, the biological behaviour of pancreatic cancer is rarely understood and there is no effective method for its early diagnosis. It has been recognized that there is a close relationship between CD26/dipeptidyl peptidase-IV (DPPIV) and tumours. Its immune regulatory function may affect the tumour immune process and can regulate the migration and invasion of the tumour cells through interaction with extracellular matrix components. To our knowledge, there is still no report about the relationship between CD26/DPPIV and PC. This study aims to elucidate the role of CD26/DPPIV in the development and progression of PC through a series of *in vivo* and *in vitro* experiments. The concentration of serum CD26/DPPIV in PC patients, as well as healthy controls was also tested to evaluate its possible diagnostic efficiency in pancreatic cancer. *Materials and Methods:* Real-time PCR was performed to examine *CD26/DPPIV* mRNA expression in pancreatic cancer tissues and cell lines. CD26/DPPIV interference plasmid was constructed with pSilencer3.1 and stable transfection was conducted on the PC cell line SW1990. A series of *in vitro* experiments were conducted to explore the effect of CD26/DPPIV on growth and migration/invasion of pancreatic cancer, including MTT cell proliferation assay, wound healing assay, plate colony forming experiment, soft agar colony formation experiment, cell cycle assay, transwell cell invasion and migration assay. In addition, *in vivo* tumourigenesis and liver metastasis models were also constructed in nude mice. We also tested the expression of several epithelial-mesenchymal transition (EMT) markers to elucidate the possible mechanism by which CD26/DPPIV promotes metastasis of PC. The enzyme-linked immunosorbent assay (ELISA) method was used to determine the sCD26/DPPIV level in pancreatic cancer patients, as well as in healthy controls. *Results:* Of 20 paired tissues, *CD26/DPPIV* mRNA expression was higher in cancer tissues than in paracancerous tissues in 16 patients, while in the other 4 patients, the results were opposite. Then, the stable RNA interference cell line of CD26/DPPIV was established on pancreatic cancer cell line SW1990. *CD26/DPPIV* knockdown decreased cell growth, colony formation, migration and invasion, and increased apoptosis in PC cells *in vitro* ($p<0.05$). *CD26/DPPIV* knockdown inhibited tumour growth and liver metastasis *in vivo* by using xenograft animal models ($p=0.05$). Thus, CD26/DPPIV promoted an aggressive PC phenotype *in vitro* and *in vivo*. In addition, CD26/DPPIV can affect the expression of several EMT related genes. ELISA results showed that the preoperative *CD26/DPPIV* level of pancreatic patients was significantly higher than that of the healthy donors ($p=0.03$) and sCD26/DPPIV level in PC patients can improve the diagnostic efficiency of carbohydrate antigen (CA)19-9 when these two markers were used together. *Conclusion:* Our results showed that *CD26/DPPIV* expression may promote tumour growth and metastasis *in vitro* and *in vivo*. Serum sCD26/DPPIV in pancreatic cancer patients was found higher than that in healthy controls.

3
FBO2A03
CHOLANGIOCARCINOMA – CAN WEST MEET EAST?

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Background/Aim: Biliary tract cancer is a rare tumour and forms 10% of primary liver tumours. Surgery for this malignancy is highly complex. This lecture will discuss the clinical background, as well as operative and non-operative modalities in the management of biliary cancer. *Conclusion:* Surgical resection, using onco-surgical principals, can achieve good long term results.

4
FBO2A04
RELATIVE COST PER LIFE-YEAR GAINED OF TREATMENTS WITH CURATIVE INTENT FOR T3NXM0 UPPER GASTROINTESTINAL CANCER

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Background/Aim: The treatment of patients with upper GI (UGI) cancer imposes a substantial financial burden on the National Health Service (NHS). Survival with best supportive care (BSC) is poor. Oesophagectomy, gastrectomy and definitive chemoradiotherapy (dCRT) are all offered to patients with locally advanced (T3) disease, with curative intent. The aim of this study was to determine the cost of each treatment per life-year gained compared with BSC. *Materials and Methods:* Costs to the NHS for 1 year of treatment from referral were calculated according to locally agreed diagnostic, staging and treatment pathways for patients undergoing oesophagectomy, gastrectomy or dCRT. Costs were calculated from national reference costs, published staff and medication costs, as well as activity-based costing. Overall survival from diagnosis was derived from a prospectively maintained database of all patients treated *via* a centralised regional multidisciplinary team (MDT). Patients with T3 oesophageal or gastric disease (n=621) were selected and grouped per treatment modality according to intention to treat: oesophagectomy with neoadjuvant chemotherapy; gastrectomy with or without peri-operative chemotherapy; or dCRT. *Results:* Median survival with T3 oesophageal cancer with BSC was 8 months (0.25-57), costing £7,426. Patients undergoing oesophagectomy survived a median of 24 months from diagnosis costing £18,270 for 1 year's treatment, and a cost per life-year gained of £8,133. After dCRT, patients survived a median of 23 months from diagnosis costing £17,462 for 1 year's treatment, and a cost per life-year gained of £8,029. Median survival for T3 gastric cancer with BSC was 5 months costing £4,861. Patients undergoing gastrectomy survived a median of 24 months from diagnosis costing £17,516 for 1 year's treatment, and a cost per life-year gained of £7,993. *Conclusion:* Both surgery and oncological therapy for T3 UGI cancer with curative intent improve overall survival considerably compared to BSC. All three treatment modalities are likely to be cost-effective at nationally accepted thresholds of willingness to pay per quality-adjusted life years (QALY).

5
FBO2A05
CDH1 (CADHERIN-1) GERMLINE MUTATIONS IN CHINESE DIFFUSE GASTRIC CANCER

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Background: Diffuse-type gastric cancer (DGC) is typical of dismal prognosis of which hereditary diffuse gastric cancer (HDGC) is a rare autosomal dominant cancer syndrome with *CDH1* germline mutations in about 30%~50% cases. As sporadic gastric cancer is more commonly seen in China, *CDH1* germline mutation data from Chinese gastric cancer population are rare. *Materials and Methods:* To investigate if *CDH1* germline mutations occur in Chinese diffuse gastric cancer patients with or without a positive family history, DNA samples were extracted from the blood of unrelated 94 Chinese diffuse gastric cancer patients. Sixteen exons of *CDH1* were amplified for each sample and the products were purified and sequenced. Germline mutation identified in a HDGC proband was then detected in her family members. *Results:* Seven *CDH1* germline mutations were identified in 94 patients (7/94=7.45%). Three missense mutations (p.T340A, p.L630V and p.V425L) were found in 3 individuals. Patients who carried p.T340A or p.L630V had very strong family history of HDGC. p.T340A was a known deleterious mutation and p.L630V and p.V425L were likely pathogenic. Three synonymous mutations were identified in 3 unrelated individuals. One splice site mutation was identified in one individual. In the pedigree whose proband carried p.T340A, 3 among 21 family members from maternal and paternal lines were individually identified as mutation carriers after resequencing. *Conclusion:* This is a preliminary report. More cases would be included to explicate the CDH1

variants in Chinese diffuse gastric cancer patients. Notably, a known deleterious mutation, which occurred in 3 members of a HDGC pedigree, both in maternal and paternal lines, needs special caution for their next step prophylactic treatment and follow-up. A genotype-phenotype correlation should be further discovered on the pathogenicity of p.L630V and p.V425L. For the strong HDGC family without *CDH1* germline mutations, *CDH1* somatic mutations, promoter methylation and loss of heterozygosity (LOH) detection should be carried out. Although the incidence is low, it is recommended that patients with DGC, especially HDGC, are offered appropriate genetic counselling and testing to decrease the gastric cancer incidence and risk in family members in China.

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FBO2A06
SURGEON LEVEL OUTCOME REPORTING FOR UPPER GI (UGI) CANCER OPERATIVE MORTALITY: A VIEW FROM OVER OFFA'S DYKE

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Background/Aim: The UK National OG Audit 2014 reports 90-day mortality rates (MR) of 4.4% and 4.5% after oesophagectomy and gastrectomy, respectively; however, controversially, the recently AUGIS-facilitated My Choices website reported median Trust MR of 1.7 % (range=0-8) and a median surgeon level mortality rate (SLMR) of 0% (range=0-20). Data from Wales was not included and the aims of this study were to compare data from the South Wales regional upper gastrointestinal (UGI) cancer network with that from England. *Patients and Methods:* Over a 3-year period between April 1, 2011 and March 31, 2014, 170 consecutive patients (median age=66 (24-86), 130 male, 80 neoadjuvant treatment) underwent surgery for UGI cancer by a multidisciplinary team (MDT) consisting of 6 specialist surgeons (3 in-reach) working at a single cancer centre from 4 National Health Service (NHS) Local Health Boards (equivalent to English NHS Trusts). The primary outcome measure was death within 30 days of surgery and, when joint consultant team operating occurred, the lead surgeon was identified (46%). *Results:* The median number of resections performed by individual UGI surgeons by year was 10 (5-25, p=0.855) and 14 (5-25) when team operating was taken into account. The median annual SLMR was zero but varied from 0 to 9.09%. Median Trust MR was 0 (0-7.14) % and overall network MR by year was 1.8% (0-3.7, p=0.389). Joint consultant procedures were not associated with any operative mortality (p=0.270). *Conclusion:* Surgeon level UGI cancer operative MRs from the South Wales UGI cancer network over a 3 year period were equivalent at both surgeon and Trust level to those reported from England. However, wide variation was observed in yearly SLMRs that might risk inappropriate target thresholds being set. Centre-based MRs appeared less sensitive to caseload effect and are potentially more representative of the prevalent MDT approach.

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FBO2A07
THE IMPORTANCE OF ANTIGENIC TARGETS IN CANCER: LESSONS FROM COLORECTAL CANCER

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Background/Aim: The role of anti-tumour T cell immune responses has been debated extensively in CRC, but a consensus is emerging that T_H1 signature (*i.e.* interferon (IFN)γ, cytotoxic) is beneficial to patients based on outcome data after "curative" resections. Furthermore, $CD4^+$ T cells appear to be fundamentally important in the generation of useful immune responses. Due consideration to the nature of the T cell antigenic target has not been robustly examined. *Materials and Methods:* Cognate $CD4^+$ T cell responses were measured by IFNγ ELISpot assays in colorectal cancer (CRC) patients either i) undergoing operative resection; ii) as part of a clinical trial* in palliative patients given an attenuated pox virus expressing 5T4 +/– cyclophosphamide. Antigenic targets included the tumour carcinoembryonic antigens CEA and the oncofetal antigen 5T4. Control antigens included influenza haemagglutinin and purified protein derivative. The phenotype of T cells was recorded using labelled antibodies and flow cytometry. *Results:* There was no evidence of immunosuppression and robust T cell responses to control antigens were measured in all patients pre-operatively. However, a response to the tumour antigen CEA identified patients with a significantly worse prognosis post-operatively due to tumour recurrence. This effect was seen independent of the TNM tumour stage. T cell responses to 5T4 appeared to reduce this effect (*i.e.* were protective). The beneficial effects of anti-5T4 responses are reflected by a significantly delayed time to tumour progression in the vaccination study of patients with inoperable CRC. *Conclusion:* Not all anti-cancer antigen T cell responses appear to be beneficial and careful selection of antigenic targets for vaccines should be given due consideration. Measured anti-CEA responses may have a clinical role in prognostication and selecting patients for additional early treatment.
(*TaCTiCC: Trovax and cyclophosphamide treatment in colorectal cancer. EudraCT Number 2010-024380-41).

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FBO2A08
THE FOCUS4 TRIAL GROUP

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Progress in drug development for advanced colorectal cancer has been slow over the last 10 years. Developments in science have resulted in the development of novel agents, which are focused on pathways more specifically than organ-specific cancer type. Recent data has identified significant colorectal cancer subgroupings, which may be amenable to specific targeted pathway interruption. Here we describe the evolution of a complex multi-arm, multi-stage phase III clinical trial work package. The UK phase III FOCUS4 trial in advanced colorectal cancer will be taken as an example of a complex but efficient novel trial design. Critical components of that design will be explored and future strategies for this and similar trials will be discussed.

9
FBO2B01
CERTIFICATION OF BREAST UNITS IN EUROPE - A RECIPE FOR IMPROVED RESULTS OF CANCER TREATMENT

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Background/Aim: The introduction of screening and multidisciplinary working has improved the treatment of breast cancer by bringing in best practice into every clinic. However, in general, there has been no auditing of individual units against quality standards in breast cancer. *Materials and Methods:* Eusoma has set up a validated European certification process that audits breast centres/units against published quality assurance (QA) indicators. Nearly 50 units have been visited in a 2-step process that involves checking 5-year retrospective results and an on-site visit with data verification. The unit has 6 months to rectify problems after an initial visit and submission of data. *Results:* The results show high compliance with QA process standards in the initial tranche of units visited and the outcomes are excellent in the patient cohorts studied. The data are excellent in the process of diagnosis, although detailed pathology and tumour markers are less well kept with examples of missing data. The Eusoma process is being used as a potential template for a future introduction of Europe-wide certification, and the European Commission (EC) is currently assembling 2 committees to produce a certification template. The implementation of compliance with the newly published quality indicators will be the responsibility of each European country using the EC template as the basis. *Conclusion:* Implementation of audited quality standards in Europe should further improve the results of breast cancer treatments.

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FBO2B02
CLINICAL OUTCOMES OF BREAST-CONSERVING SURGERY IN PATIENTS USING A MODIFIED METHOD FOR CAVITY MARGIN ASSESSMENT

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Aim: The present study describes a modified intraoperative method for cavity margin (CM) assessment in place of lumpectomy margin assessment in patients undergoing breast-conserving surgery (BCS). *Patients and Methods:* This is a retrospective review of 422 breast cancer patients undergoing BCS with intraoperative CM assessment. After an initial lumpectomy with intent to obtain C1-cm margins, separate specimens 1 9 1 cm, 0.5- cm thick were taken from the cavity margin circumferentially. These were frozen without reference to the side of the new margin as a time-saving measure and parallel sections of the resected surface were evaluated. *Results:* After a median follow-up of 55.5 months, a cumulative 5-year locoregional recurrence-free survival rate of 95.3 %, metastasis-free survival rate of 97.8 %, disease-free survival rate of 88.3 % and overall survival rate of 96.0 %, was achieved. The CM positivity rates were of no statistical difference when 7, 7-8 and 8 CMs were assessed. The second operation rate was 3.5% because of the false-negative results of the frozen section analysis on CMs. Univariate and multivariate analyses revealed that a higher pN stage and cT stage, as well as a lack of adjuvant chemotherapy or radiation, demonstrated significantly worse clinical outcomes. Locoregional recurrences and metastasis are both correlated with worse overall survival. The number of the CMs assessed was not associated with clinical outcomes. *Conclusion:* The modified CM assessment presented here is a rapid, accurate and oncologically safe approach for margin evaluation in BCS patients. Lumpectomy margin assessment might be spared when this method is used.

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FBO2B03
E-CADHERIN DEFICIENCY ASSOCIATES WITH POOR RESPONSE TO TAMOXIFEN IN ER+ INVASIVE DUCTAL BREAST CANCERS

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Background/Aim: Therapeutic resistance is a problem for a significant proportion of patients treated with endocrine agents and frequently associated with disease recurrence, often at distant sites, and a poorer prognosis. The intercellular adhesion regulator E-cadherin is known to exert tumour suppressor actions in a variety of cancers through suppression of cellular migration and invasion; recent research points to E-cadherin as a predictive marker for chemotherapeutic response. Previously, we reported the novel finding that tamoxifen-treatment of oestrogen-receptor (ER)+ breast cancer models lacking E-cadherin results in a significant gain in their aggressive, invasive capacity. Here, we have explored whether E-cadherin associates with tamoxifen response in clinical breast cancer. *Materials and Methods:* The association of E-cadherin and clinical outcome (disease-free interval (DFI), overall survival (OS) and time to metastases) was assessed in 794 ER+ breast cancer tissues comprising both patients who had received tamoxifen for 5 years (n=345) or had had no endocrine treatment (n=449). E-cadherin association with outcome was further determined in a group of ER-negative, tamoxifen-treated primary breast cancers tumours (n=93) as an internal control. *Results:* Kaplan-Meier survival analysis revealed a significant association between reduced E-cadherin expression (< median cut-off) and overall survival in the tamoxifen-treated group at 20 years (p=0.04; hazard ratio (HR)=1.51, 95% confidence interval (CI)=1.01-2.26); however, this relationship was much stronger at 5 years (p=0.02; HR=2.00, 95% CI=1.10-3.65). In the ER+, tamoxifen-untreated tumours, no relationship was observed. Reduced E-cadherin expression correlated with metastasis (regional and distant, p=0.01; HR=1.90, 95%CI=1-12-3.24) but only within the tamoxifen-treated group. In the ER-, tamoxifen-treated cohort, no adverse impact of reduced E cadherin on these parameters was observed. Subgroup analysis of the tamoxifen-treated patients revealed that the association between loss of E-cadherin expression and adverse outcome was apparent in both HER2+ (n=39) and HER2- (n=302) cohorts. However, the association was more significant within the HER2+ group (p=0.02 for OS; HR=3.45, 95% CI=1.08-11.07) (p=0.03 for metastasis; HR=2.78, 95%CI=1.02-7.57). *Conclusion:* These results support the hypothesis that low or absent E-cadherin expression in ER+, ductal breast cancers is a marker for poor outcome on tamoxifen. Patient cohorts lacking E-cadherin may benefit from other systemic endocrine therapy.

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FBO2B04
SURGICAL MANAGEMENT OF THE AXILLA IN BREAST CANCER FOLLOWING NEO-ADJUVANT CHEMOTHERAPY

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Background/Aim: Neo-adjuvant chemotherapy (NACT) has traditionally been used to downstage locally advanced or inflammatory breast cancer prior to surgery. However, more recently, NACT has had an increasing role in operable breast cancer. In this context, it is apparent that certain subgroups respond better than others. With a selective approach NACT has several advantages, including tumour down-sizing, so patients can be offered breast conservation, converting node positive patients to node negative, thus avoiding axillary node clearances (ANC) and creating time during the chemotherapy process in which gene testing can be performed and surgical choices made if proven breast cancer (BRCA) positive. Here we have assessed the value of using NACT response as means to increase the selectivity of surgical management of breast cancer patients. *Materials and Methods:* A retrospective review of the management of axillary disease in patients treated with NACT in Cardiff and Vale was carried out. Data was subdivided into two groups: a historic group where sentinel node biopsy (SNB) was performed upfront and prior to NACT followed by ANC if nodes were positive and a second group, which underwent an alternative management strategy as result of several trial outcomes. *Results:* In the first 2 years, 13/22 of the patients were node positive at diagnosis. Following NACT, they all had ANC. Subsequent analysis revealed that 6/13 were now node negative and had likely undergone unnecessary surgery. In the second group, reflecting the change in practice (n=28), a further assessment was made of response to NACT prior to performing surgery with more patients having SNB rather than ANC. *Conclusion:* NACT is playing an increasing role in operable breast cancer to down-stage both the breast and axilla. Down-staging the axillary disease reduces the number of axillary node clearances required, reducing the morbidity associated with unnecessary axillary surgery. Selective groups of patients respond well (triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2-positive (HER2+). However, this needs to be done safely backed with evidence and hence management of axilla still remains controversial.

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FBO2B05
APPLICATION OF ULTRASOUND-GUIDED VACUUM-ASSISTED PERCUTANEOUS EXCISION TECHNIQUE IN NON-PALPABLE BREAST LESIONS

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Background/Aim: Breast cancer screening programs have led to an increasing rate of detection of breast lesions; many benign lesions are treated surgically either because they fail to regress and progressively enlarge or because the female patients who suffer from the disease feel uncomfortable for observation. Open surgery for breast lesions could be associated with unfavourable cosmetic results and more extensive parenchymal dissection during the operation, especially for those non-palpable breast lesions. Non-palpable breast lesions are those that can hardly be touched clearly by hands due to such factors like too small sizes of the lesions, deep positioning, as well as high density of the mammary glands and so on. It is necessary to initiate safe and minimally invasive therapeutic operations. The excision of breast lesions with an ultrasound-guided vacuum-assisted device is a widely adopted technique for the diagnosis and treatment of breast diseases. It provides a kind of minimal invasive operation mode. We plan to explore the experience and value of ultrasound-guided vacuum-assisted percutaneous excision technique in non-palpable breast lesions. *Materials and Methods:* During January 2012 through November 2014, 452 non-palpable breast lesions diagnosed by ultra-sonography in 400 patients were treated by ultrasound-guided vacuum-assisted percutaneous excision. All patients underwent pressure dressings, which were dismantled after 48 hours and recheck after three months. *Results:* Ultrasound clearly displayed all of the breast lesions in 452 cases during Mammotome®. The operative process was successfully guided. All cases were free from either infections or pneumothorax, as well as skin lesions, of which four cases experienced postoperative local hematoma and 5 cases went with local bruising. The incidence of local hematoma was 6.0 % (27/452). One mass residue was found by ultrasound recheck after three months. *Conclusion:* Ultrasound-guided vacuum-assisted percutaneous excision for non-palpable breast lesions does have such advantages as easy operation, accurate positioning, safety, minimal invasion, fewer complications, rapid recovery and good appearance. It can be substituted for traditional operation mode and is worth to recommend as a kind of minimal invasive technique.

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FBO2B06
3D CULTURE OF HER2 OVER-EXPRESSING BREAST CANCER CELLS PROMOTES AKT TO MAPK PATHWAY SWITCHING AND LOSS OF RESPONSE TO THERAPY

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Background/Aim: The human epidermal growth factor receptor-2 (HER-2) is over-expressed in up to 25% of breast cancers and associated with a poor prognosis. Around half of HER-2+ breast cancers also express the oestrogen receptor (ER) and treatment for such tumours can involve both endocrine and HER-2-targeted therapies. However, despite preclinical data supporting the effectiveness of these agents, responses can vary widely in the clinical setting. Increasing evidence points to interplay between the tumour and its extracellular microenvironment as a significant determinant of therapeutic sensitivity and response. *Materials and Methods:* A modified version of the '3D on-top' assay was used for analysis of cellular growth in 3D culture and optimized for the breast cancer cell lines BT-474 and MDA-MB-361. Both these cell lines in 3D culture formed tightly packed spherical aggregates with a rounded (BT-474) or grape-like (MDA-MB-361) appearance compared to 2D monolayer growth. Immunocytochemical staining of cellular receptors, namely HER-2 and ER, revealed no loss or change in cellular localization in 3D compared with 2D culture. Basal growth of both cell lines showed a modest reduction in growth rate in 3D culture, which was not statistically significant. BT-474 and MDA-MB-361 cells were then grown in 2D or 3D cultures in the presence or absence of tamoxifen, fulvestrant or trastuzumab and cell counts were taken at days 0, 5 and 7. *Results:* For both cell lines, culture in 3D conditions attenuated their response to endocrine agents or trastuzumab or the combination. Comparison of signalling activation in 2D *versus* 3D culture in response to tamoxifen, fulvestrant and trastuzumab monotherapy, and in combination, was also investigated using Western blotting. For both cell lines, trastuzumab and endocrine treatments, either as monotherapy or in combination, suppressed MAPK signalling in 2D monolayer as expected. In contrast, in 3D culture, the same treatments led to MAPK activity being maintained or augmented. Finally, 3D cultures of BT-474 and MDA-MB-361 cells were treated for 10 days with trastuzumab and endocrine treatments alone, or in combination with the MEK inhibitor (U0126) or an AKT inhibitor (MK-2206), at concentrations that inhibited their

respective targets, and cell growth evaluated by coulter counting. Inhibition of MAPK significantly improved trastuzumab and endocrine response in both cell lines in 3D. AKT inhibition had no significant effect. *Conclusion:* We have shown that culture of ER+/HER-2+ breast cancer cell lines in a matrix-enriched 3D environment attenuates their response to both endocrine agents and trastuzumab. Associated with this is a matrix-induced shift from AKT to MAPK signalling; consequently, suppression of MAPK with a therapeutic inhibitor in 3D cultures restores this therapeutic response. These data demonstrate that targeting of adaptive pathways that maintain growth in 3D culture may represent an effective strategy to improve therapeutic response in breast cancer patients.

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FBO2B07
OUTCOME FOR DIFFERENTIATED THYROID CANCER – A SINGLE CENTRE EXPERIENCE

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Background/Aim: Differentiated thyroid cancer is a rare disease that carries a good prognosis for the majority of patients. The Mayo Clinic's metastasis, age, completeness, invasive, size (MACIS) score was designed to predict mortality in this disease. A MACIS score < 6 indicates an excellent prognosis, whereas a score > 8 conveys a poor prognosis. We wanted to correlate the MACIS score with patient outcome in our series. *Materials and Methods:* This was a retrospective analysis of a consecutive cohort of patients with differentiated thyroid cancer in a single institution. Data were available from a prospectively collected list of patients and follow-up data was acquired from the hospital electronic patient record. *Results:* Between July 2002 and June 2013, 137 patients underwent primary surgery for differentiated thyroid cancer. Median follow-up for 136 patients was 7 years (range=2-13). Most patients were women (78%) with a median age of 39 years (range=8-88). The majority had papillary thyroid cancer (78%) and the remainder follicular/Hurthle cell cancer (22%). The MACIS score ranged from 2.5-13.12 (median=4.67). Most patients had a score <6 (n=97, 71%). Thirteen (9%) patients had a score >8. The cause-specific mortality was 8.7% (n=12). For those patients with a MACIS <6, there was no mortality. For those patients with a score >8 and a follow-up period of five years (n=10) mortality was 90% and the sole survivor was alive with persistent disease. *Conclusion:* The MACIS score has been shown to be an effective predictor of mortality in this series. Patients with a MACIS <6 have an excellent prognosis.

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FBO2C01
MYELOID CELLS IN LIVER METASTASIS; ROLE OF FGF2

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Background/Aim: The liver is the most prevalent site for distant metastasis in colon cancer. Further patients with colorectal cancer often have metastases only to this site. Thus, therapies directed against liver metastasis would be expected to benefit patients with this malignancy in particular. Our goal in these studies was to identify candidate targets for therapy in colorectal cancer liver metastasis. *Materials and Methods:* We used murine models of liver metastasis; both murine colorectal cancer cells in syngeneic mice and human colorectal cancer cells in immunuo-suppressed mice and human colorectal cancer pathology specimens to identify and characterize myeloid cell recruitment. We depleted the recruited cells using diptheria toxin (DT) in mice with the CD11b promoter driving human diptheria toxin receptor or with antibodies. Myeloid cells were isolated from colorectal cancer tumour-bearing livers and gene expression compared to those from naïve livers. Fibroblast growth factor 2 (FGF2) was functionally blocked by antibody administration. *Results:* Inhibition of the recruitment or depletion of the macrophages recruited by murine cancer cells greatly reduced the growth of liver colonies. Inhibition recruitment of neutrophils by human colorectal cancer cells also greatly reduced the growth of liver colonies. Neutrophils recruited in the liver colonies expressed over 10X more mRNA for *FGF2* and over 5X more for heparinise, an enzyme that releases more FGF2 than naïve neutrophils. Immunostaining of human liver metastases and liver metastases in murine models of human cancer showed recruited but not naïve neutrophils to be expressing FGF2. Finally, blocking antibody to FGF2 reduced the growth of liver metastases in mice from human cells. *Conclusion:* Liver metastases recruit myeloid cells and their depletion greatly reduces liver colony growth. FGF2 derived from the myeloid cells is a potent stimulatory factor for liver colony growth and angiogenesis, thus identifying FGF2 as a potential therapeutic target in liver metastasis.

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FBO2C02
MAGI3 NEGATIVELY REGULATES WNT/β-CATENIN SIGNALLING AND SUPPRESSES MALIGNANT PHENOTYPES OF GLIOMA CELLS

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Background/Aim: Gliomas are the most common primary brain malignancies and associated with a poor prognosis. Although the underlying mechanisms remain unclear, aberrant activation of Wnt/β-catenin signalling has been shown to play an important role in gliomagenesis. This study aimed to ascertain the roles of MAGI3 in regulating Wnt/β-catenin signalling and phenotypes of glioma cells. *Materials and Methods:* Immunohistochemistry was performed to analyze MAGI3 expression in a human glioma tissue microarray. Effects of MAGI3 expression on glioma cell proliferation, migration and cell cycle were examined. In order to understand the roles of MAGI3 in regulating β-catenin expression and its downstream signalling pathway, glutathione S-transferase (GST) pull down and co-immunoprecipitation (Co-IP) were performed to reveal the binding domains of MAGI3 and β-catenin. Then, dual-luciferase reporter assays, RT-PCR and xenograft were used to confirm the regulation of MAGI3 on transcription of β-catenin and its target genes. In order to understand the relation of MAGI3 and overall survival and tumour grade, Gene Expression Omnibus (GEO) glioma datasets were analyzed. *Results:* We showed that the PDZ domain-containing protein MAGI3 was down-regulated at both mRNA and protein levels in human glioma samples. MAGI3 inhibited proliferation, migration and cell cycle progression of glioma cells and the growth of C6 tumours in mice. MAGI3 interacted with β-catenin through its PDZ domains and the PDZ-binding motif of β-catenin and suppressed β-catenin transcriptional activity in both glioma cells and xenograft tumours. Furthermore, analysis based on GEO glioma dataset showed association of MAGI3 expression with overall survival and tumour grade. *Conclusion:* We demonstrated negative correlation between MAGI3 expression and activity of Wnt/β-catenin signalling. MAGI3 expression is negatively associated with tumour grade, poor prognosis and activation of Wnt/β-catenin signalling. These results identify MAGI3 as a novel tumour suppressor and provide insight into the pathogenesis of glioma.

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FBO2C03 MOLECULAR MODELLING IN ANTICANCER DRUG DESIGN: THE DISCOVERY OF THE BCL3 INHIBITORS

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Background/Aim: B-cell lymphoma 3 (*Bcl-3*) is a proto-oncogene modulating the nuclear factor κB (NF-κB) signalling pathway, which has been first identified at the site of a t(14,19) translocation in B-cell chronic lymphocytic leukemia . In mouse models of breast cancer, Bcl3 specifically promoted the invasion and metastasis of ErbB2 driven tumours without affecting primary tumour growth, although a significant reduction in cell turnover was observed in secondary lesions. This anti-metastatic effect could potentially be used therapeutically and the aim of this project is to identify a possible Bcl-3 inhibitor using a computer-based, structure-based approach. *Materials and Methods:* Using know structural information from other members of the NF-κB protein family, we were able to build a model of Bcl-3 in complex with its partner p50. This model was then refined trough a molecular dynamics simulation. A suitable site on Bcl-3 was then identified and a virtual screening simulation was performed using a commercially available compound library (~300,000 compounds). The structures were ranked according to their binding score and the most promising compounds were pursued. *Results:* The molecular dynamics simulations have allowed us to identify a novel contact area between Bcl-3 and p50, which could be targeted by a small molecule. Using a virtual screening protocol, we were able to identify a number of potential inhibitors of the Bcl-3/p50 interaction. In particular, 10 compounds were biologically evaluated in three *in vitro* assays and one proved to be very effective in all three tests. *Conclusion:* Using a computer-aided drug design approach, we were able to identify a very potent Bcl-3 inhibitor. This compound is the first ever reported inhibitor for this new and exciting target.

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FBO2C04
CIRCULATING GALECTIN-3: METASTASIS PROMOTER AND THERAPEUTIC TARGET

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Galectin-3 is a galactoside-binding protein that is expressed by various types of human cells. Over-expression of galectin-3 is seen by most types of cancers, including colorectal, breast, lung, pancreatic, prostate and melanoma. The level of circulating galectin-3 is highly elevated in the bloodstream of cancer patients. Patients with metastatic diseases are seen to have even higher levels of circulating galectin-3 than those with only localized tumours. Recent studies have shown that the increased circulation of galectin-3 in cancer is an important promoter of tumour cell haematogenous dissemination to distant organs in metastasis. Galectin-3 binds to the cancer-associated oncofetal Thomsen-Friedenreich carbohydrate (Galβ1, 3GalNAcα-, TF) antigen on the large and heavily glycosylated transmembrane mucin protein MUC1, which protrudes 10-times higher over the cell surface than typical cell surface adhesion molecules. The galectin-3-TF/MUC1 interaction induces MUC1 cell surface polarization and the exposure of cell surface adhesion molecules. This leads to increased adhesion of disseminating tumour cells to the blood vascular endothelium in extravasation. It also leads to increased cell-cell aggregation of the tumour cells among themselves for the formation of circulating tumour emboli that enhance survival of disseminating tumour cells in the circulation. Circulating galectin-3 also interacts directly with the blood vascular endothelium causing increased secretion of several metastasis-promoting cytokines (*e.g.* interleukin (IL)-6 and granulocyte-colony stimulating factor (G-CSF)) from the vascular endothelium, which, in turn, enhances tumour cell adhesion, invasion and angiogenesis. Targeting the actions of circulating galectin-3, therefore, represents a very attractive strategy for the development of effective therapeutic agents to prevent/reduce metastasis and increase cancer survival. A number of biotech companies have started programmes to develop galectin-3-targeted anti-cancer/anti-metastasis drugs.

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FBO2C05
MICROSATELLITE INSTABILITY DETECTED IN TUMOUR-RELATED GENES IN C57BL/6J MICE WITH THYMIC LYMPHOMA INDUCED BY N-METHYL-N-NITROSOUREA

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Background/Aim: Plenty of studies confirmed the occurrence of microsatellite instability (MSI) with a variety of tumours and MSI has been found to be closely associated with the degree of malignancy and prognosis. However, whether the microsatellite loci in tumour-related genes would be induced to MSI by chemical and whether a connection exists between such MSI and tumour are still confused. *Materials and Methods:* In the present study, a total of 45 mice were injected with either N-methyl-N-nitrosourea (MNU) (90 mg/kg) or PBS (control) and MSI was determined by STR scanning and clone sequencing. Four mismatch repair (MMR) genes, containing 34 microsatellite loci, and 5 tumour suppressor (TS) genes, containing 30 microsatellite loci, were selected to explore MSI occurrence in thymic lymphoma and other tissues of MNU induced mice. *Results:* Among the 28 survived mice in the MNU-group, 19 (67.9%) mice formed thymic lymphomas. Moreover, 63.2% (12/19) of the tumours had metastasized to the other organs, including liver, spleen and kidney, determined by immunohistochemical assay. We examined the status of MSI in 110 tissues of MNU-treated mice using 64 loci optimized from 74 loci in TS gene and MMR gene. Finally, we found 8 MSI events (refer to 4 loci) in 4 types of tissues. The incidence of MSI in MMR genes was 50% (2/4) and 40% (2/5) in TS genes. The MSI occurrence ratio in either tumour and non-tumour tissues (1/17 *vs*. 0/9) or metastasis and non-metastasis tissues (2/15 *vs*. 5/69) showed no significant difference. Each MSI locus in ATM, MSH6 and P21 were found in these genes' intronic regions and 1 MSI in *Pms2* gene's 3'UTR region was found in MNU-treated mice. In addition, we found a loss of heterozygosity (LOH) at intronic dinucleotide repeats of ATM (ATM-8) in liver of one MNU-treated mouse. Four similar events occurred in *p21* gene intron (P21-1) within liver, spleen, kidney and thymus of another MNU-treated mouse without metastasis. One MSI was heterozygous mutation, which only existed in one allele of the locus Msh6-2. This intronic mutation was detected in liver with tumour metastasis of an MNU- induced mouse. We also found a homozygous 2bp insertion mutation in 3'UTR TG-repeat of *Pms2* in spleens of two MNU-treated mice without metastasis. *Conclusion:* Our study indicates that microsatellites in MMR genes and TS genes may be

sensitive to MNU and can be induced to MSI in MNU-treated C57BL/6J mice. The frequency of MSI in MMR and TS genes has no relationship with tumour and tumour metastasis.

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FBO2C06
DISULFIRAM – AN ANTI-ALCOHOLISM MEDICINE GIVING CANCER PATIENTS NEW HOPE

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Background/Aim: The medical need for better cancer therapies is undiminished, while drug development is slow (15 years/drug), risky and costly ($1.5 billion/drug). This has led to an increasing appreciation of the potential of repurposing of known drugs. We have shown that disulfiram (DS), an anti-alcoholism drug, demonstrates specific activity against a wide range of cancers. Importantly, we have been able to demonstrate that DS specifically destroys cancer-stem-like cells (CSCs) and potentiates the cytotoxicity of conventional first-line anticancer drugs. Although DS shows strong anticancer activity in the laboratory, clinical use of DS as an anti-cancer drug is limited by its rapid degradation and extensive metabolic conversion. Recently we used nano-biomaterials to encapsulate DS and extend its half-life in the bloodstream and obtained very promising *in vitro* and *in vivo* anticancer efficacy. *Materials and Methods:* Nano-encapsulated DS (NP-DS), breast cancer (MCF7, T47D, MDA-MB-231, MDA-MB-231$_{PAC10}$), liver cancer (Hep3B and PLC), lung cancer (NSCLC H801) and normal cell lines, MTT assay, combination index (CI)-isobologram, Western blot, ALDEFLUOR analysis, clonogenic assay, *in vitro* migration assay, immunocytochemistry, immunohistochemistry and mouse breast and liver cancer xenograft models were used in this study. *Results:* The chemoresistant MDA-MB-231$_{PAC10}$ and CSCs derived from MCF7 and T47D cell lines are highly resistant to a wide range of anticancer drugs, *e.g.* paclitaxel, gemcitabine, cisplatin and doxorubicin. The CSCs and MDA-MB-231$_{PAC10}$ cells are quiescent with significantly longer doubling time (64.9 h *vs.* 31.7 h). The CSCs and MDA-MB-231$_{PAC10}$ cells express high aldehyde dehydrogenase (ALDH) activity and a panel of embryonic stem cell-related proteins, *e.g.* Oct4, Sox2, Nanog and nuclealization of HIF2α and NFkBp65. NP-DS is highly toxic to breast CSCs and enhances cytotoxicity of anticancer drugs. NP-DS abolishes CSC characters, inhibits NFκB activity and completely reverses paclitaxel, doxorubicin, gemcitabine and cisplatin resistance in MDA-MB-231$_{PAC10}$ cells. NP-DS also blocked cancer cell migration *in vitro* at a very low concentration (5 nM). Intravenous administration of NP-DS showed strong anticancer efficacy in breast, liver and lung cancer xenografts. NP-DS also inhibits liver and lung cancer metastasis *in vivo*. *Conclusion:* As an FDA approved drug, nano-encapsulation may lead DS into cancer therapeutics.

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FBO2C07
ALTERING THE VASCULATURE TO PROMOTE TUMOUR IMMUNITY

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Background: Past studies have indicated that activation of tumour-specific T cells is an essential prerequisite for immune-mediated tumour control. However, the immunosuppressive tumour microenvironment often limits the activity of these cells. A population of regulatory T cells, which express the transcription factor Foxp3+ (Tregs), is highly enriched within mouse and human tumours where they are thought to contribute to tumour progression by limiting tumour-specific T cell activation. *Aims:* We aimed to examine the impact of Treg depletion on immune activity and tumour progression using a mouse model of tumourigenesis within which fibrosarcomas are induced by injection of the carcinogen methylcholanthrene (MCA). This method of tumour induction was combined with use of mice engineered to express the diphtheria toxin (DT) receptor on Foxp3$^+$ cells (Foxp3-DTR mice) whereby Tregs can be specifically and almost completely ablated through administration of DT. *Results:* We found that overall depletion of Tregs results in profound immune activity and significantly reduced tumour growth. Moreover, Treg depletion also impacts on the tumour vasculature by promoting development of intra-tumoural high endothelial venules (HEV). This is interesting as HEV are specialized in their ability to facilitate transit of T cells from the blood into lymph nodes. Importantly, presence of intra-tumoural HEV correlates with a significant increase in the number of tumour infiltrating T cells and control of tumour growth. *Conclusion:* As HEV have recently been described in several different human tumours (breast, lung, primary and metastatic melanoma), where they are linked to a good prognosis, it is likely that HEV development in tumours can have a major beneficial effect on promoting effective anti-tumour immune responses. To understand the mechanisms underpinning intratumoural HEV has, thus, immense translational potential.

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FBO3A01
TUMOURIGENIC POTENTIAL OF CIRCULATING TUMOUR CELLS IN PULMONARY VENOUS BLOOD FROM NON–SMALL CELL LUNG CANCER PATIENTS IN A XENOGRAFT MODEL

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Background/Aim: Disseminated in the blood, circulating tumour cells (CTCs) are involved in the initiation of NSCLC metastasis and recurrence and may predict cancer progression and prognosis. We here explore the clinical value and biological characteristics of circulating tumour cells (CTCs) in the pulmonary venous blood (CTCs-pv) of patients with surgically resected non-small cell lung cancer (NSCLC). *Materials and Methods:* Using the CellSearch system, we detected the number of CTCs per 7.5 ml pulmonary venous (PV) and peripheral blood from 32 patients who had undergone complete tumour resection. Xenograft assay was performed to investigate the biological characteristics of CTCs-pv after depleting the hematopoietic cells. *Results:* Peripheral blood CTCs (CTCs-peri) and CTCs-pv were detected in 8 patients (25%) and 29 patients (90.6%), respectively. Twelve patients (37.5%) had one or more circulating tumour microemboli (CTM) in pulmonary blood whereas no CTM was discovered in peripheral blood. The CTCs-pv count was positively correlated with tumour size (p=0.012) and tumour-nodes-metastasis (TNM) stage (p=0.048). Enriched CTCs-pv from 3 patients was injected into 3 immuno-deficient mice; one mouse developed xenograft tumour. Anti-human thyroid transcription factor-1 (TTF-1) immunohistochemistry and hematoxylin and eosin staining revealed that the mouse xenograft tumour was derived from CTCs-pv. *Conclusion:* This is the first report where CTCs-pv can form a xenograft tumour and that CTM are present in PV blood in NSCLC. Our results indicate that intraoperative manipulation contributes to the potential dissemination of tumourigenic CTCs and CTM and that the pulmonary vein should be ligated earlier during resection.

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FBO3A02
***EGFR* MUTATION-POSITIVE NSCLC: THE SOUTH WALES EXPERIENCE**

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Background: Oral tyrosine kinase inhibitors (TKIs) target the mutated Epidermal Growth Factor Receptor (*EGFR*) and have been shown to prolong progression-free survival in stage IIIB-IV non-small cell lung cancer (NSCLC). In addition, recent trials have shown that in patients with an exon 19 deletion, afatinib significantly prolongs overall survival in comparison to doublet chemotherapy. The prevalence of *EGFR*-mutated NSCLC varies according to histological subtype, ethnicity/geographical location, sex and smoking status. Rates as high as 64.2% in Vietnam and 50.2% in Chinese populations have been reported, whereas in a European population, rates are much lower, ranging from 4.5% in Italy to 16.6% in Spain. *Materials and Methods:* All patients with *EGFR* mutations in South Wales were identified from the database held in the Genetics Laboratory in University Hospital Wales, Cardiff. Data were collected on all patients who have been tested since the introduction of the service in 2010. *Results:* One hundred and nine patients were identified: 69 (63.3%) females and 40 (36.7%) males. Median age was 68 years (range=30-89). Ninety-two out of 109 (84%) had sensitising mutations, 8/109 (7%) had resistance mutations, 2/109 (2%) had mutations of uncertain significance and 7/109 (6%) had multiple mutations. Three out of 109 (3%) patients had exon 18 mutations, 47/109 (43%) had exon 19 deletions, 9/109 (8%) had exon 20 mutations, 43/109 (39%) had exon 21 mutations and 7/109 (6%) patients had multiple mutations. *Conclusion:* The pattern of *EGFR* mutations identified in South Wales is similar to other studies. It is difficult to estimate our exact prevalence for several reasons. Firstly, *EGFR* mutation testing is not universal. Patients undergoing potentially curative surgery, those with squamous cell carcinoma and early stage disease are not routinely tested. In addition, we have previously reported that 15% of patients have insufficient tissue for mutational analysis. Allowing for these uncertainties, we estimate that less than 10% of patients with advanced NSCLC in South Wales have an *EGFR* mutation at diagnosis.

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FBO3A03
THE COMBINATION OF A NOVEL BRUTON TYROSINE KINASE (BTK) INHIBITOR PLS123 AND mTOR INHIBITOR EVEROLIMUS SYNERGISTICALLY INDUCES ANTI-TUMOUR ACTIVITY IN MANTLE CELL LYMPHOMA

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Background/Aim: Mantle cell lymphoma (MCL) is an aggressive and incurable malignant lymphoma with a median

survival of 5 years that accounts for approximately 6% to 8% of B-non-Hodgkin's lymphoma (B-NHL) patients. Given the lack of successful treatment options for MCL, the development of new combination strategies for the treatment of this debilitating disease is urgently needed. The B-cell antigen receptor (BCR) signal pathway has gained significant attention as a potential therapeutic target in B-cell lymphoma. We recently developed a novel covalent irreversible Btk inhibitor PLS-123, which exhibits a dual-action mode of inhibition for both the catalytic activity of Btk and its own activation. This study aims to investigate whether PLS-123 potentiates the growth inhibitory effect of everolimus, a specific mTOR inhibitor, in human MCL cells. *Materials and Methods: In vitro* cell viability was analyzed using the Cell Titer-Glo Luminescent Cell Viability Assay. Induction of apoptosis and cell cycle arrest were measured by flow cytometry. Western blotting analysis was used to detect the essential regulatory enzymes within related signalling pathways post combination of PLS-123 and everolimus treatment. *Results:* Co-administration of PLS-123 and everolimus synergistically induced anti-proliferative effect in MCL cell lines (Granta519, Mino and Z138). Exposure of these MCL cells to minimally toxic concentration of everolimus and PLS-123 resulted in more significant inhibitory activity than each agent alone. Interestingly, combined treatment of MCL cells with everolimus and PLS-123 resulted in marked induction of apoptosis monitored by flow cytometry compared to single agent treatment, which were accompanied by marked up-regulation of apoptosis-related proteins (cleaved caspase-3, caspase-8, caspase-9 and PARP) and pro-apoptotic proteins Bax and Bad. The combination of PLS-123 and everolimus also substantially increased the cell population of G1 phase and decreased that of G2 in cell cycle, which indicted that combined treatment blocked cell cycle progression at the G1/S phase. The expression of CDK4 and CDK6, which regulated G1/S phase cell cycle transition, were reduced post combination treatment. Furthermore, the possible impacts of combination treatment towards related signalling cascades were next investigated by immunoblotting analysis. Combined exposure of MCL cells to everolimus and PLS-123 resulted in marked inactivation of the mTOR/AKT pathway compared to single drug treatment. The activation of p-ERK and p-AKT were also more significantly diminished after treatment of PLS-123/everolimus. *Conclusion:* The combination of novel Btk inhibitor PLS-123 and everolimus that synergistically induced anti-tumour activity in MCL cell lines suggested a new combination direction for the treatment of MCL patients.

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FBO3A04
THE BLOOD BRAIN BARRIER AND THE PREVENTION OF METASTATIC DISEASE

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Tight Junctions (TJ) have become recognised as key players in cancer metastasis. Early studies suggested a link between the reduction of TJ proteins and tumour differentiation and increasing experimental evidence has emerged to place TJs in the frontline as the structure that cancer cells must overcome in order to metastasize. The TJ is a region where the plasma membrane of adjacent cells forms a series of contacts that appear to completely occlude the extracellular space, thus creating an intercellular barrier and intramembrane diffusion fence. In epithelial cells, the TJ functions in an adhesive manner and can prevent cell dissociation, whereas TJs in endothelial cells function as a barrier through which molecules and inflammatory cells can pass. Interaction and penetration of the vascular endothelium by dissociated cancer cells is an important step in the formation of cancer metastases. TJs are the first barrier that cancer cells must overcome in order to metastasize. A considerable body of recent evidence exists on TJ and their role in a number of diseases. Recently, there has been an upsurge in studies investigating their possible role in tumourigenesis and metastasis. Early studies demonstrated a correlation between the reduction of TJ and tumour differentiation and experimental evidence has emerged to place TJs in the frontline as the structure that cancer cells must overcome in order to metastasize. Changes, somewhat similar, in both tumour and endothelial cells are necessary for successful growth and spread of cancer cells. A change in cancer cells by up-regulation or down-regulation of relevant TJ proteins results in loss of cell-cell association and cell contact inhibition leading to uncontrolled growth, loss of adhesion to and degradation of the basement. These must occur due to a concurrent loss of cell-cell association in the endothelium and modulation of TJ proteins involved in facilitating the passage of the cancer cells through this barrier. The interaction and penetration of endothelium by the metastasising tumour cell is a key step in the formation of metastasis. Metastatic brain tumours are frequently observed in patients with lung, breast and malignant melanoma and so brain metastasis (BM) remains a significant clinical issue. The blood brain barrier (BBB) is maintained and regulated by TJs. Therefore, a key step in the penetration of the BBB by cancer cells is their interaction with the endothelial cells of the BBB and the disruption of the TJ between them. As our knowledge and understanding of the molecular structure,

mechanism of action and function of TJs is expanded, the TJ can be regarded as a potentially important target for anti-cancer research and a possible area for future therapeutics.

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FBO3A05
ENDOSCOPIC REMOVAL OF ETHMOID OSTEOMAS UNDER NAVIGATION GUIDANCE

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Aim: We investigated the minimally-invasive ablation of osteomas of the ethmoid sinuses endonasally. *Patients and Methods:* A retrospective analysis was undertaken on 19 patients diagnosed with osteomas of ethmoid sinuses hospitalized from April 2005 to October 2010. All patients underwent sixteen-detector row computed tomography scan and 3D reconstruction preoperatively. All underwent operation with the help of navigation system and nasal endoscope. *Results:* All 19 patients' ethmoid osteomas were removed successfully with endoscope and navigation system. Two open procedures (1 through superciliary arch incision and 1 through labiogingival incision) were performed to remove osteomas; 17 tumours were removed under endoscopic and navigation guidance. In 5 patients whose osteomas were localized or had a diameter of no more than 2 cm, we found the osteomas with the help of navigation system and they were resected endonasally. Two patients' osteomas with narrow basilar part were relatively dissociative and were removed from the oral cavity after abscising the basilar part. Twelve patients' osteomas were with basilar part and connected with ante-meso skull base, lamina papyracea , orbital apex, cranalis opticus, fossa orbitalis bone; these neoplasms were removed using a electric drill with the guidance of navigation system. All patients are followed up to 8 to 64 months and are asymptomatic (1 who suffered from ambiopia witnessed disappearance of the symptom, 2 who suffered from prosopo-eminence, 1 is asymptomatic and 1 feeling better). Two patients underwent removal of crista galli; 1 of them suffered from postoperative cerebro-spinal rhinorrhea but recovered 15 days later after endoscopic repairing procedure and iodoform gauze packing. The 2 patients that underwent removal of crista galli suffered from anosmia and never recovered after 9 and 26 months of follow-up. One patient with enormous osteoma suffered from repeated crusting and abnormal odour but recovered after nasal flushing. *Conclusion:* Endoscopic ablation of osteomas of the ethmoid sinuses with the guidance of navigation system is an accurate, secure, minimally-invasive, calleidic procedure and preoperative computed tomography scan is a safeguard of an accurate approach of operations. Osteomas on median line and localized in ethmoid sinus is an indication of this operation. Even if the lesion affects extensively the frontal and maxillary sinuses, a combination of superciliary arch incision and labiogingval groove incision is a simple and easy and slinky option. A limited exposure of the fasciae of orbit, cerebral dura meter or optic nerve sheaths of the tumour's border is an effective way of deciding whether the tumour is to be completely resected for protecting important structures.

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FBO3B01
REMOTE-CONTROLLED ROBOTIC SURGERY FOR CANCER

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The evolution of surgery over the last 50 years has been transformed by innovation and technological advancements. In the 1980's/90's, advancements in micro-electronics and computing, in combination with improved robotic telepresence technology, allowed the introduction of "robotic surgery" with improved precision. However, it was not until about 10 years ago that such equipment became incorporated into surgical practice. Urological cancer surgery has benefited the most from robotic surgery, particularly in prostate cancer surgery. The most recent system available worldwide was introduced to Cardiff in September 2014, being the first Da Vinci XI system in the UK. We report our experience in terms of service introduction for prostate cancer and outline future developments in research, teaching and education.

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FBO3B02
A RANDOMIZED, OPEN-LABEL, MULTI-CENTER PHASE II STUDY TO COMPARE BEVACIZUMAB PLUS SORAFENIB *VERSUS* SORAFENIB FOR THE THIRD-LINE TREATMENT OF PATIENTS WITH METASTATIC RENAL CELL CARCINOMA (NCT02330783)

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Background/Aim: There is no standard treatment in patients with renal cell carcinoma that was previously treated with vascular endothelial growth factor (VEGF)-targeted therapies and mammalian target of rapamycin (mTOR) inhibitors. From the Gold study, sorafenib might be an option for the third-line treatment. The Best trial demonstrated that bevacizumab/sorafenib had best efficacy in advanced renal cancer. This study aimed to compare bevacizumab plus sorafenib *versus* sorafenib for the third-line treatment of patients with metastatic renal cell carcinoma. *Patients and Methods:* This study is an open-label, multi-centre, randomized phase II trial. Eligible patients had metastatic renal cell carcinoma with clear cell and had received first-line treatment of sunitinib and second-line treatment of everolimus before enrolment. Additional inclusion criteria included: ≥1 measurable disease, Eastern Cooperative Oncology Group Performance Status (ECOG PS) 0/1 and adequate haematological, renal and hepatic functions. Patients were randomly allocated in a 1:1 ratio to receive bevacizumab plus sorafenib (bevacizumab 5 mg/kg intravenously every two weeks plus sorafenib 400 mg twice daily) or sorafenib alone (sorafenib 400 mg, orally, twice daily). Treatment was continued for both groups until occurrence of disease progression, unacceptable toxicity, death or withdrawal of consent. The primary study endpoint is progression-free survival (PFS). Overall survival, disease control rate and safety will also be assessed. *Results:* The first patient visit was in October 1, 2013. Thirty-three of a planned 106 evaluable patients have been enrolled: 76% male, median age of 63, 100% PS 0/1 and 33% favourable/55% intermediate by Heng's criteria. Eighteen patients had received bevacizumab plus sorafenib and 15 patients had received sorafenib alone. The objective response rate was 11.1% and 0%, respectively. The median PFS was 6.5 months and 3.5 months, respectively. The median overall survival has not been reached. Treatment emergent grade 3 or 4 adverse events were fatigue (11.1%), hypertension (5.6%), proteinuria (5.6%) in the bevacizumab plus sorafenib group; palmar-plantar erythrodysaesthesia (6.7%) in the sorafenib group. *Conclusion:* Bevacizumab plus sorafenib might be beneficial for the advanced renal cancer patients who have failed to sunitinib as first- and everolimus as second-line treatment. More patients will be enrolled in the next year.

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FBO3B03
SCREENING FOR OVARIAN CANCER AND THE CHALLENGES AHEAD: RESULTS FROM THE MULTICENTRE UKCTOCS STUDY

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Background/Aim: The UK Collaborative Trial for Ovarian Cancer Screening (UKCTOCS) is the largest randomised controlled trial performed to determine the impact of screening on ovarian cancer mortality. Between April 2001 and December 2011, women in the screen arm underwent annual screening. Volunteers were followed up for a further three years till the end of December 2014. *Patients and Methods:* 279 postmenopausal women, 50-74 years old were randomised 50:50 to multimodal screening (MMS) with serum cancer antigen 125 (CA125) interpreted using a 'Risk of Ovarian Cancer' algorithm or ultrasound screening (USS). Women with abnormal screens had repeat tests. Those with persistent abnormality underwent clinical evaluation and, if appropriate, surgery. *Results:* At prevalence screen, the sensitivity, specificity and positive predictive values of the prevalence screen were 89.4%, 99.8% and 43.3% for MMS and 84.9%, 98.2% and 5.3% for USS. When the analysis was restricted to primary invasive epithelial ovarian/tubal cancers, the sensitivity was 89.5% and 75.0%, respectively. There was significant difference in specificity between the two groups but not in sensitivity. Forty two (MMS) and 45 (USS) primary ovarian/tubal cancers were detected, which included 8 borderline tumours in the MMS and 20 in the USS group. Approximately half (48.3%) (95% confidence interval (CI) =35%, 65.8%) of the invasive cancers were Stage I/II with no difference in stage distribution between the groups. *Conclusion:* The prevalence screen results demonstrate an acceptable screening strategy. Specificity was significantly lower in the USS group resulting in higher rates of repeat testing and surgery. This, in part, reflects the prevalence of adnexal abnormalities in this population. Both strategies have encouraging sensitivity with MMS showing a trend to higher sensitivity for invasive cancers. Overdiagnosis of borderline cancers appears to be more of a problem with ultrasound than multimodal screening. The results of ongoing screening are required to determine the mortality impact. Several factors affect the visualization of postmenopausal ovaries in the ultrasound arm. Their impact needs to be taken into consideration when developing quality assurance for ovarian ultrasound scanning or comparing study results as their prevalence may differ between populations.

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FBO3B04
THE COMPARISON OF BLADDERSCAN AND ULTRASOUND SYSTEM ON EVALUATING THE BLADDER CAPACITY

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Aim: To assess the feasibility and accuracy of BladderScan BVI 9400 on measuring bladder capacity and, contrastively, study the difference between BladderScan BVI 9400 and the Philips ultrasound iU22system. *Materials and Methods:* Two bladder models, I and II, were measured by BladderScan BVI 9400 and scanned by Philips BrillianceTM Big Bore CT. In 341 patients from May 2014 to August 2014 in our hospital, the bladder capacity determined by the Philips ultrasound iU22 system was compared with the bladder capacity determined by the BVI 9400 instrument. The information about age and gender was recorded for each patient. The dependence of two measurement results on patients' age and gender was evaluated. *Results:* The relative difference for model I was 2.5% between BladderScan BVI 9400 and Big Bore CT. Compared with the calibration volume, the relative difference for model II was 1.36% (BVI 9400) and 0.14% (CT). There were significant differences for 341 patients between BVI 9400 and ultrasound iU22 on evaluating bladder capacity ($p<0.001$), correlation coefficient R=0.96. As a function of ultrasound iU22 data, the coefficient of determination of correct fit of BVI9400 data was 0.91. The relative difference between BVI 9400 and iU22 decreased by increasing bladder capacity, 22.69% for less than 100 ml, 21.41% for 100-200ml and 10.50% for more than 200. The bladder capacity from BVI 9400 and iU22 has also significant difference, respectively, with gender ($p=0.002$, $p=0.003$) and without age ($p>0.05$, $p>0.05$) and there is no gender differences on the relative difference between BVI 9400 and iU22 ($p>0.1$). *Conclusion:* BladderScan BVI 9000 has the ability of quicker and more accurate bladder volume measurements than conventional two-dimensional ultrasound. Obviously, given the good stability and convenience, BladderScan BVI 9400 could be used as an auxiliary equipment to guide radiotherapy in the pelvis region.

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FBO3B05
MicroRNA CHANGES IN PTen INDUCED PROSTATE INTRAEPITHELIAL NEOPLASIA (PIN)

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Background/Aim: Prostate cancer (PCa) is the most common male cancer in the developed world and a leading cause of cancer-related deaths. Often, by the time PCa becomes symptomatic, highly invasive surgery may be the only avenue available or hormonal therapy. PCa may be successfully treated with surgical resection but when the disease invades locally and spreads to the bone, the prognosis becomes very poor and, eventually, becoming refractory to these therapies. However, these are "late-stage" therapies for PCa patients and, ideally, we would like to identify changes in the disease much earlier in its development. There is a clear need for early intervention in this disease. MicroRNAs (miRs) are small RNAs that associate with the 3' untranslated region of their target mRNA and cause their degradation or translational inhibition. MiRs may regulate several mRNAs and each mRNA may be regulated by several miRs leading to a highly complex system. MiRs regulate a diverse set of biological events from cell division and morphology to tissue development and differentiation. MiRs are often dysregulated in cancers. *Materials and Methods:* We have used a mouse model for PCa (Probasin-Cre-driven Pten deletions), which mirrors early human disease (30-60% approximately). Mice carrying prostate-specific *Pten* deletions develop high grade prostate neoplasia, which leads to invasive tumours. Using a low density array and RNA-seq we have screened all 700 mouse miRs in these tumours compared to their wild type littermates. *Results:* We found several miRs to be highly expressed and several to be completely lost in tumour cells. Using inhibitors to these over-expressed miRs, we found that they could inhibit cancer cell growth and motility *in vitro* and to reduce xenograft growth *in vivo*. Additionally, these anti-miRs showed synergistic activity with PI3K inhibitor compounds, commonly used for advanced prostate cancer. *Conclusion:* Anti-miR based compounds could be utilised as a second treatment for advanced PCa.

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FBO3B06
MANAGEMENT OF PERITONEAL METASTASES IN EPITHELIAL OVARIAN, FALLOPIAN TUBE AND PRIMARY PERITONEAL CARCINOMA

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Over 70% of epithelial ovarian cancer and fallopian tube cancer (EOC and FTC) are found in their advanced stage when tumour has disseminated all over the peritoneal cavity. Primary peritoneal cancer (PPC) is a rare tumour, which has similar clinical behaviour and treatment as EOC and FTC but without or with minor ovarian or fallopian tube involvement. The standard treatment for advanced EOC, FTC and PPC is cytoreductive surgery and adjuvant chemotherapy. Optimal surgical cytoreduction is associated with improved survival. Peritoneal carcinomatosis and its relating ascites and hypoproteinemia make optimal cytoreduction difficult to achieve. Extensive surgical procedures may be needed, including bowel resection, diaphragmatic stripping and partial hepatectomy, *etc*. These procedures may be associated with significant morbidity and

a potential delay in initiation of adjuvant chemotherapy. Neoadjuvant chemotherapy is an option for women who are not candidates for optimal cytoreduction. Patients undergoing optimal cytoreducion should be considered to be treated with postoperative intraperitoneal chemotherapy, which has been proved to improve outcome in some clinical trials. Intraoperative hyperthermic intraperitoneal chemotherapy (HIPEC) may be another option for better eliminating peritoneal disease, which has already been shown to be effective in non-gynaecologic carcinomatosis in numerous reports. However, randomized phase III clinical trials are warranted for determining the best timing and candidates for this approach.

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FBO3C01
THE TUMOUR SUPPRESSOR DLC2 ENSURES MITOTIC FIDELITY BY COORDINATING SPINDLE POSITIONING AND CELL-CELL ADHESION

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Background/Aim: Dividing epithelial cells need to coordinate spindle positioning with shape changes to maintain cell-cell adhesion. Microtubule interactions with the cell cortex regulate mitotic spindle positioning within the plane of division. How the spindle crosstalk with the actin cytoskeleton to ensure faithful mitosis and spindle positioning is unclear. *Materials and Methods:* Loss of function approaches combined with biochemical assays and live cell imaging were used to determine the role of DLC2 in mitotic Cdc42 regulation, mitotic spindle formation and function, as well as spindle positioning. *Results:* Here, we demonstrate that the tumour suppressor DLC2, a negative regulator of Cdc42, and the interacting kinesin Kif1B coordinate cell junction maintenance and planar spindle positioning by regulating microtubule growth and crosstalk with the actin cytoskeleton. Loss of DLC2 induces mislocalization of Kif1B, increased Cdc42 activity and cortical recruitment of the Cdc42 effector mDia3, a microtubule stabilizer and promoter of actin dynamics. Accordingly, DLC2 or Kif1B depletion promotes microtubule stabilization, defective spindle positioning, chromosome misalignment and aneuploidy. *Conclusion:* Our results reveal that a signalling module formed by DLC2 and the kinesin Kif1B tunes mitotic Cdc42/mDia 3 activity to guide microtubule growth and stability, actin dynamics and, thereby, astral microtubule crosstalk with the actin cytoskeleton. This regulatory network coordinates cell junction maintenance and planar spindle positioning and ensures mitotic fidelity and tissue integrity.

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FBO3C02
C-KIT OVER-EXPRESSION PROMOTES DEVELOPMENT OF COLORECTAL MUCINOUS ADENOCARCINOMA IN MURINE MODEL

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Background/Aim: Colorectal mucinous adenocarcinoma (CRMAC), one of the most malignant subtypes accounting for 15-20% of all colorectal cancer patients, shows higher risk of metastasis and poorer prognosis compared to subjects with common colorectal adenocarcinoma. However, the developmental mechanisms of CRMAC are still not fully understood. It has been suggested that receptor tyrosine kinase (RTK) family might be associated with multiple mucinous carcinomas. Here, we focused on the roles of c-kit, a member of the RTK family, in the development of the CRMAC. *Materials and Methods:* A murine model of CRMAC was successfully established by treating C57 mice (both wild type (WT) and c-kit gene mutant type ($Wads^{-/-}$) with azoxymethane + dextran sodium sulfate (AOM+DSS) for 37 weeks. Over-expression of c-kit by lentivirus transfection and inactivation of c-kit by imatinib treatment in HCT-116 cells were created. Tumour malignancy was histopathologically examined. C-kit, SCF (c-kit ligand), Math1, MUC2, p53, cyclin D1, AKT, ERK, E-cadherin, N-cadherin, vimentin, ETV4 and MMP7 expressions were analysed by Western blotting, qRT-PCR and immunohisto-chemical staining. Cell proliferation and invasion were monitored in real-time cell analyzer. *Results:* Colorectal adenocarcinoma appeared both in WT and $Wads^{-/-}$ mice 20 weeks after AOM+DSS treatment. On week 37, 8/15 of WT mice had CRMAC, while none $Wads^{-/-}$ mice (n=5) had CRMAC. Mucus is MUC2 positive in adenocarcinomas, similar to the observations in CRMAC patients. CRMAC cells were more poorly differentiated than non-CRMAC cells and invaded beyond base membrane. C-kit, p-c-kit and SCF expressions were significantly greater in CRMAC WT mice compared with non-CRMAC WT mice, non-CRMAC $Wads^{-/-}$ mice or normal WT controls. Furthermore, expressions of Math1, p53, p-AKT, p-ERK, N-cadherin, vimentin, ETV4 and MMP7 were increased, whereas cyclin D1 and E-cadherin were decreased in CRMAC WT mice. Over-expression of c-kit in HCT-116 cells accelerated cell proliferation and invasion. In contrast, imatinib treatment to block c-kit phosphorylation simultaneously resulted in proliferative and invasive suppression in HCT-116 cells. *Conclusion:* C-kit up-regulated Math1 through the AKT pathway, which possibly contributed to the tumourigenesis of CRMAC. C-kit enhanced tumour cell proliferation by decreasing p53 and increasing

cyclin D1. Interestingly, c-kit strongly promoted tumour cell invasiveness and epithelial-mesenchymal transition (EMT) by increasing ETV4 and MMP7 through the ERK pathway. Up- or down-regulating c-kit activation *in vitro* produced similar results in HCT-116 cells. Our data suggest that the c-kit signalling may contribute to the development of the malignant CRMAC. Targeting this signalling and its downstream molecules might provide potential therapeutic benefits in the treatment of patients with CRMAC.

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FBO3C03
WNT-11 AND JNK PATHWAY: THE TWO FACES OF NEUROENDOCRINE-LIKE DIFFERENTATION IN PROSTATE CANCER

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Background/Aim: The Wnt proteins are secreted cysteine-rich proteins, which have important roles in the developing embryo and in tissue homeostasis in adults. Abnormal Wnt signals can lead to many types of cancers, such as prostate, colon and liver. Wnt-11 is a member of non- canonical Wnts that modulates cell growth, differentiation and morphogenesis during development. We previously showed that Wnt-11 is up-regulated in prostate cancer (PCa). Factors, such as PCa cell survival, motility and neuroendocrine-like differentiation (NED) that are promoted by Wnt-11, induced signals. The signals downstream of Wnt-11 are not fully characterised. In this study, we investigated the relation with Jun N-terminal kinase (JNK) as potential downstream factor and Wnt-11 signalling. *Materials and Methods:* Highly metastatic PC-3 and weakly metastatic LNCaP prostate cancer cell lines were used. The cells were treated with JNKVIII, the JNK kinase inhibitor, to check its effects on cell proliferation, gene and protein expressions. Both sulforhodamine B (SRB) stain and MTT assay were carried out to assess cellular proliferation. Over-expression and knockdown cells were created to study invasion and migration. After blocking the JNK pathway, the correlation between Wnt-11 and other neuronal genes were tested at both mRNA and protein levels. *Results:* The JNKVIII treatment resulted in a 40% reduced proliferation of both cell lines PC-3 and LNCaP (p=0.002 and 0.003, respectively). Gene expression of both Wnt-11 and several neuronal genes were completely blocked when PC-3 cells were treated with the JNK inhibitor. Moreover JNKVIII treatment has been shown to reduce significantly (p<0.001) the migration of the PC-3 cells through transwell filters. *Conclusion:* It has been known that JNK can drive the expression of cytokines, which can act in a paracrine manner, to sustain the proliferation of cancer cells. In this study we have proved that the JNK pathway is indeed involved in the proliferation of both PC-3 and LNCaP cells and plays a vital role in the expression of Wnt-11 and NED. Previous studies using PC-3 cells support a role for Wnt11 in survival, NED and migration of castration-resistant prostate cancer cells. Our observations suggest that Wnt11 could be a useful therapeutic target for recurrent prostate cancer and JNK pathway target drugs can be used for dual therapy.

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FBO3C04
THE ROLE OF NHERF IN BREAST CANCER

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Background/Aim: The aberrant expression of Na^+-H^+ exchanger regulatory factor (NHERF, also known as Ezrin-radixin-moesin-binding phosphoprotein-50, EBP50) and the intragenic mutation of the *NHERF* gene were reportedly correlated with human breast cancer development. However, the impacts of NHERF on breast cancer development and its molecular mechanism are not fully understood. NHERF is related to cell cycle progression and the phosphorylation of its Ser279 and Ser301 residues during the mitosis phase can result in changes in the actin cytoskeleton. However, the biological significance of NHERF phosphorylation, which is mediated by cyclin-dependent kinase 2 (cdc2) and cyclin B, remains unclear. *Materials and Methods:* NHERF was over-expressed in the breast cancer cell line MDA-MB-231 (called EBP-231), which has low levels of NHERF protein expression, and its effects on cellular proliferation, anchorage-independent growth, apoptosis and the activity of extracellular signal-regulated kinase (ERK) were investigated. We also determined its effect on epidermal growth factor (EGF)-induced cell proliferation and activation of epidermal growth factor receptor (EGFR) signalling in the breast cancer cell lines. Finally, the phenotypes of MDA-MB-231 cells stably transfected with constructs of phospho-deficient (S279A/S301A) or phospho-mimetic (S279D/S301D) mutants were observed. *Results:* A significant decrease in cellular proliferation and colony forming ability were observed in EBP-231 cells when compared with control cells. Consistently, an increase in apoptosis of EBP-231 cells was accompanied by a decreased ERK activity. NHERF expression inhibited EGF-induced cell proliferation, ERK1/2 and AKT phosphorylation. NHERF rescue experiment further confirmed that the activation of EGF-induced downstream molecules could be specifically inhibited by NHERF expression. Furthermore, NHERF gain-of-function and loss-of-function study revealed that NHERF inhibited EGF-stimulated EGFR

phosphorylation. Meanwhile, total expression levels of EGFR were unaffected during EGF stimulation. Failure of cdc2-mediated NHERF phosphorylation significantly increased F-actin content, enhanced the adherence of cells to the extracellular matrix and caused defects in cytokinesis. Knockdown of *NHERF* S279A/S301A expression in MDA-MB-231 cells rescued the cytokinesis defect. *Conclusion:* NHERF expression can suppress breast cancer cell proliferation by promoting cell apoptosis and inhibiting ERK activity. NHERF can suppress EGF-induced proliferation of breast cancer cells by inhibiting EGFR phosphorylation and blocking EGFR downstream signalling in breast cancer cells. Cdc2/cyclin B-mediated NHERF phosphorylation may play a role in the regulation of cytokinesis by affecting actin cytoskeleton reorganization. NHERF may be a diagnostic biomarker and serve as a target for therapeutics in breast cancer.

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FBO3C05
PHYSICOCHEMICAL CHARACTERISATION OF BISPHOSPHONATES AND THEIR POTENTIAL APPLICATION FOR CANCER METASTASIS IN BONE

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Background/Aim: Bisphosphonates (BPs) are extensively used drugs for the prevention and treatment of bone mineral resorption including osteoporosis, Paget's disease, tumour induced osteolysis and hypercalcaemia. Recently, BPs have also been found to have anti-cancer property *via* stimulating γδT cells of the immune system. The pharmaceutical efficacy of BPs, in particular the anti-resorptive potency of nitrogen-containing bisphosphonates (NBPs), is determined by two factors: their affinity to target bone minerals and ability to inhibit the enzyme farnesyl pyrophosphate synthase (FPPS) after accumulation to the osetoclastic cells. The binding of BPs to bone mineral is an essential step leading to their biological effects. The typical structural motif of BPs is two phosphonic acids, P-C-P, and in NBPs a nitrogen-containing side chain, and a -OH group attached to the same carbon. The aim of this report provides an overview of the study of physicochemical property of BPs. *Materials and Methods:* The structural determinants of BPs' binding affinity were tested in greater detail using a quantitative and reproducible method of column chromatography and adsorption isotherm with hydroxyapatite ceramic spheres (20 μm). The cytotoxicity of BPs' binding hydroxyapatite spheres is also tested. *Results:* Significant differences were seen in mineral binding among the clinically-relevant BPs. Not only does the P-C-P groups but also a hydroxyl (-OH) group in the R1 position contribute to the high affinities to hydroxyapatite. In addition, some differences were seen in the rank order of BP binding between the hydroxyapatite chromatography and adsorption isotherm methods. The rank of mineral affinity of BPs is compared with their inhibitive potential to the enzyme of FPPS. The cytotoxicity of BP on macrophages was shown only on hydroxyapatite binding BPs, not the free-form in culture media. *Conclusion:* The physicochemical property of BPs may shed light in their application for the prevention and treatment of cancer metastasis in bone. However, further study using *in vivo* and 3D *in vitro* cancer metastasis models is warranted to explore the underlying molecular mechanism.

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FBO3C06
ZO-1 CONTROLS ENDOTHELIAL ADHERENS JUNCTIONS, CELL-CELL TENSION, ANGIOGENESIS AND BARRIER FORMATION

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Background/Aim: Endothelial cells (EC) cover the internal surface of blood and lymphatic vessels and play key roles in vessel formation and function. EC homeostasis requires the integration of signals from sites of adhesion to the extracellular matrix and neighbouring cells, as well as signals from circulating factors and mechanical stimuli. The integration, transmission and regulation of mechanical forces at sites of adhesion are of fundamental importance as they drive vessel development and progression of diseases. Adherens junctions are crucial for mechanotransduction; however, whether tight junctions contribute to the regulation of cell-cell tension is unknown. *Materials and Methods:* We used primary human microvascular endothelial cells and specific protein down-regulation by RNA interference. We assessed protein expression by Western blot and localization by immunofluorescence. To evaluate functional consequences on angiogenic behaviour, we used *in vitro* angiogenesis assays - capillary-like formation on Matrigel and microcarrier based fibrin gel assays - and an in *in vivo* angiogenesis assay

- Matrigel plug assay in mice. Cell-cell tension was measured with a fluorescence resonance energy transfer (FRET)-based tension sensor and laser ablation assays. These methods were complemented by Rho activity measurements, endothelial permeability and apoptosis assay. *Results:* We demonstrate that the tight junction protein ZO-1 regulates cell-cell tension acting on VE-cadherin-based adherens junctions, as well as cell migration, barrier formation of primary endothelial cells and angiogenesis *in vitro* and *in vivo*. ZO-1 depletion led to redistribution of active myosin II from junctions to stress fibers, reduced tension on VE-cadherin and loss of junctional mechanotransducers, such as vinculin and PAK2, that correlated with induced vinculin dissociation from the alpha-catenin/VE-cadherin complex. Claudin-5 depletion only mimicked ZO-1 effects on barrier formation, whereas the effects on mechanotransducers were rescued by inhibition of Rho-associated protein kinase (ROCK) and phenocopied by JAM-A, JACOP and p114RhoGEF down-regulation. ZO-1 was required for junctional recruitment of JACOP, which recruited p114RhoGEF. *Conclusion:* ZO-1 is, thus, a central regulator of VE-cadherin-dependent endothelial junctions that orchestrates the spatial actomyosin organization, tuning cell-cell tension, migration, angiogenesis and barrier formation.

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FP0102
STRATEGIES AND CONTROVERSY IN THE SURGICAL TREATMENT OF GASTRIC CANCER

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Gastric cancer still remains a major health problem. It is the most frequent cause of cancer-related deaths worldwide, although its incidence has steadily declined during the last decades in Western countries. Gastric cancer is often diagnosed at an advanced stage in China. Despite improvements in local control and empirical chemotherapy, prognosis -particularly for these patients- remains poor. Surgery still is the cornerstone in the treatment of gastric cancer. Although a D2 lymph node dissection is the generally accepted standard surgical procedure worldwide because of its acceptable safety profile and superior treatment outcome, there is still a lot of controversy in both the extent of surgery and the value of perioperative adjuvant treatment, such as splenectomy for upper gastric tumour, bursectomy for SS/SE gastric cancer, D3 gastrectomy for bulky N2 or clinical N3, and so on. Tools, such as sentinel lymph node biopsy and genomic profiling, are seen to be promising developments enabling more tailored treatment. Until the use of these tools is validated, we must rely solely on the evidence for large groups of patients originating from quality-controlled trails.

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FP0103
THE STATUS AND PROSPECT OF SCREENING AND TREATMENT OF CERVICAL CANCER IN CHINA

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Cervical cancer is one of the most common gynaecological malignant tumours in China seriously endangering the health and lives of women. Cervical cancer screening is an important means to early diagnosis. The China government has implemented "the cervical cancer, breast cancer screening program" for women in rural areas by raising funds. Recently, the incident of cervical cancer has increased gradually in China, including younger cases and multiple pathology types. Treatments for this cancer are mainly surgery, radiotherapy and chemotherapy. The reform and innovation of treatment, such as radical trachelectomy, nerve-sparing radical hysterectomy, laparoscopic radical hysterectomy, neoadjuvant chemotherapy, concurrent chemotherapy and radiotherapy, intensity-modulated radiation therapy, molecular target therapy, *etc*., contribute continuously to improvements. As most cervical cancers are detected in last stages and the current radiotherapy approach needs regulation, in China, cervical cancer has a totally undesirable outcome. Although the government has put efforts to popularize the cervical cancer screening program and standardized treatment, there is a long way to go as there is still a huge gap between cities and rural places. Firstly, screening programs are still needed to decrease late stage cervical cancer; secondly, the fundamental research on the mechanism(s) and prognosis of cervical cancer should be enhanced; thirdly, upgrading the overall therapy levels is of outmost importance. This article introduced the status and prospects of diagnosis and treatment of cervical cancer in China.

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FP0104
OPTIMISING SURGICAL RESECTION OF COLORECTAL CANCER LIVER METASTASES

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Background/Aim: Colorectal cancer (CRC) is a major public health concern in both Europe and, in particular, China where the incidence is increasing at 5% per year, especially among the rapidly emerging middle classes as they 'Westernise' their

diets. While half of all patients who develop CRC will be cured by appropriate surgery to remove their primary tumour, half will either present with metastatic disease or will, subsequently, develop metastases after apparently curative surgery for their primary tumour. Fifty per cent of those who are found to have metastatic disease will have disease confined to the liver and, if this disease can be surgically resected, will afford a 50% chance of surviving 5 years and 30% probability of being alive 10 years later and cured. However, historically, only 10% of patients with liver limited metastases have been considered suitable for such surgery. *Materials and Methods:* Systematic reviews of the literature over the last 25 years, combined with population and institutional-based analyses examining morphological and molecular tumour characteristics, correlated to survival after surgery, in addition to the use of peri-operative systemic therapy to convert inoperable disease to operability and improve post-hepatectomy disease-free survival (DFS). Such characteristics include: interval between diagnosis of primary CRC and detection of metastases; differentiation of the primary CRC; lymph node involvement; size of largest liver metastasis; number of liver metastases; presence of resectable extra-hepatic metastases; elevated serum carcinoembryonic antigen (CEA) levels; resection margin of resected metastases; *KRAS* and *BRAF* mutational status of resected metastases. *Results:* All morphologic and molecular factors reviewed had a relative negative impact on survival after liver surgery with hazard ratios ranging from 1.5 to 2.02 (the latter for an inadequate surgical resection margin). Both genetic mutations were prognostic for poorer outcome after surgery compared to wild type *KRAS* and *BRAF*. Effective systemic therapy with a high response rate (>70%) could bring >40% of people with initially inoperable liver-limited CRC metastases to liver resection with curative intent, while the addition of peri-operative systemic therapy to resectable CRC metastases is associated with a 9% improvement in DFS 3 years after liver resection. *Conclusion:* If feasible, liver resection for CRC metastases offers long-term survival and an opportunity for cure. It is possible to predict those patients with a good prognosis after such surgery and those whose tumour's morphologic and molecular characteristics indicate a poorer prognosis might then benefit from more intensive systemic treatment both before and after liver surgery.

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FP0106
THE ROLE OF CULTURE IN IMPROVING CANCER SERVICES IN A TIME OF AUSTERITY

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Cancer treatment is an archetype of a complex system, one that involves many different elements all of which have to be in balance for the goal of good evidence-based treatments to be effective. In most developed countries, organising effective cancer treatment poses a significant challenge, given the inherent complexity arising from its typology, presentation and diagnosis, as well as treatment options. In all cases, variations in demand, even over quite short time cycles, can create large backlogs of patients who then queue for the next stage of their pathway to be delivered. The demand volatility is also affected by new and developing treatment or diagnostic capabilities. At a time when the National Health Service (NHS) is facing the triple effect of an ageing population (in part a tribute to improved cancer treatments), the consequences of lifestyles that are inherently less healthy and impacting as public services are experiencing an across the board tightening of the resources available to confront these challenges. It would, thus, be reasonable to assume that cancer service performance might be showing signs of this system stress with resultant lengthening treatment times and growing service delivery problems. In Wales, and specifically at Cardiff and the Vale cancer, waiting times are improving and patient satisfaction is at an all-time high. The literature on improvement science is a large one and there have been many successful attempts internationally to adapt and implement such systems of thought and practice to the health care environment. Frequently, the sites that have adopted this methodology have used the production systems that are concerned with process control and combined these with work on human factors and measurement to help visualise and then monitor care processes, often using a simple Plan Do Study Act (PDSA) mechanism. The experience at Cardiff has confirmed that these practices are useful and will certainly contribute to a better understanding of the system and how it performs and responds to improvement interventions. However, we believe the most important driver for this improved performance has been an explicit shift in culture away from a weary attitude of compliance with targets and casual and all too common references to failures of care as 'breeches' and 'carry forwards', usually expressed as percentages or numbers with every last artefact of humanity drained from the language. Our attempt to rise to the challenges we faced was to begin to express what we needed to do in plain language that is patient-specific, to flatten our organisational structures and encourage the right conversations to take place between people who are in a position to help and to align these conversations towards the goal of improved cancer treatment. We believe that improving cancer services is first and foremost a challenge about complexity and that the appropriate response to complexity is to avoid assuming that it is possible to 'plan' a way out of a dynamic system. Instead, the aim is to engage people in conversations in order to encourage real participation so that the choices we all make about what we do and how we do it are shaped by our collective wish to see patients with cancer or suspected cancer treated well.

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SBO4A01
THE DUAL PI3K/mTOR INHIBITOR BEZ235 EXERTS PROMINENT ANTITUMOUR ACTIVITY IN HER2-POSITIVE GASTRIC CANCER AND HAS SYNERGY WITH TRASTUZUMAB

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Aim: To investigate the antitumour activity of the dual PI3K/mTOR inhibitor BEZ235 in HER2-positive gastric cancer *in vitro* and *in vivo* and the synergism between BEZ235 and trastuzumab. *Materials and Methods:* Human epidermal growth factor receptor 2 (HER2)-positive (NCI-N87 and SNU216) and HER2-negative (MKN45) gastric cancer cells were used in this study *in vitro*. Cell viability, cell cycle and HER2 downstream pathway were measured using MTS assay, flow cytometry and Western blot, respectively. NCI-N87 cells and gastroscopic biopsies from HER2-positive gastric cancer patients were inoculated subcutaneously into nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice to establish HER2-positive NCI-N87 xenografts and patient-derived xenografts (PDX) *in vivo*. HER2-positive gastric cancer PDX were treated with BEZ235 or trastuzumab alone or combined to assess the antitumour activity. *Results:* Both BEZ235 and trastuzumab inhibit the growth of NCI-N87 and SNU216 cells in a dose-dependent manner *in vitro* by inducing cell cycle arrest at G1 phase. BEZ235 alone has superior inhibitory effect than trastuzumab *in vitro* cells ($p<0.01$). *In vivo*, HER2-positive gastric cancer xenograft models, using NCI-N87 cells and gastroscopic biopsies, were successfully established and passaged. The histological characteristics, HER2 expression and chemosensitivity of the patient-derived tumour xenograft (PDTX) models were highly consistent with primary tumour. In contrast to control group or trastuzumab group *in vivo*, the growth of tumours treated by BEZ235 alone or combined with trastuzumab was significantly suppressed both in NCI-N87 xenografts and in two PDX ($p<0.01$). Moreover, compared to BEZ235 alone, BEZ235 combined with trastuzumab had synergistic inhibitory *in vitro* and *in vivo* xenografts. The synergistic inhibitory effect of BEZ235 with trastuzumab against gastric cancer *in vitro* and *in vivo* was achieved through inhibiting HER2 downstream important pathways as shown by repression of phosphorylated AKT, S6 and ERK. *Conclusion:* BEZ235 was demonstrated for the first time to exert powerful activity against HER2-positive gastric cancer in PDX and had synergy with trastuzumab, which provided solid evidence for future clinical trials.

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SBO4A02
MODULATING THE STEM CELL NICHE AND NEOPLASIA IN THE MURINE INTESTINE: CHARACTERISING THE ROLES OF THE WNT AND PI3-KINASE PATHWAYS

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Deregulation of the Wnt pathway, principally through loss of function mutations of *APC*, is known to underlie nearly all human colorectal tumours. In the mouse, colorectal cancer has similarly been modelled using various mutations of the *Apc* gene. We have used both constitutive and conditional alleles in combination with other candidate loci to further develop these models with two aims in mind, first to understand better the relationship between these mutations and the pool of normal stem cells/cells of origin of neoplasia; second, to identify and validate novel therapeutic targets. Thus, we have generated mice with an inducible Cre-Lox deletion of *Apc*, which has allowed us to model the very early steps of Wnt driven neoplasia. We have investigated the role of both Apc2, Brg-1 and Cited-1 in these models, proteins that have previously been implicated in regulation of the Wnt pathway. For *Brg-1*, we show that this gene is essential for the maintenance of the intestinal stem cell and that deficiency of *Brg-1* compromises Apc-driven neoplasia. For Cited-1, we show that this is induced following Apc loss and is up-regulated in human colorectal tumours and that ApcMin/Cited double mutants show reduced adenoma predisposition. We have also analysed the effects of Cited-1 deficiency upon the immediate phenotype of *Apc* loss, finding that deficiency de-represses beta catenin and enhances Wnt signalling and all the immediate phenotypes we observe following *Apc* loss, including the 'crypt progenitor' phenotype. Critical, amongst these, is elevated cell death, which we believe underpins the observed reduction in adenoma formation. For Apc2, we show an expansion of stem cell markers in the absence of gene function but failure of this to translate into enhanced tumourigenesis in the intestine. By contrast we show marked synergy between Apc and Apc2 in mammary epithelium tumourigenesis. We have also investigated the role of PI3-kinase pathway, deregulation of which is another key molecular event underpinning human colorectal cancer. Previously, it has been assumed that such tumourigenesis is driven out of the epithelial compartment, in part as a consequence of increasing the size of the stem cell/cell of origin pool. However, we have shown that epithelial specific deletion of *Pten* does not immediately impinge upon either adenoma formation or the stem cell compartment. This raises the possibility that PI3-kinase pathway disruption may regulate the stem cell pool and tumour predisposition in a non-

cell autonomous manner out-with the epithelial compartment. By using stromal specific deletion of Pten, we have been able to show changes in the epithelial stem cell compartment, enhanced epithelial-mesenchymal transition (EMT) and ultimately enhanced adenoma formation. These changes all clearly derive from altered epithelial-stromal crosstalk, which ultimately predisposes to enhanced tumourigenesis. Taken together, our studies reveal a complex interplay between the deregulation of these key pathways, the stem cell compartment and tumourigenesis. For Wnt, our data is consistent with a 'just right' level of Wnt activity, which expands the stem compartment in a cell autonomous fashion and leads to enhanced tumourigenesis. Levels of Wnt activity above or below this level appear to attenuate neoplasia. For the PI3-kinase pathway, we show a non-cell autonomous mechanism, which regulates both the stem cell niche and tumourigenesis.

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SBO4A03
COMPARISONS OF STRESS AND HUMORAL IMMUNOLOGIC RESPONSES EARLY AFTER LAPAROSCOPY-ASSISTED DISTAL GASTRECTOMY PERFORMED AS A COMPONENT OF FAST TRACK SURGERY FOR GASTRIC CANCER

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Background/Aim: With progress in diagnostic and therapeutic techniques, there is increased concern for faster and more comfortable recovery. The aim is to investigate the stress and humoral immunologic responses early after laparoscopy-assisted distal gastrectomy (LADG) performed as a component of fast-track surgery (FTS) for gastric cancer. *Patients and Methods:* Eighty-eight eligible patients were randomly assigned into four groups comprising twenty-two patients each: (i) FTS + LADG, underwent LADG and FTS; (ii) LADG, underwent LADG with traditional perioperative management; (iii) FTS + open distal gastrectomy (ODG), underwent ODG and FTS; and (iv) ODG, underwent ODG with traditional perioperative management. The clinical and humoral immunologic variables were compared between them. *Results:* Compared with the ODG group, in the other three groups, C-reactive protein (CRP) were lower (all $p \leq 0.001$), C3, C4, IgG and IgA were higher and had less pronounced variations after surgery, especially in the FTS + LADG group. CRP in the FTS + LADG group were lower than in the FTS + ODG group on D4 and D7 and C3 and C4 in the FTS + LADG group were higher than in the ODG group On D1 (all $p<0.05$). As to the amplitude of variation between the various time points, it was smaller in the FTS + LADG than the FTS + ODG group at CRP and C3 from D1 to D4 ($p<0.05$, $p<0.01$) and at CRP from D4 to D7 ($p=0.05$). At C4 from D0 to D1, it was smaller in the FTS + LADG and FTS + ODG groups than the ODG group (both $p<0.001$) and smaller in the FTS + LADG than the LADG group ($p<0.05$). From D4 to D7 it was smaller in the FTS + LADG than the ODG group ($p<0.05$). *Conclusion:* FTS and laparoscopic surgery can both individually reduce postoperative inflammatory responses and improve early postoperative humoral immune function. A combination of FTS and LADG does it further, but to a limited degree. FTS with traditional open gastrectomy achieves similar results as laparoscopic surgery for gastric cancer.

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SBO4A04
PULMONARY METASTASECTOMY FOR COLORECTAL CANCER: ARE WE DOING THE RIGHT THING?

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Surgical resection is widely employed as a conventional treatment option for patients with lung metastases originating from a wide range of primary tumours. Of these, colorectal cancer is the most common. However, lung metastasectomy relies on no higher level of evidence than case series and metastasectomy registries. Not only the lack of evidence leaves space for a large variability in practice but, under a deeper analysis, the whole rationale for the surgical resection of lung metastases vacillates. In fact, after a critical examination, much of the apparent benefit may be due to selection of patients rather than the resection itself. This issue was raised for the first time in 1980 by Aberg who argued whether the survival rates of about 30% at 5 years were actually due to the surgery or to the involuntary careful selection of patients affected with cancers with more favourable biologic behaviour. The commonly accepted selection criteria for lung metastasectomy are: (i) control of primary tumour, (ii) disease limited to lung (±liver), (iii) ability to resect all metastatic disease, (iv) sufficient cardiopulmonary reserve. The majority of thoracic surgeons would probably agree on adding to the previous list a limited number of metastases (the lesser the better, but no cut-off has been defined). The abovementioned selection criteria happen to be prognostic or predictive factors, hence affecting cancer survival by themselves, regardless of the treatment the patient receives. This leads to the bias of selecting the long-term survivors ahead of the treatment and, then, to the mistake of attributing that survival to the treatment. In order to define the actual role of lung metastasectomy in the treatment of colorectal cancer, a randomized controlled trial (RCT) is necessary. For years the idea of a RCT to solve the question

was considered utopian and impractical. In 2007, Professor Tom Treasure reopened the debate on the lack of evidence for lung metastasectomy. In 2010, the PulMiCC trial was launched. PulMiCC is a randomised trial that will compare active monitoring with active monitoring and pulmonary metastasectomy in patients with colorectal cancer. It has been designed as a "real life" feasibility study. In stage 1 of the trial, patients with treated primary colorectal cancer metastatic to the lungs are invited to consent for protocol-based evaluation of their suitability for metastasectomy. The evaluation is as in current practice and includes positron emission tomography/computed tomography (PET/CT). A decision for or against metastasectomy may be based on the opinion of the clinicians and the preference of the patient. If there is uncertainty, the patient is invited to consent to have the treatment arm assigned by randomization in stage 2 of PulMiCC. More than 300 patients have entered stage 1 and more than 70 are in stage 2. PulMiCC trial is going to be amended from feasibility study to phase III study and is still recruiting throughout UK.

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SBO4A05
CYCLOPHILIN J IS A NOVEL PEPTIDYL-PROLYLISOMERASE AND TARGET FOR REPRESSING THE GROWTH OF HEPATOCELLULAR CARCINOMA

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Background/Aim: Cyclophilin J (CYPJ) is a new member of the peptidyl-prolyl *cis-trans* isomerase (PPIase) with up-regulated expression in hepatocellular carcinoma (HCC). We aimed to study the role of CYPJ in HCC carcinogenesis and its therapeutic potential. *Materials and Methods:* We determined the expression of CYPJ in 56 HCC/adjacent normal tissues using Northern blot and semi-quantitative RT-PCR, analysed the biochemical characteristics of CYPJ and resolved the 3D-structure of CYPJ/CyclosporinA (CsA) complex. We also studied the roles of CYPJ in cell cycle, cyclin D1 regulation, *in vitro* and *in vivo* tumour growth. *Results:* Results showed that CYPJ was up-regulated in over 60% HCC tissues. The PPIase activity of CYPJ could be inhibited by the widely used immunosuppressive drug CsA. CYPJ promotes the transition of cells from G1 phase to S phase by activating cyclin D1 promoter in a PPIase-dependent manner. CYPJ over-expression accelerated liver cell growth *in vitro* (cell growth assay, colony formation) and *in vivo* (xenograft tumour formation). Inhibition of CYPJ by its inhibitor CsA or CYPJ-specific RNAi diminished the growth of liver cancer cells *in vitro* and *in vivo*. *Conclusion:* Up-regulation of CYPJ plays a role in HCC carcinogenesis and is a potential target for the development of new strategies to treat HCC.

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SBO4B01
ASSOCIATION BETWEEN *BRCA1* AND *BRCA2* MUTATIONS AND RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN WOMEN WITH BREAST CANCER

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Background/Aim: The optimal neoadjuvant chemotherapy regimens for *BRCA1* and *BRCA2* carriers remain unclear and the survival of *BRCA1* and *BRCA2* carriers is not fully elucidated. To investigate the efficacy of various neoadjuvant chemotherapy regimens in BRCA1 carriers, in BRCA2 carriers and in non-carriers in terms of pathologic complete response (pCR); and to compare five-year recurrence-free survival and distant recurrence-free survival rates in *BRCA1* carriers, *BRCA2* carriers and non-carriers. *Patients and Methods:* Two thousand eight hundred and seventy-one Chinese women with operable primary breast cancer were treated with neoadjuvant chemotherapy between 2003 and 2011. All subjects underwent genetic testing for *BRCA1* and *BRCA2* mutations. Pathologic complete response (pCR), five-year recurrence-free survival and five-year distant recurrence-free survival were analysed. *Results:* One hundred and thirty-four mutation carriers were identified (68 BRCA1 carriers and 66 BRCA2 carriers). The pCR rate was 40% for *BRCA1* carriers, 27% for BRCA2 carriers and 18% for non-carriers. Among women treated with an anthracycline-based regimen, the pCR rate was 42% for BRCA1 carriers, 35% for *BRCA2* carriers and 14% for non-carriers (BRCA1 *vs*. non-carriers, $p<0.001$; *BRCA2 vs*. non-carriers; $p=0.008$). Among women treated with a taxane-based regimen, the pCR rate was 25% for BRCA1 carriers, 17% for *BRCA2* carriers and 19% for

non-carriers (BRCA1 *vs*. non-carriers, p=0.66; BRCA2 *vs*. non-carriers, p=1.00). *BRCA2* carriers had a significantly better recurrence-free survival than non-carriers (adjusted hazard ratio (HR)=0.22; 95% confidence interval (CI)=0.06-0.89, p=0.033). The recurrence-free survival for BRCA1 carriers was similar to that of non-carriers (adjusted HR=0.76; 95%CI=0.36-1.63; p=0.49). *Conclusion: BRCA1* and *BRCA2* carriers are more likely to respond to neoadjuvant anthracycline-based chemotherapy than are non-carriers; BRCA2 carriers have a superior recurrence-free survival than non-carriers.

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SBO4B02
BONE MORPHOGENETIC PROTEINS IN TRIPLE NEGATIVE BREAST CANCER AND POTENTIAL LINK WITH EPIDERMAL GROWTH FACTOR

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Breast cancer is the most common cancer in women in the UK and US. Currently, surgery, chemotherapy, hormone therapy, radiotherapy and Herceptin® are cornerstones for the treatment of breast cancer. However, patients may face treatment failure, particularly for those with triple-negative breast cancer (TNBC) that does not express oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), which can be targeted by Herceptin®. Another epidermal growth factor (EGF) receptor (EGFR) is frequently over-expressed in the TNBC. The expression of EGFR and HER2 is inversely associated with ER expression in breast cancer and these are associated with increased metastatic potential of breast cancer cells. Targeting EGFR, using tyrosine kinase inhibitors (TKI), can suppress EGFR-mediated epithelial mesenchymal transition (EMT) in breast cancer. However, little benefit has been evident from the previous and current clinical trials for testing TKIs in breast cancer. This suggests that a complex cascade operating downstream of EGFR or an alternative pathway may tend to be more active when EGFR is targeted by the TKIs. Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-beta (TGF-β) superfamily and actively involved in the EMT events during cancer progression and metastasis. EGF/EGFR signalling has been implicated in the regulation of BMPs expression and also their signalling. EFGR can direct regulate the expression of certain BMPs and also affect Smad signalling of BMPs. BMP can synergistically work together with EGF to promote proliferation of breast cancer cells. On the other hand, some BMPs and BMP intracellular signalling may tend to be more active when EGF/EGFR is repressed as the negative regulation of BMP signalling by EGF has been evident. These suggest that crosstalk/interaction between EGF and BMPs play important role(s) in the disease progression of TNBC, particularly in the regulation of EMT. Further study of the role played by BMP in TNBC will expand our current understanding of the disease and may provide a proof of concept for future personalised disease management of the TNBC.

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SBO4B03
ANTI-METASTATIC AND CYTOTOXIC PROPERTIES OF FRANKINCENSE AND SCENTED MYRRH

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Background/Aim: The anticancer properties of Somali frankincense *(Boswellia frereana)* and scented myrrh *(Commiphora guidotti)* have not been previously reported. Both aromatic oleoresins are comprised of a resin and an essential oil component and both contain a bouquet of unique terpenic chemicals. The frankincense extracts contain mainly non-volatile chemical components, whilst scented myrrh extracts mainly volatile terpene compounds. This study aims to ascertain the effect of these two distinctly different oleoresins on various cancer cell lines both *in vitro* and *in vivo*. *Materials and Methods:* The ethanol extract of frankincense resin and the volatile essential oil of scented myrrh were solubilised in ethanol and analysed for their constituents by gas chromatography-mass spectrometry (GC-MS). Extracts were added to cultures of murine mammary cancer and non-cancer cell lines (4T1 and EpH4) or human breast cancer cell lines (MCF-7, SKBR3, BT474 and MDA-MB-231, PC3, BT-549, BT-20, DU-145, UMRC-5, HRT-18) and non-cancer cell lines (MCF-10A). The effect of frankincense on cancer cells was assessed using *in vitro* assays for invasion, adhesion and wounding. MMP9 expression and activity were monitored using gelatin zymography. The cytotoxic activity of scented myrrh was assessed by MTS-based assays, induction of apoptosis

measured by caspase-3/7 activity and annexin V- propidium iodide (PI) flow cytometric analysis. The cytotoxic activity of a terpene present in scented myrrh was evaluated in a 4T1 mouse model of breast cancer. *Results:* GC-MS analysis showed the frankincense resin extract to be comprised mainly of pentacyclic triterpenes, whilst the volatile oil of scented myrrh primarily of monoterpenes and sesquiterpenes. The frankincense extract was cytotoxic to MCF-7 breast cancer cells only at relatively high concentrations. It was, however, highly effective in inhibiting invasion in six cancer cells; in particular triple-negative breast cancer cells (MDA-MB-231) and prostate cancer cells (PC-3). In contrast, scented myrrh and selected chemical components were found to be cytotoxic to cancer cells at much lower concentrations and to induce high levels of apoptosis when analysed by flow cytometry. *In vivo* studies, with 4T1 tumour bearing mice, showed that treatment with a chemical present in scented myrrh significantly reduced the rate of tumour growth. *Conclusion:* This is the first report on the use of these aromatic oleoresins in cancer treatment. Both frankincense and scented myrrh were found to have contrasting mechanisms of action useful for cancer therapy. Scented myrrh and its components may have a useful role as cytotoxic agents, whilst frankincense maybe useful in inhibiting the growth and metastasis of cancer cells.

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SBO4B04
DOWN-REGULATION OF KLOTHO BETA IS RELATED TO BREAST CANCER PROGRESSION

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Background: Klotho beta (KLB) is a co-receptor and key regulator of the fibroblast growth factor receptor 4 (FGFR4) pathway. KLB interacts with FGFR4 to induce apoptosis and inhibit the proliferation of hepatoma cells; however, little is known regarding the role of KLB in breast cancer. *Materials and Methods:* We performed analysis of breast tissue arrays (82 cases) through an immunohistochemical approach and examined the condition of the *KLB* gene allele to determine the loss of heterozygosity (LOH) in 42 cases of paired micro-dissected breast specimens. *Results:* We found very low KLB expression in breast cancer samples compared with paired adjacent non-tumourous breast tissues. In cancer tissues, KLB expression was negatively associated with pathological grade and lymph node metastasis. Meanwhile, we found selective LOH at the KLB locus in 57.1% of primary tumours. *Conclusion:* These data suggest that *KLB* may be associated with the progression and metastasis of breast cancer and, therefore, have clinical and therapeutic importance.

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SBO4B05
OSTEOPROTEGRIN (OPG) RECEPTOR ACTIVATOR OF NUCLEAR-κB (RANK) AND RANK LIGAND (RANKL): A COMPLEX INTERPLAY BETWEEN BREAST CANCER PROGRESSION AND THE BONE

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Background/Aim: Since their discovery in the 1990's osteoprotegrin (OPG) receptor activator of nuclear-κB (RANK) and RANK ligand (RANKL) have revolutionised our understanding in a key aspect of bone turnover. As a result of Paget's discovery that breast cancer has a predisposition to metastasise to the bone, research -both at the laboratory bench and at the bed side- have strived to find suitable diagnostic and therapeutic interventions. *Materials and Methods:* Using a clinical breast cancer cohort (n=127), the molecular transcript profiles of OPG, RANK and RANKL were screened using quantitative polymerase chain reaction (qPCR) and analysed against clinical-pathology including tumour, node, metastasis (TNM) scoring, Nottingham prognostic index (NPI) and outcome after 10 years follow-up. Subsequent *in vitro* work was carried out in the immortalised breast cancer cell line MDA-MB-231 targeting OPG and RANK using ribozyme transgenes to examine cancer cell behaviour traits, including cell migration and invasion. *Results:* In the clinical cohort, *RANK*, *RANKL* and *OPG* transcript levels were shown to be reduced in the tumour samples compared to paired normal samples, though none of these reductions reached statistical significance. Low transcript expression levels of *RANK* were significantly associated with the development of metastases ($p<0.05$), including bone metastases ($p<0.05$), and those that died of the disease ($p<0.01$) compared to those patients who remained disease-free. In contrast, RANKL transcripts levels were significantly increased in patients who developed bone metastases ($p=0.05$) but were significantly decreased in patients who developed local recurrence ($p<0.05$) and those who died of the disease ($p<0.05$) compared to those who had remained disease-free. This was also seen in the Kaplan-Meier survival curves in which low *RANK* or *RANKL* expression were associated with significantly poorer overall survival ($p<0.05$). In contrast, *OPG* transcript levels correlated to a significantly better overall survival ($p<0.05$). *In vitro* targeting of *OPG* expression

using ribozyme transgenes, resulted in significant increases in MDA-MB-231 breast cancer cell motility and invasion ($p<0.05$). Exposure of these cells to a bone matrix-like environment reduced these behavioural trends; however these did not reach statistical significance. *Conclusion:* The complexity and vast number of cell types, soluble factors and signalling pathways, which influence molecular features of osteotropic cancer cells, have progressed dramatically in the last decade allowing for the licensing of therapeutics, such as Denosumab. However, further efforts to elucidate all the pathways and cell types, which are affected, could potentially lead to the identification of patients most at risk of developing breast cancer-associated bone metastases and therapeutic intervention being curative-or management-driven rather than palliative.

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SBO4B06
BRMS1L SUPPRESSES BREAST CANCER METASTASIS BY INDUCING EPIGENETIC SILENCE OF FZD10

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BRMS1L (breast cancer metastasis suppressor 1 like, BRMS1-like) is a component of Sin3A–histone deacetylase (HDAC) co-repressor complex that suppresses target gene transcription. Here we show that reduced BRMS1L in breast cancer tissues is associated with metastasis and poor patient survival. Functionally, BRMS1L inhibits breast cancer cells migration and invasion by inhibiting epithelial–mesenchymal transition. These effects are mediated by epigenetic silencing of FZD10, a receptor for Wnt signalling, through HDAC1 recruitment and histone H3K9 deacetylation at the promoter. Consequently, BRMS1L-induced FZD10 silencing inhibits aberrant activation of WNT3-FZD10-β-catenin signalling. Furthermore, BRMS1L is a target of miR-106b and miR-106b upregulation leads to BRMS1L reduction in breast cancer cells. RNA interference-mediated silencing of BRMS1L expression promotes metastasis of breast cancer xenografts in immunocompromised mice, whereas ectopic BRMS1L expression inhibits metastasis. Therefore, BRMS1L provides an epigenetic regulation of Wnt signalling in breast cancer cells and acts as a breast cancer metastasis suppressor

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SBO4C01
PROTIDES AS A NEW ANTI-CANCER DRUG FAMILY

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Background/Aim: Nucleosides, such as gemcitabine and the fluoropyrimidines continue to be important drugs in the therapy of both solid tumours and leukaemias but they have several limitations, including inherent and acquired resistance and toxicity. Several problems rise from the absolute necessity for intracellular phosphorylation to the active phosphates (nucleotides). The nucleotides are not useful therapeutics as their charge prevents cell entry. Over 20 years, this lab has have developed masked membrane-permeable nucleotide pro-drugs ('ProTides') to solve this problem and 4 ProTides have now entered clinical trial, including 2 for cancer. *Materials and Methods:* (i) Identification of a known or novel nucleoside analogue; (ii) Synthetic organo-phosphorus chemistry to generate a family of 5'-ProTides; (iii) Varying the ProTide motif in aryl, ester and amino acid regions; (iv) *In vitro* assay *versus* a series of sensitive and resistant tumour cell lines; (v) *In vivo* pharmacokinetics (PK) and toxicology; and (vi) Pre-clinical work up and clinical evaluation. *Results:* We have prepared over 5,000 ProTides for cancer and viral indications. The leading cancer ProTide is Acelarin, derived from gemcitabine and licenced to Nucana Biomed. Acelarin entered phase 1 clinical trial in October 2013 at Hammersmith hospital London and a phase 2 efficacy trial closed in December 2014. We will update on this trial, phase 3 plans and a new 2nd in class agent due to enter phase 1 in Q2/2015 at the meetin and overview cancer ProTides in general. *Conclusion:* ProTides represent a promising family of new anti-cancer agents, which overcome many limitations of existing agents. They overcome resistance mechanisms arising from cell entry, phosphorylation and deamination and, based on early clinical studies, appear to be well tolerated and effective in disease control.

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SBO4C02
P16 METHYLATION IS A USEFUL PREDICTOR FOR CANCER DEVELOPMENT FROM ORAL EPITHELIAL DYSPLASIA IN A MULTICENTRE PROSPECTIVE STUDY

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Background/Aim: Silencing of P16 through methylation and

locus deletion is the most frequent early events in carcinogenesis. The aim of this study was to prospectively and experimentally determine if P16 methylation is a driver in cancer development. *Materials and Methods:* Patients (N=181) with mild or moderate oral epithelial dysplasia (OED) were recruited into the multicentre prospective cohort. P16 methylation was analyzed using MethyLight and bisulfite-DHPLC/-sequencing. Progression of OEDs was monitored during a 3-year follow-up period. A DNA methyltransferase was constructed using a P16 promoter-specific engineered zinc finger protein fused with the catalytic domain of DNMT3A and used to study roles of P16-specific methylation in cancer development. *Results:* P16 methylation-informative cases (n=152) were enrolled in the prospective cohorts with an ultimate compliance of 96.7%. OED-derived squamous cell carcinomas were observed in 21 patients (14.3%) during the follow-up (median=41.0). The OED to cancer progression rate from the P16 methylation-positive patients was significantly increased when compared to P16 methylation-negative patients (27.1% *vs*. 8.1%; adjusted odds ratio =4.6; p=0.006). Using P16 methylation-positive criteria, as a biomarker for early prediction of cancer development from OEDs, showed a sensitivity and specificity 62% and 76%, respectively. Experimentally, the engineered methyltransferase induced P16-specific methylation and transcription inactivation in tumour cells. Migration and invasion were obviously enhanced in cell lines following P16-specific methylation *in vitro* and *in vivo*. *Conclusion:* P16 methylation is a useful biomarker for determining the malignant potential of OED early in the course of the disease (NCT01695018).

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SBO4C03

NEW TARGETS FOR CLINICAL EVALUATION IN GLIOBLASTOMA MULTIFORME, A TUMOUR WITH LIMITED THERAPEUTIC OPTIONS

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Background/Aim: Glioblastoma multiforme (GBM), the most common intrinsic malignant brain tumour in adults, despite intensive clinical investigation, still has a very poor prognosis with overall survival usually less than a year. New approaches are needed and we have been investigating new targets in GBM cells *in vitro* with a view to developing more effective therapies for these tumours. Tumour-specific tumour necrosis factor (TNF)-related apoptosis ligand (TRAIL) has attracted extensive interest in cancer therapy. Histone deacetylase (HDAC) inhibitors, such as panobinostat (PANO), have been shown to restore dysregulated gene expression in tumour cells *in vitro* and we hypothesize that combination of PANO with TRAIL could be a useful strategy for GBM therapy. *PDE1C* is a proliferation-associated gene and over-expression is associated with poor prognosis in GBM patients; thus, interference with *PDE1C* gene expression may be clinically exploitable. *Materials and Methods:* We have used a panel of short-term cultures derived from patient samples of GBM that have been extensively characterised genomically in these studies. Real-time Q-PCR analysis was used to determine gene expression and copy number analysis. The effects of TRAIL and PANO on cultures were assessed both in isolation and combination using drug synergy assays, while a pathway reporter assay was used to determine which pathways were affected by treatment. siRNA transfections were performed using the DharmaFECT-1 transfection reagent. Cell proliferation was determined using a sulforhodamine B (SRB) assay and migration and invasive potential was assessed using a CytoSelect 24-well cell migration and invasion assay. *Results:* We detected significant levels of expression of DR5 compared to DR4, and DcR2 and significant levels of osteoprotegerin (OPG) compared to DR4 and DcR1 in these cultures. In biopsy specimens, DR5 expression was greater than DR4, while levels of DcR1, DcR2 and OPG expression was comparably similar to DR4. Drug synergy analysis showed that cultures are sensitive to TRAIL (EC_{50}=3-50 ng/ml) and PANO (EC_{50}=6-120 nM). However, in combination with TRAIL (1-3 ng/ml), the PANO EC_{50} was significantly reduced to 3-53 nM. The increased effectiveness of TRAIL-PANO combination was associated with increased reporter activity for NANOG, retinoic acid response element (RARE), Wnt (T-cell factor/lymphoid enhancer factor (TCF/LEF)) and xenobiotic aryl hydrocarbon receptor (AhR). We confirmed gain of PDE1C in GBM cultures and that gain of PDE1C is not associated with increased expression in these cultures. *PDE1C* depletion consistently reduced proliferation by 45-54% when compared to *PDE1C* expressing cells in all but one culture. siPD cells demonstrated reduced capacity to both migrate through the polycarbonate membrane and invade through the extracellular matrix (ECM)-coated polycarbonate membrane when compared to siNT cells. *Conclusion:* These results demonstrate that PANO improves response to TRAIL in GBM *in vitro* and that *PDE1C* seemed to play an essential role in driving proliferation, migration and invasion in GBM cultures. They also suggest that proliferation, migration and invasion of GBM cells could also be regulated downstream of *PDE1C*. New therapies based on these observations may be of clinical utility.

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SBO4C04
DISCOVERY OF A REGULATORY MOTIF OF HUMAN SATELLITE DNA TRANSCRIPTION RESPONSIVE TO BATF2/SARI OVER-EXPRESSION

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Background: Basic leucine zipper transcription factors (BATFs) play profound roles in the gene expression regulatory network in tumour cells. BATF2, also known as SARI (suppressor of AP-1, regulated by interferon), was able to suppress both growth and migration of various cancers. *Materials and Methods:* We used chromatin immunoprecipitation and DNA sequencing (ChIP-seq) to identify the BATF2 binding sites across the entire human genome. MACS software was utilized to identify the specific BATF2 interaction sites with high frequencies. We also validated the effects of the located sites by luciferase reporter assays and the transcript abundance determined by RT-qPCR. *Results:* From the bioinformatics analyses, the most significant motif discovered as TTCCATT [CT]GATTCCATTC[AG]AT was primarily distributed among the chromosome centromere regions and within type II satellite DNAs. It was confirmed, as determined by luciferase reporters driven by the motifs in tandems, that the identified motifs were able to prime the transcription with directional and asymmetrical features. *Conclusion:* Our findings provided important clues for understanding the role of BATF2 in tumours and also shed light on the conceivable role of satellite DNAs in the global regulation for genome stability maintenance.

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SBO5A01
THE DEVELOPMENT OF LAPAROSCOPIC COLORECTOMY IN CHINA

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It is now 21 years since the first laparoscopic colectomy that was performed in 1993 in China. It started in few hospitals in the late 90s' with less than 10 surgeons who could perform it independently. From 2000, laparoscopic colorectomy became more popular; it was estimated that more than 2,000 surgeons could perform it in 2013 and it will reach 5,000 until 2020. But the development is not balanced. In Southeast cities, some large minimally invasive surgery (MIS) centres can perform this procedure at a rate of more than 100 cases per year but in some Northwest hospitals, this number is less than 10. Besides this, because of the lack of certification, some surgeons never received systematic training. Facing this, the Chinese Anti-Cancer Association launched several training programs since 2007. Through efforts, until last year, nearly 5,000 surgeons received standard training in different levels. Due to its difficulty in manipulation and lack of evidence, it appears harder to perform a laparoscopic rectal cancer resection. Recently, a large randomized controlled trial (RCT) was launched by Chinese researchers in order to confirm the long-term outcomes of laparoscopy in rectal cancer. In the future, Chinese doctors will obtain these results. Laparoscopic colorectomy in China, if used properly, can provide additional benefits for those patients.

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SBO5A02
RISK FACTORS FOR AIN III AND PROGRESSION TO ANAL CANCER IN SOUTH WALES

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Anal intraepithelial neoplasia (AIN) is a precursor to the development of anal cancer. It is known that human papilloma virus (HPV) types 16 and 18 are associated with the development of AIN. It is also recognised that AIN can progress through stages AIN I to AIN III and on to invasive anal cancer. However, little is known about the proportion of patients who progress to anal cancer and the rate at which they do so. This project studied a group of patients with AIN III in South Wales over 5 years in order to determine the rate of progression from AIN III to anal cancer and the factors associated with progression. Twenty six patients were followed for a mean of 3 years. Eleven patients (42%) presented with invasive anal carcinoma on a background of AIN. Three patients (20%) progressed from AIN III to invasive anal cancer during the study. Female gender, smoking, a history of anogenital warts or other sexually transmitted diseases and a previous malignancy were associated with the incidence of AIN III. Immunosuppression was associated with progression of AIN III to invasive anal cancer.

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SBO5A03
BIOMARKERS IN COLORECTAL CANCER

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Background/Aim: The adenoma-carcinoma sequence in the carcinogenesis of colorectal cancer (CRC), first proposed by Basil Morson, has been confirmed by advances in molecular genetics. An understanding of these molecular events may be used for earlier diagnosis to guide treatment, as prognostic markers in predictive genetic testing and genetic counselling and in prevention as in pre-implantation genetic diagnosis of CRC. *Materials and Methods:* A review of the literature was made on biomarkers in CRC. Results and *Conclusion:* Recent advances in molecular biology have shown that CRC develops as a result of multi-step events, which transform normal epithelium to adenoma and invasive carcinoma and its metastases. The majority of CRCs arise following an accumulation of chromosome abnormalities known as the chromosome instability pathway (CIN) and accounts for about 85% of CRC. Such cancers have a poor prognosis. About 15% of CRC show microsatellite instability (MSI). This is due to mutations in mismatch repair (MMR) genes *MLH1, MSH2, MSH6* and *PMS2* as in Lynch syndrome or in aberrant DNA methylation of *MLH1* causing sporadic CRC. MSI CRCs have lower recurrence rates and better survival despite being resistant to alkylating agents and cisplatin. Epigenetic instability causing CRC due to hyper-methylation of gene promoters containing CpG islands is known as CpG island methylator phenotype (CIMP). A subgroup of hyperplastic polyps, known as sessile serrated adenomas, may develop into carcinoma involving CIMP and mutation in the BRAF gene. Global DNA hypomethylation as a cause of cancer has been found in many CIN CRC. Aberrant microRNAs are also involved in the initiation and progression of CRC. The advent of next-generation sequencing technology facilitates discovery of molecular pathways involved in the carcinogenesis of CRC, thus opening opportunities for the development of screening strategy, earlier diagnosis, targeted therapies as in the application of *KRAS* status in anti- epidermal growth factor receptor (EGFR) monoclonal antibodies therapy, prognostic markers, genetic counselling and in pre-implantation genetic diagnosis. A review of the potential applications of colorectal biomarkers in the management of colorectal cancer is presented.

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SBO5A04
CYTOREDUCTIVE SURGERY IN THE MANAGEMENT OF PERITONEAL MALIGNANCY FROM COLORECTAL CARCINOMA

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Colorectal carcinoma is a common cancer diagnosis in the Western world and is the second commonest cause of cancer death in the UK. Approximately 2% of colorectal cancers develop peritoneal disease as the sole site of metastatic deposits. Traditionally, these would be considered untreatable and palliative treatments would have been the only course of action. Despite advances in systemic chemotherapy, which are invaluable in the treatment of extra-peritoneal metastases, such as those of the liver or lung, chemotherapy has not been demonstrated to improve survival in cases of isolated peritoneal malignancy from colorectal carcinoma. More recently, cytoreductive surgery followed by hyperthermic intraperitoneal chemotherapy (HIPEC) has been used with good success in selected patients to achieve long-term cure. We present the role of cytoreductive surgery and HIPEC in the management of peritoneal malignancy from colorectal carcinoma. We also present evidence for its effectiveness and guidelines on case selection and technical factors for the successful management of peritoneal malignancy secondary to colorectal carcinoma.

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SBO5A05
THE KEY ROLE OF HBV MUTATIONS IN CARCINOGENESIS

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Background/Aim: Primary liver cancer is one of the most common malignancies. It is second cancer-related death in China. Over 80% liver cancers in China were caused by hepatitis (hepatitis B virus (HBV)). Development of the HBV rtA181T/sW172* mutant could occur during prolonged lamivudine (LAM) therapy conferring cross resistance to adefovir. Studies demonstrated an increased oncogenic potential of this mutant in small group of hepatocellular carcinoma (HCC) cohort and *in vitro* cells. In this study, we aimed to investigate the clinical significance of this finding with 12,000 specimens who took the anti-HBV drug resistant assay in the last five years (2009-2014) in Beijing Institutes of Hepatology and 36% of them had *AVR* resistance. *Materials and Methods:* Serum samples from chronic hepatitis B patients were run for virological drug resistant assays. PCR products from the HBV virus were sequenced and mutation assay with *HBV* mutation software from Stanford University. Virological factors assay including HBV-DNA level, genotype, S-antigen and E antigen, as well as clinical variables. The covalently closed circular DNA (cccDNA) of *HBV* levels were analysed in HCC tissues with qPCR. Functional assays were performed with HepG2 cells. *Results:* In total, 4,300 spacemen were detected with anti- HBV drug

resistance and the ratio of the drug resistant is 36% (4300/12,000). One thousand three hundred and twenty of them had rtA181 mutation, including 812 cases of rtA181T and 508 cases of rtA181V. The ratio of rtA181T is 18% and rtA181V is 12% in total mutation cases. Twenty-six of the 812 (3.2%) rtA181T-positive patients and 9 of the 508 (1.8%) rtA181V-positive- patients developed HCC. Kaplan-Meier analysis indicated that the presence of rtA181T mutation ($p<0.05$) and liver cirrhosis ($p<0.001$) was significantly associated with subsequent occurrence of HCC. Eighty percent of HCC patients belonged to the cirrhosis group. The cohort study with 50 cases of rtA181T and 50 cases of rtA181V showed that the genetic evolution of *HBV* mutated in the rtA181T is different from the rtA181V in peripheral blood. *In situ* and qPCR assays confirmed that more cccDNA and HBV virus RNA in the liver cells from specimens that carried rtA181T mutated *HBV*. HepG2 cells transfected with pCDNA3-HBVrtA181T and pCDNA3-HBVrtA181V showed that HBV virus in HepG2 cells transfected with pCDNA3-HBVrtA181T were 50 times higher than that transfected with pCDNA3-HBV rtA181V. Further studies showed the autophagy and endoplasmic reticulum (ER) stress levels were significantly induced in HepG2 transfected with pCDNA3-HBVrtA181T. *Conclusion:* The rtA181T/sW172* mutant in LAM-resistant patients increased the risk of HCC and could be due to more oxidative DNA damage induced by high level rtA181T. Further work is required to identify the mechanism.

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SBO5A06
FBXW7 ORCHESTRATES A FAILSAFE PROGRAM AGAINST COLORECTAL CANCER PROGRESSION

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Background/Aim: FBXW7 (F-box and WD repeat domain-containing 7, also known as FBW7, AGO, SEL10, CDC4) and TP53 are widely-known tumour suppressors and their genetic alterations lead to malignant transformation, metastatic spread and poor survival of cancer sufferers. They are among the most commonly mutated genes after *K-RAS* and *APC* in colorectal cancer (CRC). FBXW7 constitutes one of the four subunits of SKP1-cullin-F-box (SCF)-E3 ubiquitin protein ligase complex, which functions in phosphorylation-dependent ubiquitination. To date, a wide array of SCF^{FBXW7} E3-ligase substrates have been identified and characterized, including cyclin E, c-Myc, c-Jun, Notch, Presenilin, SREBP and Aurora-A, Krüppel-like factor 5 (KLF5). These and several other FBXW7 E3-ligase substrates play central roles in cell division, growth, differentiation, cell-fate determination and maintenance of the phenotype of a variety of types of stem cells. We aim to elucidate how FBXW7 maintains the epithelial homeostasis *via* orchestrating a variety of signalling pathways and gene networks *Materials and Methods:* The murine *Fbxw7* alleles are flanked with the LoxP sites ($Fbxw7^{fl/fl}$) and *Fbxw7* is subsequently specifically knocked out in the murine intestinal epithelia (*i.e.* $Fbxw7^{\Delta G}$) *via* crossing with Villin-Cre mice. Crypt cells were derived from $Fbxw7^{fl/fl}$ (*i.e.* control) and $Fbxw7^{\Delta G}$, followed by 2D mass spectrometry analysis to identify novel FBXW7 substrates and molecular network. $Fbxw7^{\Delta G}$ was further crossed with ApcMin mice to investigate its tumour suppressor function in the context of loss of Apc, the gate keeper of the gut. *Results:* The proto-oncogene *DEK* is a direct target of FBXW7 in a GSK-3β phosphorylation-dependent manner. An Apc^{Min} background mouse lacking of *Fbxw7* in the gut could not survive one month due to a large quantity of adenomatous polyps in the murine intestine. Furthermore, for the first time, Fbxw7's onco-suppressor role has been extended to negative regulation of epithelial-mesenchymal transition (EMT), a crucial mechanism modulating the initial steps of metastatic progression. Finally, *FBXW7*-mutated colorectal cancer cells exhibit aberrant expression of phosphorylated-p53 at Serine-15. *Conclusion:* FBXW7 orchestrates a broad array of signalling pathways and gene networks for tumour suppression. Furthermore, aberrant phosphorylation, in the context of *FBXW7* mutation, may shed light on the targeted therapeutics for CRC.

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SBO5B01
A STUDY FOR EARLY NSCLC RESECTION OPERATION CHOICE

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Lung cancer is a serious threat to people's lives and health. Lung cancer mortality ranks first in malignant tumours. Currently, surgical lobectomy plus regional lymph node dissection is established as the standard treatment in early stage non-small cell lung cancer (NSCLC) patients. Peripheral lung cancer with less than 2 cm in diameter is diagnosed since multi-slice spiral computed tomography (CT) is widely used in clinical practice. Evidence suggests that elderly lung cancer patients with poor cardiopulmonary function cannot accept lobectomy. Asian lobectomy, including anatomical pulmonary resection and lung wedge resection, received similar treatment with lobectomy. Therefore, whether Asian lobectomy is an appropriate treatment

for peripheral lung cancer patients with less than 2 cm in diameter, who can tolerate lobectomy, is a hot topic in the field of thoracic surgery today. This study aimed to explore the lung sub lobe resection for a diameter of 2 cm or less early peripheral NSCLC comparing with lobectomy. The objective was to determine whether the sub lobe resection is suitable for the treatment of early peripheral NSCLC. This study may further improve the effect of early surgical treatment for NSCLC and improve patient quality of life after surgery.

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SBO5B02
ACTIVATION OF THE BMP-BMPR PATHWAY CONFERRED RESISTANCE TO EGFR-TKIS IN LUNG SQUAMOUS CELL CARCINOMA PATIENTS WITH EGFR MUTATIONS

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The empirical criteria for defining a clinical subtype of lung cancer are gradually transiting from histopathology to genetic variations in driver genes. Targeting these driver mutations, such as sensitizing epidermal growth factor receptor (*EGFR*) mutations, has dramatically improved the prognosis of advanced non-small lung cancer (NSCLC). However, the clinical benefit of molecularly targeted therapy on NSCLC appears to differ between lung adenocarcinomas and squamous cell carcinomas (SqCC). We report here that lung SqCC harbouring *EGFR* mutations show resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) that result from the activation of BMP-BMPR-Smad1/5-p70S6K. The combined treatment of these tumour cells with EGFR-TKI together with inhibitors specific to BMPR or downstream mammalian target of rapamycin (mTOR) effectively reversed the resistance to EGFR-TKI. Moreover, blocking the whole PI3K-AKT-mTOR pathway with the PI3K/mTOR dual inhibitor BEZ235 also showed efficacy in treating this subtype of lung SqCC. This study details the empirical basis for a feasible clinical solution for squamous cell carcinomas with *EGFR* mutations.

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SBO5B03
CIRCULATING PROGENITOR CELLS IN LUNG AND OTHER CANCERS

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Background/Aim: Multipotent or lineage-specific progenitor cells are either associated with, or critical for the invasion, metastasis or prognosis of lung cancer and other malignancies. Circulating endothelial progenitor cells (CEPCs) are a subset of primitive cells, which are derived from the bone marrow, circulate into blood stream and differentiate into endothelial cells for angiogenesis with response to pathological conditions, such as malignant tumour growth. In lung cancer, it is a challenge to access tumour response to chemotherapy and antiangiogenic treatments because certain targeted therapies may not give rise to rapid and measurable tumour shrinkage for imaging-based clinical assessment. CEPCs could be a promising surrogate marker for the effectiveness of lung cancer treatment and therapeutic target, although it is prerequisite to understand their functional activities in tumour-associated environment. We, therefore, aimed to investigate the interaction of CEPCs and lung cancer cells and the underlying signalling mechanisms. *Materials and Methods:* Tissues were collected from lung cancer patients following the local ethic procedures. Gene expression of the circulating progenitor cells were determined by qRT-PCR. Expression of angiogenesis-associated miRNA was evaluated by Sybr-Green miRNA assay. Functions of angiogenesis-associated miRNAs were examined using miRNA mimics or inhibitors. Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood using Histopaque gradient separation. Cell migration and wound healing were monitored on the electric cell-substrate impedance sensing (ECIS) system. *Results:* We have established a method to enumerate CPCs from human peripheral blood using flow cytometry. CEPCs can be selected from isolated PBMCs by cell culture. The preliminary data showed that it was possible to distinguish strand selection of angiogenesis-associated miRNA in lung cancer cells. *Conclusion:* It is extremely important to understand the involvement of CEPCs in altered tumour-associated vasculogenesis. However, the characteristics of CEPCs have not been well defined. We have observed some promising clues and will continue our investigation of the interaction between the CEPCs and lung cancer cells using the techniques we have established. A medium-sized cohort study of lung cancer screening is under way.

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SBO5B04
THE LONG-TERM RESULT FOLLOWING LUNG RESECTION COMBINED WITH OFF-PUMP CORONARY ARTERY BYPASSES GRAFTING

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Coronary artery disease (CHD) and lung cancer are among the leading causes of death in China. In clinical practice, patients having both diseases have been markedly increased in the past 20 years. In 2003, we began lung resection combined with off-pump coronary artery bypasses grafting surgery for those who have lung tumour and severe CHD simultaneously. Recently, we tried some new methods in order to improve the long-term result, including thoracoscopy assistance for lung cancer resection and adjuvant chemotherapy. Thirty patients, in total, having lung cancer, mostly at stage I or II, and simultaneous two-or three-vessel disease of severe CHD underwent combined off-pump coronary artery bypass grafting (OPCAB) and pulmonary resection. The biopsy of lung tumour was carried out initially, then myocardial revascularization performed followed by lobectomies or partial lung resections. Five patients accepted chemotherapy before or after surgery (2 both new-adjuvant and adjuvant chemotherapy and 3 adjuvant chemotherapy only). All patients survived the operation. No new myocardial infarction (MI) occurred in the perioperative period. Mean operating room time was 294.80±64.30 min. The average number of anastomosed coronary vessels was 2.3. After myocardial revascularization approach, twenty-five patients who were diagnosed as non-small cell lung cancer underwent lobectomy or segmentecnomy through the same sternotomy incision with assistance of thoracoscope (n=12), lobectomy through another lateral thoractomy (n=11) and bypass to left anterior descending (LAD) and lobectomy through left thoractomy only (n=2). The other five patients with benign disease underwent partial lung resections through sternotomy. The most frequent complications were cardiac arrhythmias (5 cases), atelectasis (3 cases) and pulmonary infections (2 cases). All the patients were followed up for 12-129 months. Within this period, 5 patients (20%) died due to cancer recurrence. The 3-year and 5-year survival rates were nearly 70% and 60%. We conclude that combined OPCAB and pulmonary resection is a safe and effective treatment for patients with simultaneous lung cancer and severe CHD, especially those with three-vessel disease who are usually not candidates for percutaneous coronary intervention.

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SBO5B05
LUNG METASTASIS: EMERGING TARGETS AND OPPORTUNITIES FOR THERAPEUTIC INTERVENTION

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Metastases from lung cancer present a major clinical problem, particularly as typical sites of metastatic relapse for lung cancers include the brain, bones and liver. An increasing amount of evidence points to a role for receptor and non-receptor tyrosine kinases as mediators of the metastatic phenotype and, significantly, the development of a drug-resistant phenotype. Consequently, inhibitors of the epidermal growth factor receptor and the c-Met receptor have been identified as potential therapeutic targets in both primary and metastatic tumours. Importantly, the advent of recent molecular profiling approaches has revealed lung cancers as a group of diseases with diverse pathological features. As such, a number of tyrosine kinase inhibitors also present attractive treatment options within defined lung cancer subsets.

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SBO5B06
HSA-MIR-182 DOWNREGULATES RASA1 AND SUPPRESSES LUNG SQUAMOUS CELL CARCINOMA CELL PROLIFERATION

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Aim: This work was undertaken to identify the differences in expression of miRNAs, which would predict early-stage non-small cell lung cancer prognosis and recurrence. The results may be helpful to demonstrate one mechanism of lung squamous cancer proliferation. *Materials and Methods:* Lung squamous cell carcinoma specimens were collected at the time of surgery. Microarray of expression of specific miRNAs in lung squamous cell cancer tissue, were assessed by qRT-PCR. *Results:* We found that the hsa-mir-182 family are highly expressed in lung squamous cell carcinoma. Paradoxically, our study reveals that mir-182 suppresses cell proliferation *in vivo*. *RASA1* is related to cell apoptosis. We further show that mir-182 down-regulates RASA1. *Conclusion:* We provide first time evidence through tissue microarray and quantitative PCR validation of mir-182 in the expression of lung squamous cell carcinoma. Our data provide a possible mechanism for lung cancer cell proliferation in lung squamous cell cancer and may be helpful in discovering a new strategy to reveal lung squamous cell carcinoma progress.

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SBO5B07
CANCER STEM CELLS (CSC) METABOLISM AND HYPOXIA AS A NOVEL TARGET FOR TREATMENT OF THORACIC MALIGNANCIES

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Background: Lung Cancer (LC) and Malignant Pleural Mesothelioma (MPM) are cancers that share hypoxia as a common characteristic. Hypoxic niches are also a common finding in many human tumours therefore "stemness" in cancer cells has been attracting raising interest as a novel therapeutic strategy to target both mature tumour cells and CSC. We have previously demonstrated mitochondrial functional and morphologic abnormalities in MPM cells. Moreover other groups showed that CSC proteomics shows massive expression of mitochondrial related proteins. On the other hand Carbonic Anhydrase IX (CAIX; an enzyme well-known for being involved in the reponse to hypoxia) has progressively been recognized as a potential target for LC and MPM. *Methods:* Tumour Cell Sphere Assay was conducted to identify CSC whereas constitutive and hypoxia-induced CAIX expression in LC and MP cell lines and tissue was evaluated by immune-blotting and immuno-histochemistry, respectively. Mito-chondrial Biogenesis and CAIX activity was inhibited with specific inhibitors and silencing. *Results:* Our results have revealed that CSC proliferation counts on mitochondrial biogenesis because its specific inhibition *i.e.* with Doxycicline, induced a highly significant reduction (> 3 fold) of CSC spheres number and that CAIX was strongly expressed in tumour cell lines and tumour tissue. In addition, inhibition of CAIX either by silencing, or treatment with specific antagonists, exerted strong *in vitro* and *in vivo* antineoplastic effects. *Conclusion:* CSC and hypoxia show a driving role in carcinogenesis and tumour development of LC and MPM. This looks like a common mechanism among human tumours that could impact on therapeutic approaches to cancer and has prompted us to translate these results in clinical settings. The preliminary translational clinical results will be briefly presented.

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SBO5B08
COMBINED LAPAROSCOPIC AND THORACOSCOPIC *VERSUS* OPEN IVOR LEWIS OESOPHAGECTOMY FOR OESOPHAGEAL CANCER: A RETROSPECTIVE COHORT STUDY

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Aim: To assess the feasibility and safety of combined laparoscopic and thoracoscopic oesophagectomy followed by intrathoracic oesophagogastric anastomosis. *Patients and Methods:* Retrospective comparative analysis of 51 cases of oesophageal cancer at the author's department from March 2010 to October 2014. Patients were divided into two groups based on the type of operative procedures: minimally invasive oesophagectomy group (MIE group, n=56) and open Ivor Lewis oesophagectomy group (open group, n=67). *Results:* There was no statistically significant difference between the MIE group and open group in terms of operation duration (289.2±70.0 min *vs*. 262.2±47.1 min, p=0.114), perioperative bleeding volume (409.6±556.8 ml *vs*. 296.0±145.7 ml, p=0.803), total number of lymph nodes dissected (281 vs.304, t=1.474, p=0.147), postoperative complications (19.2% *vs*. 28.0%, p=0.460), time to intake *via* oral (8.3±3.2 d *vs*. 10.0±8.3 d, p=0.339), postoperative hospital days (12.3±7.0 d vs.12.3±8.1 d, p=0.990). The MIE group is further stratified into two subgroups: earlier period group (n=10) and later period group (n=16) based on the dates when the operations were performed and the outcomes of the two subgroups were compared. The operation time on average of the later period group was significantly shorter than that of the earlier period group (265.3±58.1 min *vs*. 327.5±73.0 min, p=0.024) but did not statistically differ from that of the open group (p=0.852). The bleeding volume among the later period group was significantly lower than that among the earlier period group (215.6±143.4 ml *vs*. 720.0±808.0 ml, p=0.000), yet not significantly different as opposed to that among the open group (p=0.052). The MIE group incurred significantly higher hospitalization expenses than the IL group (p=0.000). *Conclusion:* Our experience in combined laparoscopic and thoracoscopic Ivor Lewis oesophagectomy provided preliminary evidence that such a procedure is safe and feasible for oesophageal carcinoma.

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SBO5C01
COMPLEMENT, INFLAMMATION AND CANCER – A DOUBLE-EDGED SWORD?

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Complement is a key inflammatory driver and important protector from infection; however, it carries an unfortunate propensity to cause tissue damage when over-activated and is implicated in many inflammatory and degenerative diseases. This has led to its frequent description as a double-edged sword, both protective and damaging depending on the context. Roles of complement in cancer surveillance have received relatively little attention and the data that are available serve to confuse. In some contexts, complement protects by killing cancer cells, whilst in others it does nothing or even exacerbates by accelerating proliferation. I will describe the system, its roles in inflammation and relevance to cancer, noting in particular the need for knowledge as anti-complement drugs are more widely used.

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SBO5C02
DECREASED HEPATIC STIMULATOR SUBSTANCE (HSS) EXPRESSION PROMOTES HEPATOCELLULAR CARCINOMA METASTASIS *VIA* ERK ACTIVATION

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Hepatic stimulator substance (HSS), also named augmenter of liver regeneration (ALR), is a newly identified factor that can promote liver regeneration and stimulate hepatoma cell growth. A recent study indicated that HSS expression may be altered in hepatocellular carcinoma (HCC) tissue suggesting a potential link between HSS and HCC. However, whether HSS plays a role in the development of HCC and what its function is during HCC metastasis remain unclear. Here, we demonstrated that a notable decrease in HSS expression was clearly found in metastatic HCC tissues. To verify the possible role of HSS in HCC metastasis, hepatoma cell lines in which the HSS gene was up- or down-regulated were established. *HSS*-knockdown cells presented abundant spikes on their cell surfaces and more pseudopodia. Transwell assay also showed that these cells exhibited greater invasion capabilities when *HSS* was knocked down. An orthotopic tumour transplant model demonstrated that the inhibition of HSS expression promoted notable distal metastasis of tumour cells. Moreover, mice that were administered *HSS*-knockdown cells became emaciated and died earlier compared with those mice that were administered *HSS*-expressing cells. To explore the possible mechanism of HSS involvement in HCC migration, epithelial-mesenchymal transition (EMT) was investigated. In the *HSS*-expressing cells, the expression of a few major epithelial cell markers was obviously down-regulated, whereas the expression of a few mesenchymal cell markers was up-regulated. Interestingly, the phosphorylation of extracellular signal-regulated kinase (ERK) and its downstream signal Snail significantly increased, accompanied by EMT occurrence. The inhibition of ERK signalling resulted in a reduction in HCC invasion. Taken together, our results provide a novel insight into understanding the involvement of HSS in HCC metastasis partially through EMT progression *via* ERK activation.

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SBO5C03
CLINICAL SIGNIFICANCE OF OVER-EXPRESSION OF THYMOSIN B4 IN BREAST CANCER-ASSOCIATED ENDOTHELIAL CELLS

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Background/Aim: Previous studies have shown that thymosin β4 (Tβ4), related to many critical biological processes, including cell proliferation, migration and differentiation, is frequently over-expressed in cancers (*e.g.* breast cancer) and that such over-expression correlates to malignant progression. However, the localization of Tβ4 in human cancers has not been thoroughly investigated. In breast cancer, there is a considerable heterogeneity in the cellular distribution of Tβ4. In most breast tumours examined, cancer cells exhibit low or intermediate levels of Tβ4, while the stromal cells, such as endothelial cells, leukocyte and macrophages, showed intense reactivity of Tβ4. In this study, we ascertained the distribution of Tβ4 in breast cancer-associated endothelial cells and assess the role of Tβ4 in breast tumour progression. *Materials and Methods:* In this study we adapted a novel spheroid co-culture system to establish an *ex vivo* interaction model between breast cancer and endothelial cells, which were labelled with different fluorescent dyes, in order to acquire breast cancer-associated endothelial cells. The sphere formation, after 48h, was dissociated by trypsinization, followed by FASC sorting. PCR-based subtraction analysis was performed to identify differentially high expression of genes in breast cancer-associated endothelial cells. Tβ4 expression in human breast tumour tissues was analysed by tissue microarrays. In order to confirm, real-time PCR assays for Tβ4 in human breast specimens were carried out and normalised against the corresponding tumour vascular marker-VE-cadherin. In murine breast cancer xenograft models, we tested the effects of *in vivo* siRNA knockdown of Tβ4 in breast cancer progression. *Results:* The sphere formation co-culture system provide robust generation of breast cancer-associated endothelial cells. *TMSB4X* that encodes Tβ4, was found to be up-regulated in breast cancer-stimulated endothelial cells *via* our PCR-subtract screen. Both tissue microarray and real-time PCR analysis in human breast specimens indicated that high expressed levels of Tβ4 are significantly associated with tumour vasculature. Moreover, siRNA knockdown of Tβ4 significantly inhibited tumour growth in an *in vivo* animal model. By using photoacoustic microscopy, we also observed a significant reduction in tumour vasculature. *Conclusion:* We identified Tβ4, a novel molecular biomarker for breast tumour angiogenesis, as differentially highly expressed in cancer-associated endothelial cells. A specific function of Tβ4 in the pathogenesis of breast cancer progression might be explored as a potential anti-breast cancer therapy.

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SBO5C04
POTENTIAL IMPACT OF ANAESTHETICS ON CANCER CELL BIOLOGY

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Cancer is the second leading cause of death worldwide. In particular, solid organ tumours derived from breast, lung, colorectal, prostate and gynaecological tissues account for a major proportion of the patient mortality. Despite the increasing efficacy of chemotherapy and the enormous investment into developing targeted pharmacological therapies to treat cancer over recent decades, surgical resection remains the most popular first-line treatment option for most patients with solid tumours offering the best chance of cure. It has long been recognised that surgery itself has a direct impact upon tumour biology, with reports focussing principally on how the surgical stress response-associated immunosuppression may increase the risk of post-operative metastatic spread. However, the impact of anaesthetics/ techniques on post-operative cancer outcome has been largely neglected. Our novel preliminary findings indicate that anaesthetics do exert a strong effect on cancer cell biology. These cumulative data also suggest that some anaesthetics may do a favourable job for cancer patients but some do not. This lecture will cover the impact of anaesthetics on cancer growth and metastasis and provide novel insight into molecular mechanisms. The importance is that this area of research will likely change clinical practice of anaesthesia and *in vivo* cancer research.

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SBO5C05
THE ASSOCIATION OF *ALK* EXPRESSION TO *ALK* GENE ALTERATIONS IN NEUROBLASTOMA

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Background/Aim: Neuroblastoma (NB) is the most common solid extracranial paediatric tumour that exhibits a wide clinical spectrum ranging from spontaneous regression to widespread metastatic fatal disease. *ALK* aberrant expression in NB tissues was associated with patients' worse prognosis; however, the potential mechanism underlying *ALK* aberrant expression was largely unknown. This study aimed to detect *ALK* gene alterations, including rearrangement, mutation, promoter methylation and to associate the ALK genes alterations with ALK protein expression and patients' clinical information. *Materials and Methods:* ALK expression was examined in 3 NB cell lines and 43 tumour tissues using immunohistochemistry. *ALK* rearrangement and copy number changes were detected by fluorescence-based *in situ* hybridization (FISH). *ALK* mutation was identified by sequencing. Methylation state of *ALK* promoter was analyzed through bi-sulfite treatment followed by sequencing. *Results:* ALK cytoplasmic staining was observed in NB cell lines SH-SY5Y and SK-N-SH but not in SK-N-AS. Of 43 NB tissues, 60.5% (26/43) showed ALK positive predominantly in membranous. There were no breakages of *ALK* found in any of NB cell lines and tissues. *ALK* amplification was observed in 4.6% (2/43) of cases but *ALK* gain was detected in 69.7% (30/43) of cases. Extra copies of *ALK* (including amplification and gain of *ALK*) was associated with ALK expression (p=0.02). However, there is no association between extra copies of *ALK* and patient age (p=0.328), tumour sites (p=0.775) and differentiation (p=1.000). Kaplan-Meier survival analysis indicated that there is no difference of the average survival time between patients with and without extra copies of *ALK* (p=0.497). Of 3 NB cell lines and 43 NB tumour tissues, targeted region of *ALK* (exon 23-28) was sequenced and only one synonymous mutation was found in one case in exon 23 of ALK at base 4552, a G>C substitution but not causing the change of encoded amino acid (G4552C, A1200A). Methylation state of *ALK* promoter in 3 NB cell lines and 6 NB tumour tissues (3 ALK positive and 3 ALK negative) were examined. There was no methylation found in promoter region of *ALK* closed to the transcription start site including 26 CpG dinudeotide sites. *Conclusion:* The extra copies of *ALK* gene (including amplification and gain of ALK) are a frequent genetic aberration in NB, which associates with ALK expression; however, mutation, promoter methylation and rearrangement of *ALK* gene rarely occur in NB.

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SBO5C06
DESIGNING DRUGS TO TACKLE UNMET MEDICAL NEED IN BREAST CANCER

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Background/Aim: Advances in breast cancer research have established the existence of clinical disease sub-types, each with distinct pathologies, course of disease progression and response to therapeutic intervention. Survival outcomes have improved dramatically for oestrogen receptor (ER)-positive disease largely due to the routine use of tamoxifen or related anti-oestrogens, such as anastrozole. On the other hand, disease sub-types, such as "triple-negative" breast cancer (lacking expression of ER, progesterone or human epidermal growth factor receptor 2 (HER2) receptor) present a difficult challenge with standard chemotherapy having little impact on overall survival. Treatment of HER2-positive breast cancer, a sub-type characterised by frequent metastatic progression, is also clinically challenging. Treatment of HER2-positive disease can be partially addressed by agents, such as the monoclonal antibody trastuzumab, albeit with disease relapse and progression in many cases. Previous research at Cardiff has established the important role of Bcl3 in metastatic progression of HER2-positive breast cancer within *in vitro* and *in vivo* models. Bcl3 is a facilitator protein of the nuclear factor κB (NF-κB) signalling system with significant potential as a target for cancer drug design. This presentation will report the design, synthesis and anticancer evaluation of the first anti-metastatic Bcl3 inhibitors. *Materials and Methods:* Structure-based molecular modelling methods (within Molecular Operating Environment (MOE)) were used to study the protein-protein interaction domain between crystal structures of Bcl3 (seventh ankyrin repeat) and binding partner p50. A pharmacophore model was built describing the electrostatic interactions between the binding partners, which was then screened against the virtual SPECS database (360K molecules). A process of filtering and further docking, refinement and re-scoring allowed the selection of 10 virtual hit molecules for evaluation. Compounds were checked *in vitro* for absence of overt cytotoxicity in the metastatic breast cancer cell line MDA-MB-231 and were then evaluated for anti-Bcl3 activity using an NF-κB cell reporter, Bcl3-p50 ELISA assay and a cell migratory (Boyden chamber) assay. *In vivo* evaluation was carried out in a mouse model bearing the metastatic MDA-MB-231 cell line, where animals were treated with JS6 daily for 10 days following metastatic seeding. *Results:* Docking and refinement of virtual hit compounds led to the identification of ten distinct compounds for anticancer evaluation. One of these compounds (JS6) exhibited potent (sub-micromolar) inhibitory activity in a range of relevant breast cancer models including an NF-κB reporter cell line, a Bcl3-p50 ELISA assay and in cell migration assays, without displaying cytotoxic properties. Crucially, JS6 effectively suppressed metastatic progression in *in vivo* models bearing human metastatic MDA-MB-231 cells. These results have led to patent filing and subsequent licensing of IP to Tiziana Life Sciences. Further pre-clinical development studies are ongoing. *Conclusion:* The first small molecule inhibitor against the metastatic facilitator protein Bcl3 has been discovered in the Cardiff laboratories. The potent *in vitro* and *in vivo* anti-metastatic activity of the lead compound JS6 means that further pre-clinical evaluation is warranted. Further development work leading to an anti-metastatic clinical candidate is ongoing, facilitated by formation of Tiziana Life Sciences and subsequent commercial fundraising.

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SBO5C07 THE GENOME-WIDE BINDING PROFILE OF NOTCH FAMILY PROTEINS IN PANCREATIC CANCER CELLS

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Background/Aim: There are four members of Notch family proteins, Notch1-4. Notch proteins have no DNA binding domain; they usually go through other transcriptional factors. CBF1/Suppressor of Hairless/LAG-1 (CSL) is thought to be a key downstream co-factor for Notch binding to the chromatin. These Notch proteins have different intracellular domains, thus possibly having different target gene profiles. However, the differences in the genome binding sites among different Notch proteins are still not known. Pancreatic cancer cell BxPC3 expresses all four Notch proteins and, hence, is used for the current study. *Materials and Methods:* Plasmids coding for each Notch1-4 intracellular domain sequences or CSL with a 3xFLAG tag were constructed and transfected to BxPC3 pancreatic cancer cells. The chromatin immunoprecipitation (ChIP)-seq was performed with anti-FLAG antibodies. Illumina Hiseq-2000 was used for sequencing. *Results:* We obtained 598, 745, 51, 250 and 99 genome binding sites for Notch1, Notch2, Notch3, Notch4 and CSL, respectively. The total binding sites are 1,551. Only a minority of genes were found to be overlapped between any two of these five different transcription factors. For example, there are 48 overlapping genes, such as *CDCA7, C5orf13, TM9SF4, TFE3, BAZ2B* and *IL4* between Notch1 and Notch2. Notch1 target genes have higher expression level than that of Notch2. It seems that Notch1 and Notch2 have different regulatory effects on target gene expression: Notch1 has stimulatory but Notch2 has repressive effect on the expression of cancer-related genes. *Conclusion:* Only a minority of genome-binding sites overlap between any two of five transcription factors, Notch1-4 and CSL; thus, Notch family proteins possess different gene target profiles. Most Notch regulatory

activities do not depend on CSL; other unknown Notch-interacting transcription factors remain to be identified.

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SBO5C08
UNREGULATED MICRO RNA-21 LEVELS IN EXOSOMES FROM CEREBROSPINAL FLUID OF GLIOMA PATIENTS ASSOCIATED WITH POOR PROGNOSIS OF INVASIVE RECURRENCE

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Background/Aim: Exosomes contain a variety of proteins and nucleic acids consistent with the cells from which they are derived. Such special extra-cellular vesicles (EV) play an important role in mediating cell-cell communication and exert a substantial potential on cell phenotype. The aim of this study was to determine the expression levels of specific cancer-related exosomal microRNA in the cerebrospinal fluid (CSF) of patients with recurrent glioma and investigate its significance for clinical prognosis. *Patients and Methods:* Forty five in-ward patients with previous glioma surgery history were included. Both CSF and serum samples were collected, together with a group of non-tumour brain trauma patients as the controls. The exosomes were isolated and the microRNA-21 (miR-21) levels were measured. The quality of the exosome preparations was examined with morphological and biomedical assays, where transmission electron microscopy (TEM) and Western-blot were used. The extracted RNA was quality-controlled by Bioanalyzer for purity and integrity. Quantitative TaqMan-PCR was performed to determine the miR-21 expression levels. Additional correlation and statistical analyses by cross-referencing other clinical data of the patients were conducted. *Results:* The measures of exosomal miR-21 from CSF in glioma patients were significantly higher than non-tumour controls. Exosomal miR-21 appeared to be as a better indication than the total miR-21 from CSF. No significant difference in serum-derived exosomal miR-21 was detected between the patient and normal healthy control volunteer group. The correlation of CSF-derived exosomal miR-21 levels with some pathological or demographic characteristics of glioma, including the anatomical site of tumour recurrence and spinal/ventricle spreading/metastasis, was suggested. *Conclusion:* The results from this study indicated that the exosomal miR-21 from CFS could be a strong reference for glioma diagnosis and prognosis to separate tumours from other brains diseases or to predict reoccurrence or metastasis during the progression of glioma.

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SC0602
GETTING CANCER RESEARCH INTO PRACTICE: THE ROLE OF NICE

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The aim of cancer research is to improve the understanding of cancer biology in order to develop and test new, more effective treatments and, ultimately, to improve outcomes for patients. The gap between research and clinical practice is notoriously difficult to bridge for a variety of reasons, with some interventions taking a long time to get into practice and others being introduced too rapidly or at too great a cost for the health system. The National Institute for Health and Care Excellence (NICE) was set up in 1999 with the aim of providing authoritative, evidence-based guidance to the National Health Services (NHS) in England and Wales about the most effective and cost effective care. Cancer topics have been an important part of the work ever since. Over 200 pieces of guidance have been published, including 20 clinical guidelines and over 100 appraisals of individual technologies (mainly drugs). This guidance has helped to transform cancer care by making new interventions widely available and helping to standardise clinical practice across the countries. A number of core principles underpin all NICE guidance: appraisal of available research evidence, consideration of cost effectiveness, independent advisory committees involving all relevant health professionals and consumers, transparency of the decision-making process and genuine, open consultation. In this talk I will explain how NICE develops its recommendations and how it has had to respond to a number of difficult challenges over the years.

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P01-S-BRC
THREE NOVEL NA+/H+ EXCHANGER REGULATORY FACTOR 1 GENE MUTATIONS IN HUMAN BREAST CANCER

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Background/Aim: The Na^+/H^+ exchanger regulatory factor 1 (NHERF1) was reported to interact with a number of other molecules involved in cell growth and cancer progression, such as β-catenin, Yes-associated protein 1 (YAP1), epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR). We have recently identified three *NHERF1* novel gene mutations (Y24S, E43G, A190D) in breast tumours. *Materials and Methods:* To investigate the functional properties of NHERF1, wild-type and cancer-derived *NHERF1* mutations (Y24S, E43G, A190D) were expressed in *NHERF1* knockdown cells (MCF7ΔNHERF1) and SKMES-1 cells. Functional testing included cell growth, adhesion, invasion and migration. Glutathione S-transferase (GST) pull-down assays and Western blotting were performed to study if *NHERF1* mutations could affect their interaction with cancer-related proteins. *Results:* In contrast to wild-type *NHERF1*, expression of the mutations failed to suppress cellular malignancy in human cancer cells. Pull-down experiments showed that both E43G and A190D mutations could weaken the interaction of NHERF1 and EGFR. In addition, E43G mutation could enhance interaction between NHERF1 and β-catenin. We also fund that theE43G mutation inactivate NHERF1 inhibition of AKT and ERK activation. *Conclusion:* These results further demonstrated the functional consequences of breast cancer-derived *NHERF1* mutations (Y24S, E43G, A190D) and suggested the causal role of *NHERF1* in breast tumour development and progression.

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P02-S-BRC
RELATIONSHIP BETWEEN HSP70 AND ERBB-2 IN HUMAN BREAST CANCER CELL LINES ON DRUG RESISTANCE

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Background/Aim: Heat shock protein 70 (Hsp70) is over-expressed in human breast cancer and plays an important role in the progression of this cancer. Hsp70 is known to be downstream of ErBb-2 but little is understood regarding the relationship between Hsp70 and drug resistance mediated by ErbB-2 in breast cancer. *Materials and Methods:* After infecting breast cancer cells with lentivirus-mediated Hsp70-specific shRNA (Lenti-ShHsp70) and Lenti-ShErBb-2, we detected the expression of Hsp70 and ErbB-2 by real-time PCR and Western blotting, respectively. *Results:* Compared with the control groups, *Hsp70* mRNA expression was decreased in the infected ones, while Western blotting indicated a concordant protein reduction. On the other hand, ErbB-2 was significantly down-regulated by Hsp70 silencing in SK-BR-3 at both mRNA and protein levels. Expression of Hsp70 in transfected cells was also decreased by Lenti-ShErBb-2. CCK8 assay indicated that the inhibition of Hsp70 can increase the sensitivity of SK-BR-3 cells to fluorouracil treatment. *Conclusion:* Overall, these findings presented evidence that unveil the role of Hsp70 in affecting ErbB-2 and ErbB-2-mediated drug-resistance of cancer cells.

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P03-S-BRC
REDUCED EXPRESSION OF CANCER-ASSOCIATED SM-LIKE 1 IN HUMAN BREAST CANCER IS ASSOCIATED WITH POOR PROGNOSIS AND ITS REGULATION OF INVASIVENESS OF CANCER CELLS

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Background/Aim: Cancer-associated Sm-like 1 (CaSm1) also known as LSm1 belongs to a family of RNA-binding proteins comprising eight LSm proteins (LSm1-8). LSm proteins are located at both nucleus and cytoplasm and play a role in RNA metabolism. CaSm1 (LSm1) protein forms a complex with other LSm proteins. It was initially identified as a gene over-expressed in human pancreatic cancer. Higher expression of CaSm1 has been subsequently found in other malignancies, such as prostate, breast, lung cancer and mesothelioma, which is related to cancer progression. The current study aims to further expand our understanding of the role played by CaSm1 in breast cancer. *Materials and Methods:* Breast cancer tumour samples (n=111) and normal background tissues (n=30) were collected immediately after surgery and stored at -80oC until use. The clinical follow-up was routinely performed after surgery with a median follow-up period of 120 months by June 2004. Expression of CaSm1 in breast cancer tumour samples was determined using real-time PCR. Anti-CaSm1 ribozymes were designed based on the secondary structure of *CaSm1* mRNA and constructed into a mammalian expression plasmid vector. Knockdown of *CaSm1* in MDA-MB-231 was verified using conventional PCR, real time PCR and Western blot. Functional tests, including cell growth, adhesion, migration and invasion assay were employed to assess the

effect of *CaSm1* knockdown on cellular function of MDA-MB-231 cells. *Results:* CaSm1 expression is reduced in the breast cancer tumours, p=0.025, compared with its expression in the background tissues. It expression appears to be reduced in tumours at more advanced stages according to the TNM staging. Patients with tumours expressing lower level of CaSm1 had shorter overall survival (median=115.1 months), p=0.01, compared with that of patients who had higher expression of CaSm1 (median survival=147.8 months). MDA-MB-231 cells with *CaSm1* knockdown exhibited significantly reduced growth (p<0.001) and migration (p<0.001) compared to the control cells, respectively. Moreover, CaSm1 knockdown resulted in increased invasiveness of the cancer cells, which was associated with an up-regulation of MMP2. *Conclusion:* Reduced expression of CaSm1 in breast cancer is associated with shorter overall survival the patients. Moreover, knockdown of *CaSm1* promotes the invasion of MDA-MB-231 cells but reduces their *in vitro* growth and migration. It suggests that CaSm1-regulated invasion may be involved in the disease progression of the breast cancer, which requires further investigation using both *in vitro* and *in vivo* experimental models.

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P04-S-BRC
TARGETING αB-CRYSTALLIN (CRYAB): A POSSIBLE NOVEL STRATEGY TO IMPEDE TRIPLE-NEGATIVE BREAST CANCER GROWTH

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Background/Aim: Approximately 15-20% of breast cancers are classified as triple-negative and tend to be of an aggressive nature and associated with poor prognosis. There are currently no molecular-based targeted therapies for triple-negative breast cancer (TNBC) making the development of new and improved treatment options for TNBC one of the highest priorities in breast cancer research. Previous therapies include Avastin®, which target vascular endothelial growth factor (VEGF); a stimulator of angiogenesis. Whilst tumour growth inhibition was initially observed, this was commonly followed by tumour regrowth. αB-crystallin (CRYAB), a molecular chaperone of VEGF has been found to be predictive of poor survival in breast cancer patients. Whilst *CRYAB* knockdown studies have shown encouraging results, its role as a cardioprotection adaptive response element indicates directly targeting that may cause harmful side effects. The ability of 3-Methyl Glutamic acid (3-MGA) to block VEGF and CRYAB interaction was revealed to suppress cell proliferation and angiogenesis *in vitro* and tumour growth *in vivo*. This project aims to further investigate the feasibility of 3-MGA as a therapeutic option for TNBC through the synthesis of multiple 3-MGA analogues. *Materials and Methods:* The cytotoxicity of 3-MGA analogues was evaluated in TNBC cell lines (MDA-MB-231 and MDA-MB-436), epithelial breast cell line (MCF10A) and microvascular endothelial cell line (HMVEC-D) with the MTT assay. Suitable MGA analogues were selected for subsequent assays to determine their effectiveness in blocking VEGF and CRYAB using the PathHunter® Enzyme Fragment Complementation cell-based assay platform and ELISA. Additionally, these selected MGA analogues were to be used to treat endothelial cells co-cultured with TNBC cell lines to evaluate its *in vitro* effects, including angiogenesis and cell proliferation. *Results:* Eight of the thirteen MGA analogues had IC_{50} values similar to 3-Methyl Glutamic acid. GA11 was selected as the analogue with the most suitable IC50 value of 53.2±3 μM. *Conclusion:* Targeting of VEGF in TNBC has shown disappointing results, whilst direct interference with CRYAB activity is undesirable due to possible harmful side effects. Approaches to block VEGF and CRYAB activity utilising 3-MGA has shown promising results and the work outlined here aims to identify MGA analogues with similar effects as proof of concept. This will enable progression of this study to preclinical trials and will prove that approaches that interfere with VEGF and CRYAB interaction are viable therapeutic options for TNBC.

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P05-S-BRC
DEVELOPMENT OF CARBON MONOXIDE (CO) AS SYNERGISTIC AGENT TO IMPROVE ANTI-VEGF STRATEGY FOR TRIPLE-NEGATIVE BREAST CANCER

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Background/Aim: For a long time, carbon monoxide (CO) has been best known for its potent toxic effect as an air pollutant because of its strong affinity (>210-fold greater than that of oxygen) for haemoglobin. More recently, CO was found to behave as an important endogenous signalling molecule. Accordingly, CO gas and CO donors, as exogenous CO sources, have been developed and utilized as therapeutic agents for reactive oxygen species (ROS)-related and other inflammatory diseases. ROS are potent carcinogens because they can cause oxidative DNA and protein damage, damage to

tumour suppressor genes and enhance expression of proto-oncogenes. Oxidative stress has been shown to induce malignant transformation of cells in culture. More recently, we have shown that CO suppressed vascular endothelial growth factor (VEGF)-stimulated VEGF receptor 2 phoshorylation, as well as fibroblast growth factor-2 (FGF-2) and VEGF-mediated Akt phosphorylation. Given the anti-VEGF drugs as a few available targeted therapies for triple-negative breast cancer, there remains an urgent and unmet need for improving anti-VEGF therapies that could be done *via* screening existing compound and tailoring to new combinational strategy. *Materials and Methods:* Two triple-negative breast cancer cell lines (MDA-MB-231 and MDA-MB-436) were treated with CO-release compound (CORM-2) at different concentrations that was added to the medium in the presence of bevacizumab (Avastin®). MTT assays were carried out on these cells. The effects of CORM-2 on cell migration was analysed by electric cell-substrate impedance sensing (ECIS). An extracellular flux analyser was used to determine *in vitro* oxygen consumption rate, oxidative phosphorylation, oxidation levels. *Results:* Exposure of the breast cancer cells to CORM-2 resulted in a significant decrease in proliferation in dose-dependent manner compared to Avastin group. ECIS analysis showed that CORM-2 treatment significantly enhanced the inhibitory effects of Avastin on the migration ability of the breast cancer cells. Furthermore, CORM-2 exerted a dephosphorylation effect on VEGFR2 expressed by the breast cancer. Intriguingly, Avastin alone did not exhibit a detectable effect on the VEGFR2 phosphorylation status in the breast cancer cells. By using extracellular flux analyzers, oxidative stress of the breast cancer cells was alleviated by introducing CO into the medium. *Conclusion:* CO exhibited inhibitory effects on triple-negative breast cancer cells progression. To our knowledge, this is the first report showing that CO can act as a synergistic agent for anti-VEGF therapy in triple-negative breast cancer cells. These data point to a potential therapeutic use for CO as a new targeted therapy for triple-negative breast cancer.

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P06-S-BRC
BMP, SMAD AND EMT MARKER EXPRESSION IN EGF-TREATED TRIPLE-NEGATIVE BREAST CANCER CELLS

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Background/Aim: Bone morphogenetic protein (BMP) signalling shares a complex relationship with epidermal growth factor (EGF) resulting in changes to the metastatic properties of cancer cells *via* the coordination of epithelial mesenchymal transition (EMT). The role played by BMPs in triple-negative breast cancer (TNBC) remains largely unknown. Due to the important role played by EGF/EGFR in TNBC cells, this study aims to investigate EGF-regulated BMP signalling and BMP-induced EMT regulators in TNBC cells. *Materials and Methods:* BMP-2, 4, 6 and 7, SMAD1, 3 and 4 and EMT marker (Slug and Snail) expression was analysed by conventional PCR in three TNBC cell lines, MDA-MB-231, BT-20 and BT-549. Cells were treated with a time course of EGF from 0 to 4 hours in one group. In another group, cells were treated with EGF in combinations of the EGFR and HER2 inhibitors Gefitinib and CP724714 (CP), respectively. All cells were treated after overnight serum starvation and cells kept in complete medium were included as additional controls. *Results:* In MDA-MB-231 cells, BMP-2 showed up-regulation with EGF treatment in a time dependant manor, BMP-2 was also up-regulated by EGF + CP and such up-regulation was blocked by the addition of Gefitinib. BMP-4, conversely, showed up-regulation when treated with Gefitinib alone. SMAD1 and 4 were both up-regulated by the addition of CP alone in these cells. Slug and Snail remained unchanged in all treatment groups of MDA-MB-231 cells. In BT-20 cells, BMP-6 was down-regulated upon serum starvation, which was rescued by EGF treatment. Increased expression of SMAD1, 3 and 4 was seen in the BT-20 cells deprived of serum compared with the cells in medium with 10% FCS. Snail was down-regulated by treatment with CP alone and Slug showed relative consistency in all treated BT-20 cells. In BT-549 cells, BMP-4 was up-regulated by the addition of EGF, EGF + Gefitinib and Gefitinib alone. SMAD1, 3 and 4 expression was down-regulated in BT-549 cells when treated with CP alone. Down-regulation of SMAD3 and 4 was also witnessed in these cells upon treatment with EGF + Gefitinib. Snail was down-regulated at 1 hour and 4 hours of EGF treatment, whereas Slug remained consistent in all treated BT-549 cells. *Conclusion:* BMP signalling *via* SMADs can affect EMT, a key part of the metastatic cascade. Our results suggest that EGF can be effective in altering BMP signalling in TNBC cells. However, more work needs to be done to further clarify this relationship.

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P07-S-BRC
THE IMPACT OF TIMM17A ON AGGRESSIVENESS OF HUMAN BREAST CANCER CELLS

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Background/Aim: The mitochondrial protein translocase of inner mitochondrial membrane 17 homolog A (TIMM17A) has been identified as a biomarker of breast cancer. The highest *TIMM17A* mRNA expression has also been found in breast cancer tissues compared to normal. TIMM17A is related to mitochondrial function, which has associated with the pathogenesis of breast cancer. The present study aimed to investigate the biological role of TIMM17A in human breast cancer cells. *Materials and Methods:* A panel of human breast cancer cells were used in the present study. The expression profile of TIMM17A in the cells was evaluated using RT-PCR. We constructed a set of anti- TIMM17A transgenes, which were used to stably transfect breast cancer cells in order to generate *TIMM17A* knockdown cells. The impact of *TIMM17A* knockdown on the migration and invasion were evaluated using the respective cell models. *Results:* The mRNA expression of the *TIMM17A* gene was detected in breast cancer cells, MCF-7, MDA MB-231 and ZR 75-1. These cells were subject to transfection with the TIMM17A ribozymes, followed by established *TIMM17A* knockdown cell lines. Decreased expression of TIMM17A in MCF-7 cells and MDA MB-231 cells resulted in reduction of cell migration using electric cell-substrate impedance sensing compared to vector control ($p<0.05$). It was also found that decrease of TIMM17A expression resulted in reduction of cell invasion compared to vector control ($p<0.05$). *Conclusion:* It is concluded that TIMM17A, a mitochondrial protein highly expressed in cancer, including breast cancer, has a profound impact on the cellular function of breast cancer cells. Decrease of TIMM17A expression is associated with the reduction of the aggressiveness of breast cancer cells. TIMM17A is, therefore, a potential therapeutic target in breast cancer.

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P08-S-BRC
HAVCR1 IS A POTENTIAL PROGNOSTIC FACTOR IN HUMAN BREAST CANCER

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Background/Aim: HAVcR1, also known as TIM-1 and KIM-1, is a molecule that acts as a cellular receptor for hepatitis A and has also been indicated in atopic and allergic diseases, as well as in kidney regeneration. The recent discovery that HAVcR1 is the same as the Rho Guanine exchange factor-5 (RHOGEF5) suggests that it may also play a role in cancer. The current study examined the expression pattern of HAVcR-1 in human breast cancer and its potential link with clinical outcome. *Results:* The HAVcR1 protein was found in normal mammary epithelial cells but more prominently in breast cancer cells from tumour tissues. Using quantitative analysis of the *HAVcR1* transcript, it was revealed that breast tumour tissues (n=121) expressed significantly higher level that normal tissues ($p=0.0099$). Node positive tumours and tumours from patients with predicted prognosis had significantly higher levels than node negative tumours and patients with good prognosis ($p=0.004$ and $p=0.02$, respectively). Patients with metastasis and patients who died of breast cancer had significantly higher levels of HAVcR1 compared with patients who remained disease-free over a 10-year follow-up ($p=0.027$ and $p=0.0098$, respectively). Survival analysis has further revealed a significant relation between high level of HAVcR1 and poor overall survival (103.7 (77.4-129.7) months for those with high level of HAVcR1 *vs*. 140.6 (131.6-149.6) months, $p=0.0057$), as well as disease-free survival (100.1 (73.1-127.0) months *vs*. 133.0 (123.9 *vs*. 144.1) months, $p=0.0279$). *Conclusion:* The current study has revealed that HAVcR1 is potentially involved in the development and progression of human breast cancer. High levels of HAVcR1 are strongly linked to poor clinical outcome. HAVcR1 is, therefore, a potential prognostic factor and therapeutic target in human breast cancer.

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P09-S-BRC
SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-METHYL GLUTAMIC ACID ANALOGUES ACTING AS INHIBITORS OF THE INTERACTION BETWEEN αB-CRYSTALLIN/VEGF165 FOR TRIPLE-NEGATIVE BREAST CANCER TREATMENT

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Background/Aim: Triple-negative breast cancer (TNBC) is characterized by the lack of oestrogen receptors, progesterone

receptors and human epidermal growth factor receptor 2 (HER-2). Thus, common treatments like hormone therapy and drugs that target oestrogen, progesterone and HER-2 are ineffective. Recent studies reported the high presence of αB-crystallin (CRYAB) in TNBC. CRYAB binds to and corrects the unfolded/misfolded vascular endothelial growth factor (VEGF) under conditions of cellular stress of tumour microenvironment. CRYAB may be a key component in the activation of the intracrine VEGF pathway by protecting VEGF from intracellular degradation in the tumour endothelial cells. Therefore, focusing on the disruption of these two proteins (CRYAB and VEGF) could be a potential new targeted therapy for TNBC. The present communication will report the design, synthesis and the growth inhibition of several compounds, which have been designed to target the binding of these two proteins. *Materials and Methods:* Using structure-based molecular docking of CRYAB, it has been found that 3-methyl glutamic acid can block the interaction between CRYAB and $VEGF_{165}$. This compound has two acid groups and an amine functional group, which can reduce the bioavailability of the drug in the organism. For this reason, 12 analogues of the 3-methylglutamic acid have been designed and synthesized. These new derivatives are based on the conversion of the amine and acid functional group to an amide and ester protecting groups. These analogues could work as pro-drugs that should show enhanced cell permeation compared to the lead compound. In order to determine the growth inhibition of the synthesized compounds, the MTT assay in 4 different cell lines, 2 TNBC (MDA-MB-231 and MDA-MB-436) and 2 healthy cell lines (HMVECd and MCF10A), has been employed. *Results:* Eleven of the 12 designed analogues and also 2 more compounds have been synthesized, which are sub-products of some of the reactions that have been carried out. Comparing the cytotoxicity of the 13 compounds to the lead compound, there are 6 compounds that inhibit the MDA-MB-231 cell growth more than the 3-methyl glutamic acid; however, in MDA-MB-436 cells, the most cytotoxic drug is the lead compound itself. *Conclusion:* Taking into account the IC50 of the healthy cells, at least 2 compounds have been found that can be eligible for further evaluation. These two compounds inhibit more the growth of TNBC cells than in the healthy cell lines and are more cytotoxic than the lead compound in the MDA-MB-231 cell line. The following step will be to evaluate if there is a reduction in the levels of VEGF gene expression, VEGF protein and tube formation in the endothelial cell line HMVECd.

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P10-S-BRC
POTENTIAL ROLE OF FOLLISTATIN IN BREAST CANCER PROGRESSION AND BONE METASTASIS

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Background/Aim: Follistatin (FST), an activin and bone morphogenetic protein (BMP) antagonist, plays an integral role in embryogenesis and postnatal development, with studies demonstrating disrupted growth, diminished muscle mass and respiratory distress in FST null mice. Interestingly, whilst *FST* was found to be among the genes over-expressed in the bone metastasis signature, it has also been shown to suppress the production of experimental metastasis. This seems to correlate with quantitative real time PCR and immunohistochemistry (IHC) analyses of breast cancer and normal background tissues performed in our laboratory, which demonstrated lower *FST* transcript levels in more advanced and aggressive breast cancers. This suggests FST as a possible negative regulator of tumour growth and breast cancer progression. The current study aims to ascertain the role of FST in breast cancer-related bone metastasis. *Materials and Methods:* Breast cancer cell lines, MDA-MB-231, MCF-7, ZR-751, BT-549 and BT-20 were screened for the expression of *FST* variants, *FST344* and *FST317* and their cognate BMPs, by RT-PCR. Over-expression cell models were created by amplifying the *FST344* coding sequence from human prostate tissue cDNA, whilst knockdown cell models were generated using ribozyme transgenes based on the secondary structure of *FST344* mRNA. The *FST344* coding sequence and ribozyme transgene products were both cloned into the pEF6/V5-His TOPO TA vector and transfected into suitable breast cancer cell lines. Verification of over-expression/knockdown was then assessed by qPCR and growth and invasion assays were performed to assess the effect on cell function. *Results:* Screening results demonstrated higher levels of BMP-4 in MCF-7, MDA-MB-231 and ZR-751 cells, whilst BMP-2 and BMP-6 showed higher levels in MDA-MB-231 cells. BMP-7 was highly expressed in MCF-7 cells only. Since MCF-7 also demonstrated very low *FST344* transcript levels, this cell line was opted for transfection with the generated *FST344* over-expression plasmid and the BMP7 ribozyme transgene (BMP7 Rib 1). Additionally, MDA-MB-231 cells, which expressed high levels of *FST344*, were opted for the knockdown of FST. MCF-7 BMP-7 Rib 1 cells and MDA-MB-231 FST Rib 2 cells demonstrated successful knockdown and MCF-7 FST344 cells showed successful *FST344* and *FST317* over-expression. Functional assays resulted in a significant increase in cell growth in MCF-7 FST344 cells and decrease in invasion ($p<0.01$). *Conclusion:* FST increases *in vitro* growth of breast cancer cells but has an inhibitory effect on the invasion of the cells tested. This suggests a role for FST in early tumour growth, which can be different in cancer progression.

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P11-S-BRC
REGULATORY ROLE OF IDO1-TSP1 LINK IN BREAST CANCER DORMANCY

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Background/Aim: Metastasis is responsible for breast cancer relapse many years or even decades after treatment. This occurs because breast cancer cells that migrate and invade distant sites remain dormant and undetectable for a long period of time. Thrombospondin 1 (TSP1), an extracellular protein, is secreted by endothelial cells (ECs) and surrounds cancer cells, inducing their growth arrest. On the other hand, breast cancer cells over-express indoleamine 2,3-dioxygenase (IDO1), which can degrade intracellular L-tryptophan, a key amino acid of TSP1. IDO1 has also been reported in breast cancer exosomes. We hypothesize that a crosstalk between breast cancer and endothelial cells (ECs) modulates cancer dormancy *via* a possible IDO1-TSP1 link, leading to a gradual reduction in TSP1, thus abolishing its inhibitory effect on cancer growth. *Materials and Methods:* The proliferation of MDA-MB 231 cells was determined by flow cytometry (ki-67 marker) when cells were cultured alone or co-cultured with ECs. IDO1 expression and exosome secretion were analysed by Western blot and Nanosight technology, respectively, in MDA-MB 231 cells cultured with standard medium or low glucose medium (1 mM D-glucose). ECs were also co-cultured with non-malignant breast cells (MCF10A) or with malignant breast cancer cells (MCF7 and MDA-MB 231) and IDO1, TSP1 and Ang2 (angiogenesis marker) expression were assessed in the ECs by Western blot. Angiogenesis was assessed by a Matrigel assay under different treatments: (i) full conditioned medium from MDA-MB 231 cells; (ii) exosome depleted conditioned medium (centrifuged at 100,000 x g for 1h 30 min); or (iii) the re-suspended pellet from the exosome depleted medium. *Results:* Co-culturing MDA-MB 231 cells with ECs inhibited their proliferation. IDO1 expression and exosome secretion were significantly increased under low glucose conditions. MDA -MB 231 cells induced an increase in IDO1 expression in ECs and a decrease in TSP1 protein level, along with an increase in Ang2. Full conditioned medium from MDA-MB 231 cells promoted tubule formation but there was no difference between exosome depleted conditioned medium (no exosomal IDO1) and the control group. *Conclusion:* Taken together, our data suggest that breast cancer cells might up-regulate IDO1 in ECs through the secretion of exosomes whilst under stress conditions in order to disrupt the inhibitory effect of TSP1.

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P12-S-BRC
IL-21 PLAYS A DIRECT ROLE IN MIGRATION AND INVASION OF BREAST CANCER CELLS

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Background/Aim: Interleukin 21 (IL-21) is a cytokine produced predominantly by CD4+ T cells and natural killer T (NKT) cells. IL-21 has been implicated in various immunological processes but its roles in the pathogenesis of cancer are diverse depending on cancer types. We herein investigated the expression of IL-21 receptor and the effect of IL-21 on breast cancer cells. *Materials and Methods:* We evaluated the expression of IL-21 receptor (IL-21R) in breast cancer cells and prostate cancers by RT-PCR, sequencing and Western blotting. We investigated the effect of recombinant human IL-21 on behaviours of IL-21R^{+} breast cancer cells using migration, invasion and growth assays. *IL-21R* was knocked down using Dharmacon siRNA SMART^{pool} reagents for functional studies. *Results:* The expression of IL-21R was stronger in MDA-231, weaker in MCF7 and negative in ZR-75.1 cells, which were demonstrated by the data from RT-PCR, Western blotting and sequence analysis. The invasion and migration capacity of IL-21R^{high} MDA-231 cells was enhanced by IL-21 in a dose-dependent manner. After *IL-21R* was knocked down with 100 nM siRNA reagent, the response of MDA-231 to the treatment of IL-21 was attenuated. It appeared that IL-21 had no significant effect on proliferation of MDA-231; however, siRNA silencing of *IL-21R* suppressed cell proliferation spontaneously. *Conclusion:* IL-21 promotes migration and invasion of breast cancer cells that express IL-21R strongly. It appears that IL-21R is involved in the tumour cell growth spontaneously, although the underlying mechanism remains to be further investigated.

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P13-S-BRC
OSTEOCYTES HAVE AN EFFECT ON MIGRATION, PROLIFERATION AND INVASION OF BREAST AND PROSTATE CANCER CELLS

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Background/Aim: Most of breast and prostate metastatic cancers metastasize to the bone, which leads to the majority of cancer-related deaths. Osteocytes constitute over 90% of adult bone cells. Osteocytes orchestrate bone re-modelling through determining osteoclast activity and affecting osteoblasts.

Osteocytes are enriched in hypoxia-resistant proteins and apoptotic osteocytes recruit osteoclasts to initiate targeted bone resorption. The osteocyte lacuno-canalicular network is also intimately associated with the blood vessel network in the bone matrix. We, therefore, investigated the roles of osteocytes in behaviours of breast and prostate cancer cells. *Materials and Methods:* Conditioned medium (CM) was collected from MLO-Y4 osteocytic cells following culture for 2 days. Cell migration and wound healing were monitored on the electric cell-substrate impedance sensing (ECIS) system. Invasion of cancer cells *in vitro* was measured as the capacity of cells to pass through a Matrigel-coated transwell insert (8-um pore size). Cell proliferation was determined using the alamarBlue® assay. *Results:* CM from both monolayer- and 3D-cultured osteocytes stimulated proliferation of DU145 ($p<0.01$) and PC3 ($p<0.01$) prostate cancer cells but not LNCaP cells compared to control medium. Osteocyte CM also stimulated proliferation of MDA231 ($p<0.01$) and MCF7 ($p<0.01$) breast cancer cells. Osteocyte CM inhibited migration and wound healing capacity of oestrogen receptor positive (ER^+) MCF7 ($p<0.01$) and ZR-75.1 ($p<0.01$) breast cancer cells but not MDA-MB-231 cells, which are triple negative. However, in prostate cancer cells, CM from osteocytes promoted the migration and adhesion capacity of PC3 ($p<0.01$) and DU145 cells ($p<0.05$) but had reverse effect on PZHPV7, a normal prostate epithelial cell line. Moreover, osteocyte CM stimulated transwell chemotactic migration of MDA231 cells instead of MCF-7 and ZR-75.1 cells. *Conclusion:* CM from osteocytes has diverse effect on behaviours of breast cancer cells and prostate cancer cells. This may be associated with cancer-specific metastatic progress. In particular, breast cancer metastasis tends to be more osteolytic, while prostate cancer metastasis can be more osteoblastic. Further study will be focused on the mechanisms of deregulated function of osteocytes in the tumour microenvironment.

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P14-S-BRC
IMPEDING OUTGROWTH OF CANCER CELLS BY INTERFERING THE INTERPLAY BETWEEN PSMA AND MDM2

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Background/Aim: Both mouse double minute 2 homolog (MDM2) and prostate-specific membrane antigen (PSMA) play integral roles in angiogenesis and metastasis in many cancer types. Furthermore, the expression of both proteins has been linked to vascular endothelial growth factor (VEGF) and hypoxia-induced angiogenesis. MDM2 is heavily implicated in hypoxia, with an ability to bind to HIF1α in a p53-independent manner and, therefore, increase the expression of VEGF and other hypoxia-related genes. *In vivo* studies of mice show elevated expression of PSMA in hypoxic regions of a tumour mass. Also, a recent study shows that *PSMA* silencing leads to decreased expression of MDM2. We, therefore, investigated the hypothesis that the tumour-cell outgrowth and tumour-associated angiogenesis can be halted by targeting the signalling pathways that mediate the interplay between MDM2 and PSMA. *Materials and Methods:* Knock down of *PSMA* and *MDM2* was performed using siRNA, ribozymes or CRISPR plasmids. Over-expression of the two genes was undertaken using pEF6/V5-His TOPO-PSMA and MDM2p-Mdm2-YFP plasmids, respectively. Gene expression was determined by qRT-PCR. Cell migration and wound healing were monitored on the electric cell-substrate impedance sensing (ECIS) system. Cell proliferation was determined using the alamarBlue® assay after treatment for 72 hours. *Results:* Ribozymes did not knock down gene expression of *MDM2* in MDA-MB-231 and MCF7 breast cancer cells. However, treatment of cells with MDM2 ribozymes resulted in down regulation of PSMA in MCF7 cells. Gene silencing of *PSMA* by siRNA resulted in reduction of MDM2 expression compared to scrambled siRNA control ($p<0.0001$). Interestingly, siRNA silencing of *MDM2* in breast cancer cells also led to down-regulation of *PSMA* ($p<0.001$). This striking phenomenon was also observed in LNCAP prostate cancer cells, which are $PSMA^+$. Transfection of siRNA did not cause an adverse effect on cell proliferation. *Conclusion:* Our data indicated for the first time that there is a signalling pathway to connect PSMA and MDM2. This signalling pathway may control both proteins' involvement in angiogenesis and affect activation of downstream targets, such as VEGF and HIF-1, which remains to be investigated further. The relative difficulty of *MDM2* knockdown in cancer cells may be due to the autoregulatory positive feedback loop between p53 and MDM2.

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P15-S-BRC
FALSE-NEGATIVE FROZEN SECTION OF SENTINEL LYMPH NODE BIOPSY IN A CHINESE BREAST CANCER POPULATION

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Background/Aim: The biggest drawback of a frozen section (FS) of sentinel lymph node biopsy (SLNB) is the false-negative result, which necessitates patient for reoperations. This study aimed to investigate the accuracy of FS in

diagnosis of SLN metastases and analyse the predictive factors for false-negative patients. *Patients and Methods:* Patients with operable breast cancer and clinically negative axillary were recruited for SLNB. The SLNs were identified by a combination of blue dye and isotope. All nodes were examined by intra-operative FS and underwent further paraffin sectioning. *Results:* A total of 1,219 patients received SLNB over a 7-year period. Of these patients, 283 had nodal metastases on FS. A total of 45 patients had false-negative FS (false-negative rate=13.72%) and 71.11% of metastases were less than 2 mm in size. Univariate analysis revealed that age, stellate mammographic pattern, oestrogen receptor (ER) and progesterone receptor (PR) status were statistically different when compared to the 52-patient cohort who was negative for SLNB ($p<0.05$). ER status remained significant on multivariate analysis ($p=0.005$). After clinical follow-up, recurrence-free survival rates of 45 false-negative SLNB patients did not differ from control group ($p=0.057$), while false-negative patients had a poorer overall survival ($p=0.029$). *Conclusion:* FS was useful for the detection of nodal metastases in the SLNs. The main failure of FS was in the detection of micro-metastases. Univariate analysis revealed that age, stellate mammographic pattern, ER and PR status were predictors for FS false-negative patients. ER status remained significant on multivariate analysis. Axillary lymph node dissection (ALND) can be avoided in most patients with intraoperative, false-negative SLNB.

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P16-S-BRC
THE PERIPHERAL BLOOD NEUTROPHIL-TO-LYMPHOCYTE RATIO IS BETTER THAN THE LYMPHOCYTE-TO-MONOCYTE RATIO FOR PREDICTING THE LONG-TERM SURVIVAL OF TRIPLE-NEGATIVE BREAST CANCER PATIENTS

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Background/Aim: The peripheral hematologic parameters of patients with breast cancer display prognostic value, although their value has not been studied in different molecular subtypes of breast cancer. We investigated the prognostic significance of the neutrophil-to-lymphocyte ratio (NLR) and the lymphocyte-to-monocyte ratio (LMR) in different molecular subtypes of breast cancer. *Patients and Methods:* A retrospective cohort of 1,570 operable breast cancer patients was recruited between January 2000 and July 2010. The counts of peripheral neutrophils, lymphocytes, monocytes and platelets were collected and used to calculate the NLR and the LMR. Univariate and multivariate Cox proportional hazard analyses were applied to evaluate the associations of the NLR and the LMR with disease-free survival (DFS) and overall survival (OS). *Results:* Univariate analysis revealed that lower NLR (≤2.0) and higher LMR (>4.8) were significantly associated with superior DFS in all patients (NLR, $p=0.005$; LMR, $p=0.041$) and in the triple-negative breast cancer (TNBC) patients (NLR, $p=0.007$; LMR, $p=0.011$). However, multivariate analysis showed that only lower NLR was a significant independent predictor of superior DFS and OS in all breast cancer patients (DFS, hazard ratio (HR)=1.50 95% confidence interval (CI)=1.14-1.97; OS, HR=1.63, 95% CI=1.07-2.49) and in TNBC patients (DFS, HR=2.58, 95% CI=1.23-5.42; OS, HR=3.05, 95% CI=1.08-8.61). Both univariate and multivariate analysis showed that neither the NLR nor the LMR significantly predicted DFS and OS among the patients with other molecular subtypes of breast cancer. *Conclusion:* An elevated pre-treatment peripheral NLR significantly and independently indicated a poor prognosis for breast cancer and TNBC and this measurement displayed greater prognostic value than a reduced LMR. The NLR was not a prognostic factor for other breast cancer subtypes.

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P01-S-CRC
DOK7 VARIANT EXPRESSION AND FUNCTION IN HUMAN COLORECTAL CANCER

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Background/Aim: The downstream of tyrosine kinase (DOK) protein family has 7 members, DOK1-7. The most recently identified member of this family, DOK7, is hypermethylated in breast cancer, even pre-diagnosis, suggesting a potential tumour suppressor role and raising the possibility that it may be used as a biomarker. However, there is no published work investigating its role in colorectal cancer. This study aimed to ascertain the expression of DOK7 in colorectal cancer, to determine its effect on colorectal cancer cell function and to establish if there is an association between DOK7 expression and clinical outcome. *Materials and Methods:* DOK7 expression in human colorectal cancer was analysed using PCR in three human colorectal cancer cells lines (RKO, HT115 and HRT18). Q-PCR analysis was used to analyse tissue obtained

from a cohort of patients with colorectal cancer (tumour=94; normal colorectal tissue=80). Patients were followed up clinically, radiologically and endoscopically with a median follow-up time of 65 months. Transfected knockdowns and over-expressing cells were created using electroporation. *Results:* DOK7 has several isoforms encoded by different transcript variants. The expression of DOK7 variants 1, 2 and 3 has been characterised in both the colorectal cancer cell lines and in the clinical cohort: DOK7V1 expression is significantly reduced in colorectal cancer tissue compared with normal colorectal tissue and appears to be related to T stage. By comparison, the expression of both DOK7V2 and DOK7V3 is significantly increased in colorectal cancer compared with normal colorectal tissue. *Conclusion:* DOK7 expression in colorectal cancer is complex and our results suggest that different variants play different roles in the development and progression of human colorectal cancer. Current work has focused on investigating the effect of DOK7V1 and DOK7V2 on cell function and its involvement in cell signalling pathways.

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P02-S-CRC
NOV IS CORRELATED WITH DISEASE PROGRESSION OF COLORECTAL CANCER AND HAS A ROLE IN THE REGULATION OF CELL PROLIFERATION AND INVASION

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Background/Aim: NOV (Nephroblastoma OVerexpressed) belongs to the CCN family and plays an important role in certain solid tumours. However, the role played by NOV in colorectal cancer (CRC) remains unclear. In the current study, we analysed the relationship between NOV expression and the disease progression of CRC and investigated the functions of NOV in CRC cells. *Materials and Methods:* The expression of NOV in a cohort of 259 CRC tissues and 174 normal colorectal tissues was determined using real time PCR and the association with the pathological Dukes' stage was analysed using SPSS statistics. The expression of NOV was determined in four colorectal cancer cell lines. *NOV* over-expression and knockdown was established in HT115 and RKO cells using NOV over-expression and anti-NOV ribozyme plasmid vectors, respectively. *NOV* mRNA and protein expression in cells were subsequently determined by RT-PCR and Western blot analysis. Functional tests included cell proliferation, adhesion, invasion and migration that were employed to assess the impact of *NOV* on these cellular functions of the colorectal cancer cells. A flow cytometric method was employed to analyse the effect on apoptosis of CRC with modified expression of *NOV*. Alteration of caspases was determined using RT-PCR. *Results: NOV* transcripts were found at lower levels in the CRC samples compared to the paired normal colorectal tissues ($p<0.01$). Lower levels of *NOV* transcripts were seen in patients with distant metastasis compared to that of patients remained disease-free ($p<0.05$). NOV expressed at lower levels in patients at the most advanced stage (Dukes' stage D) compared to Dukes' stage A, B and C ($p<0.01$). Knock down of *NOV* increased cell proliferation and invasion of RKO cells, whilst an opposite effect was seen in the HT115 NOV over-expressing cells. The alterations of *NOV* expression, neither over-expression nor knockdown, exhibited an effect on adhesion and migration of the colorectal cancer cells examined. *NOV* knockdown resulted in a decreased apoptotic population in RKO cells in which a reduced expression of Caspase-8 was also observed, whilst an increase was seen in the HT115 cells with *NOV* over-expression. *Conclusion:* NOV is down-regulated in CRC tumours, which is associated with the disease progression. NOV inhibits the proliferation and invasion of CRC cells *in vitro*. The inhibition of cell growth is mediated by regulating extrinsic signalling pathway for apoptosis *via* Caspase-8. It suggests that NOV is a putative proliferation and metastasis suppressor molecule in CRC.

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P03-S-CRC
VILIP-1 PLAYS A SUPPRESSIVE ROLE IN THE MOTILITY OF COLORECTAL CANCER CELLS AND THE INHIBITION INVOLVES THE REGULATION OF MMPS *VIA* JNK SIGNALLING PATHWAY

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Background/Aim: VILIP-1 is suggested as a novel metastasis suppressor for several human solid tumours, including squamous cell carcinoma. However, the role of VILIP-1 in colorectal cancer cells remains largely unknown. Therefore, the aim of this study was to investigate the role and signal transduction of VILIP-1 in colorectal cancer cells. *Materials and Methods:* Two colorectal cancer cells were chosen

(HT115 and RKO). Based on the high *VILIP-1* mRNA expression levels screened in HT115 and the low levels in RKO, sublines of cancer cells with differential expression of *VILIP-1* were created, using ribozyme transgenes to knock down and over-express the expression of *VILIP-1* in HT115 and RKO cells, respectively. The stabilized transfected cells were used to deduce the effect of VILIP-1 on the function of colorectal cancer cells compared with the control (pEF cells) using functional assays (growth, adhesion, wound healing and invasion assays) and electric cell-substrate impedance sensing (ECIS) assay. To further explore the signalling pathway downstream of VILIP-1, ERK and JNK inhibitors were also used in HT115 *VILIP-1* knockdown cells. The influence of VILIP-1on MMP-9 and MMP-2 were detected using by a zymography assay. *Results:* VILIP-1significantly inhibited the invasion and migration of colorectal cancer cells ($p<0.05$), while the effect of KISS-1 on the growth and adhesion of colorectal cancer cells did not show significant difference in both HT115 and RKO cells compared with the control cells ($p>0.05$). The increased enzyme activity of MMP-2 was caused by *VILIP-1* knockdown and the reduction was caused by JNK inhibitor, shown by a zymography assay. *Conclusion:* The present study has demonstrated that VILIP-1 may play a pivotal tumour suppressor role in colorectal cancer cells. The inhibitory effect on motility of cancer cells involves the regulation of MMP-2 *via* JNK signalling pathway.

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P04-S-CRC
VALIDATION OF THE MSKCC NOMOGRAM TO PREDICT OVERALL SURVIVAL AFTER CURATIVE COLECTOMY IN A CHINESE COLON CANCER POPULATION

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Background/Aim: Colon cancer nomogram designed by Memorial Sloan-Kettering Cancer Centre (MSKCC) is an online prediction tool to predict overall survival for individual patient after curative resection. However, this model was never externally validated. We evaluated the accuracy of this nomogram in an independent external Chinese cohort. *Materials and Methods:* Clinical data from 1,005 patients who underwent primary curative-intent surgery at Peking University Cancer Hospital and Institute between 1996 and 2008 were used for external validation. Clinicopathological characteristics and the performance of the MSKCC nomogram for prediction of overall survival were evaluated for 985 patients with complete data by using concordance index (C-index) and calibration plot. *Results:* The C-index for the MSKCC nomogram was 0.71 in the Chinese cohort, compared with 0.67 for the American Joint Committee on Cancer (AJCC) stage ($p<0.0001$). This suggests that the nomogram discriminates overall survival better than the AJCC staging system. Calibration plot showed a good calibration of the nomogram in the validation cohort. Furthermore, the MSKCC nomogram prediction illustrated the heterogeneity for survival of Chinese patients within each AJCC stage. *Conclusion:* The MSKCC nomogram for colon cancer provides more accurate survival predictions than the AJCC staging system when applied to an external Chinese cohort. The MSKCC nomogram improved individualized prediction of survival and, thus, may aid in more accurate patient counselling, selection of various treatment options and follow-up scheduling.

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P01-C-GC
CIRCULATING CHROMOGRANIN A AS A MARKER FOR MONITORING RESPONSE IN ADVANCED GASTROENTEROPANCREATIC NEUROENDOCRINE TUMOURS TREATED WITH IRINOTECAN PLUS CISPLATIN

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Background/Aim: Irinotecan plus cisplatin (IP) regimen was first reported to be effective and well-tolerated in advanced gastroenteropancreatic neuroendocrine tumours (GEP-NETs) in our previous study, and circulating chromogranin A (CgA) was reported to be a marker for diagnosis of GEP-NETs. This study was aimed to investigate the role of Chromogranin A (CgA) for monitoring response of advanced GEP-NETs treated with IP regimen. *Patients and Methods:* Eighty patients with advanced GEP-NETs treated in Peking University Cancer Hospital from September 2011 to May 2014 and 65 healthy individuals were included in this study. Serum CgA levels were analyzed for relationship with patient's characteristics and clinical outcome. *Results:* The median CgA levels were significantly higher in patients with

advanced GEP-NETs than in healthy individuals (93.75 ng/ml *vs*. 37.1 ng/ml; $p<0.01$), as well as significantly higher in patients with carcinoid syndrome or liver metastasis than in those without carcinoid syndrome (298.8 ng/ml *vs*. 82.9 ng/ml; $p=0.011$) or liver metastasis (137.0 ng/ml *vs*. 64.4 ng/ml; $p=0.023$). A CgA cut-off value of 46.15ng/ml was used in this study with sensitivity 78.8% and specificity 73.8%, respectively. Serial CgA levels could indicate the clinical response of chemotherapy with IP regimen. Patients with CgA levels higher than 46.15 ng/ml had a poorer survival than patients with CgA levels lower than 46.15 ng/ml ($p=0.048$). *Conclusion:* Serum CgA level functions as a potential marker for monitoring the response in patients with advanced GEP-NETs treated with IP regimen, which would be validated in future larger cohorts.

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P02-C-GC
POST-OPERATIVE IMATINIB IN PATIENTS WITH INTERMEDIATE RISK GASTROINTESTINAL STROMAL TUMOUR - A MULTICENTER RETROSPECTIVE CONTROLLED STUDY

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Background/Aim: Imatinib adjuvant therapy improves recurrence-free survival (RFS) in patients with gastrointestinal stromal tumour (GIST) despite the associated high recurrence risk. Nevertheless, it remains uncertain whether imatinib adjuvant treatment is effective in GIST patients with an associated intermediate risk. This study aimed to determine whether adjuvant treatment with imatinib improved RFS in cases of GIST with intermediate risk. *Patients and Methods:* Patients that had undergone complete tumour resection with intermediate risk of recurrence followed by imatinib adjuvant therapy at least one year or only observation were enrolled in this multi-centre, controlled, retrospective study. RFS and safety of imatinib adjuvant therapy were subsequently evaluated. *Results:* Of 192 patients, 99 made the adjuvant group and 93 patients were in the control group. Median follow-up was 39.0 months (95% confidence interval (CI)=35.0-43.1). Kaplan-Meier analysis showed RFS rates at 1, 2 and 3 years to be higher in the adjuvant group than in the control group (100% *vs*. 98.9% in 1st year; 100% *vs*. 96.1% in the second and 98.2% *versus* 90.2% during the 3rd year) ($p=0.004$). Subgroup analysis revealed that imatinib adjuvant therapy had significantly improved the RFS in GIST patients of intestinal or rectal location ($p=0.009$) and with c-kit exon 11 deletion mutation ($p=0.039$). Multivariate analysis of RFS showed stomach location was significantly associated with good RFS. Gastric GIST reduced recurrence risk by 85.0% (hazaed ratio (HR)=0.150, $p=0.034$) compared with intestinal or rectal GIST. Imatinib adjuvant therapy was well tolerated. *Conclusion:* Adjuvant imatinib improves the level of RFS in cases of GIST with an intermediate risk of recurrence after complete tumour resection, particularly observed in GIST with intestinal and rectal location or c-kit gene exon 11 deletion mutation.

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P03-C-GC
SERUM MICRORNAS PROFILE PREDICT POSTOPERATIVE CHEMOTHERAPY RESPONSE IN GASTRIC CANCER PATIENTS AFTER SURGICAL TREATMENT

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Background/Aim: Adjuvant chemotherapy after surgery is an important component in gastric cancer patients' treatment. However, some patients do not benefit from adjuvant chemotherapy due to chemotherapy resistance. Furthermore, a set of biomarkers that can discriminate patients with good outcomes from those with poor outcomes after adjuvant chemotherapy has not been established. We aim to develop a practical serum microRNA-based profile that can predict the effectiveness of adjuvant chemotherapy for gastric cancer after surgical treatment. *Materials and Methods:* Microarray technologies were used to screen a group of miRNAs related to chemotherapy sensitivity from gastric cancer cell SGC7901 and SGC7901/DDP. The association of expression patterns of identified serum microRNAs with overall survival was confirmed in 68 gastric cancer patients. Furthermore, we also validated the serum microRNAs signature in an independent cohort of 60 gastric cancer patients. *Results:* Among screening phase by microarray, 11 miRNAs were up-regulated more than 2-fold in SGC7901/DDP cell line, while 18 miRNAs were less than the threshold level (0.5-fold) set. Through the bioinformatics analysis, we found that miR-15a, miR-15b and miR-93 were the most valuable down-regulated miRNAs in SGC7901/DDP, while the miR-27a, miR-106a and miR-664 were the most valuable up-regulated miRNAs. Then, we confirmed that only serum miR-106, miR-15a, miR-93 and

miR-664 could predict prognosis of patients who received postoperative chemotherapy. We identified a four-serum microRNA signature (miR-106, miR-15a, miR-93 and miR-664) as a risk score for overall survival in gastric cancer patients who received surgery and adjuvant chemotherapy. The higher the risk score, the worse the prognosis ($p<0.05$). In the independent cohort of gastric cancer patients, the predictive value of this four-microRNAs signature also showed that high risk score means poor prognosis. *Conclusion:* Our risk score derived from four serum microRNAs was closely associated with overall survival in gastric cancer patients after surgery and adjuvant chemotherapy. This prognostic risk score could be applicable to guide individual chemotherapeutic regimen.

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P01-S-GC
ACTIVATION OF PI3K/AKT SIGNALLING PATHWAY IN GASTRIC CANCER THROUGH ACTIVE SPHINGOSINE KINASE-1

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Background/Aim: Sphingosine kinase 1 (SphK1) is a biologically active lipid, which plays a significant role in growth, survival, migration of cell, as well as in anti-apoptosis and it has been shown to enhance cell immortalization. SphK1 is involved in one of crucial signalling pathway, S1P signalling pathway, which can catalyse sphingosine phosphorylates to S1P in an ATP-dependent manner. S1P is a lysophospholipid that has been implicated as an important regulator in many physiological, pathophysiological and cancer processes. Expression of SphK1 has been linked with tumour progression. However, there is little research focusing on its potential function in gastric cancers, as well as the possible signalling pathway in which SphK1 is involved. The present study is to examine the effect of Sphk1 on a gastric cancer cell line, its clinical significance in gastric cancer progression together with the prediction that SphK1 is a potential pharmacologic target. *Materials and Methods:* The correlation of Sphk1 expression and clinical features of gastric cancer was studied *via* immunohistochemistry analysis of 263 paraffin-embedded archived gastric cancer specimens and quantitative RT-PCR analysis of 515 fresh gastric tumour tissues. The cellular functions of Sphk1 were examined using *in vitro* knockdown of *Sphk1* in gastric cancer cell lines. Functional tests, including cell growth, adhesion, invasion and cell cycle were conducted. Western blotting was performed to examine the impact of Sphk1 on the PI3K/AKT signalling pathway. *Results:* Strong immunostaining of Sphk1 was detected in gastric cancer tissues, compared with normal gastric tissues ($p<0.01$). *Sphk1* mRNA levels in gastric cancer tissues were significantly elevated when compared with their paired adjacent noncancerous tissues ($p=0.030$). Over-expression of Sphk1 was positively associated with depth of invasion, lymph node metastasis, distant metastasis and TNM stage ($p<0.01$). Kaplan-Meier survival curves revealed that patients with Sphk1 positive expression had a significant decrease in overall survival (OS) and progress-free survival (PFS) in gastric cancer patients ($p<0.001$). Multivariate analysis indicated the expression of Sphk1 was an independent prognostic factor in gastric cancer patients ($p<0.001$). *In vitro* experiments showed that knockdown of *Sphk1* in AGS gastric cancer cells resulted in a decrease in cell proliferation (2-fold), invasion (2-fold) and migration (1.5-fold). Inhibiting Sphk1 expression could lead to AGS cell apoptosis. Furthermore, we proved that silencing *Sphk1* could result in de-phosphorylation of AKT, indicating that Sphk1 might enhance the gastric tumour cell survival through PI3K/AKT signalling pathway. *Conclusion:* Our results demonstrated that the inhibition of Sphk1 expression and/or its kinase activity could down-regulate PI3K/AKT survival signalling pathway, leading to apoptosis of gastric cancer cells, suggesting that Sphk1 might be a potential novel target for the treatment of gastric cancer.

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P02-S-GC
PI3K/AKT/mTOR PATHWAY IS ACTIVATED AFTER IMATINIB SECONDARY RESISTANCE IN GASTROINTESTINAL STROMAL TUMOURS (GISTs)

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Background/Aim: Phosphatidyl-inositol-3-kinase (PI3K)/AKT/mammalian the target of the rapamycin (mTOR) pathway activation may be related to imatinib resistance; however, no study has focused on whether signal conduction of this pathway will change after imatinib resistance. *Patients and Methods:* A total of 111 gastrointestinal stromal tumour (GIST) samples from 91 patients were used in this study, including 20 pairs of samples before and after imatinib treatment. Immunohistochemistry was performed on tissue for p-KIT (phospho-KIT), PTEN (phosphatase and tensin homolog deleted on chromosome ten), PI3K, phospho-AKT (p-AKT), phospho-4EBP1 (p-4EBP1) and phospho-S6 (p-S6RP). *Results:* The activation of AKT/mTOR activation was

significantly higher in imatinib secondary resistant GIST (53.1%) than in imatinib sensitive (27.1%) and primary resistant GIST (33.3%) (p=0.049). Analysing 20 pairs of samples, comparing pre-imatinib GIST with on-treatment ones, the PI3K status was changed from inactivated to activated in 4 cases each in 8 patients with effective imatinib and 12 patients with secondary resistance, respectively. AKT/mTOR status was inactivated in pre-imatinib and on-treatment samples in 8 patients with effective imatinib; however, the status of 6 patients was changed from inactivated to activated in 12 patients at the time of tumour progression. The negative expression of p-KIT was accompanied with PI3K pathway and/or AKT/mTOR pathway activity in some GISTs with secondary resistance. *Conclusion:* PI3K/AKT/mTOR pathway can be partly activated after imatinib secondary resistance in GIST. In this pathway, activation of AKT/mTOR is a more crucial factor and, thus, PI3K activation may be the early part of secondary resistance.

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P03-S-GC
MIR-215 PROMOTES MALIGNANT PROGRESSION OF GASTRIC CANCER BY TARGETING RUNX1

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Background/Aim: miR-215 was reported to be down-regulated and function as a tumour suppressor in several kinds of cancers; on the contrary, miR-215 was preferentially up-regulated in gastric cancer based on our previous results. This study was undertaken to investigate the potential biological function of miR-215 in gastric cancer (GC). *Materials and Methods:* miR-215 expression was detected in 77 paired GC tissues and adjacent non-tumour tissues. The biological functions of miR-215 were analysed by cell viability, colony formation, migration, invasion, cell cycle and apoptosis and luciferase assays *in vitro*, as well as *in vivo* tumourigenicity and metastasis analysis. *Results:* miR-215 was significantly up-regulated in 7 gastric cancer cells and 77 GC tissues compared to their adjacent non-tumour tissues (p<0.05); miR-215 expression was higher in advanced gastric cancer (stage III/IV) than early stage (stage I/II) gastric cancer (p<0.05). Ectopic expression of miR-215 in GES-1 and HGC-27 cells (miR-215 low expression) promoted cell growth, migration, invasion and metastasis abilities, which were reversed in NCI-N87 cells (miR-215 high expression) after down-regulation of miR-215. Potential target genes of miR-215 were predicted and RUNX1, a transcription factor and a tumour suppressor, was confirmed as one of the potential targets by luciferase assay. RUNX1 was down-regulated in gastric cancer tissues compared to their adjacent non-tumour tissues (p<0.05); RUNX1 could reverse partial functions of miR-215 *in vitro*. *Conclusion:* miR-215 promotes malignant progression of gastric cancer by targeting RUNX1, while RUNX1 could partially reverse miR-215's properties.

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P04-S-GC
RECURRENT *KIT*/*PDGFRA* MUTATIONS AND HETEROGENEITY IN WILD-TYPE GASTROINTESTINAL STROMAL TUMOURS BY NEXT-GENERATION SEQUENCING

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Background/Aim: Gastrointestinal stromal tumours (GISTs) with no mutations in exons 9, 11, 13, and 17 of *KIT* gene and exons 12, and 18 of *PDGFRA* gene were defined as wild-type GISTs accounting for about 15%. However, a few of wild-type GISTs with *KIT* mutations in other exons were occasionally reported. This study was performed to understand the whole genomic genotypes of *KIT* or *PDGFRA* genes in large-scale wild-type GISTs. *Materials and Methods:* A cohort of 185 wild-type GISTs from a total of 1,080 cases from our Department were eligible for inclusion. Enough genomic DNAs from 175 wild-type GISTs (one focus per patient was detected) were analyzed by targeted next-generation sequencing (NGS) followed by validation using Sanger sequencing (SS). *Results:* For the above hot spots of *KIT* and *PDGFRA* genes, 29 out of 175 wild-type cases (16.6%) were identified to carry mutations by NGS (exons 11 and 17 of *KIT*: 17 and 5 cases, respectively; exons 12, 14, and 18 of *PDGFRA*: 1, 1, and 5 cases, respectively); these mutations were exclusive. Intra-tumoural *KIT* mutational heterogeneity was observed in 5 samples (one sample carried W553G-mutated and wild-type cells; one sample carried N822K-mutated and wild-type cells; two samples carried L576P-mutated and wild-type cells; one sample carried W557R-mutated and wild-type cells), which potentially triggered the mechanisms of polyclonal evolution and metastasis, as well as different imatinib sensitivity. *Conclusion:* A subset of GISTs regarded as wild-type tumours using SS could be redefined as mutant tumours by NGS, which provided comprehensive understanding of *KIT*/*PDGFRA* genotypes.

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P05-S-GC
FAMITINIB EXHIBITS POWERFUL ANTITUMOUR ACTIVITY IN HUMAN GASTRIC CANCER CELLS AND XENOGRAFTS

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Background/Aim: Famitinib (SHR1020), a novel multi-targeted tyrosine kinase inhibitor, exerts antitumour activity in several solid tumours by mainly targeting vascular endothelial growth factor receptor 2 (VEGFR2), c-kit, platelet-derived growth factor receptor (PDGFR), *etc*. This study was designed to investigate the potential activity against human gastric cancer cells *in vitro* and *in vivo*. *Materials and Methods:* The IC_{50} values of drugs were determined by MTS assay. Cell cycle and cell apoptosis were analyzed using flow cytometry, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and Western blot. CD34 staining was employed to evaluate the microvessel density. BGC-823-derived xenografts in nude mice were established to assess the efficacy of drugs *in vivo*. *Results:* Famitinib could inhibit cell proliferation by inducing cell cycle arrest at G_2/M phase and cell apoptosis in a dose-dependent manner in gastric cancer cell lines *in vitro*. *In vivo*, in BGC-823 xenograft models, famitinib retarded tumour growth significantly through inhibiting angiogenesis. Compared to the common used chemotherapeutic drugs (5-fluorouracil, cisplatin or paclitaxel alone), famitinib exerted the highest tumour suppression with an inhibitory ratio >85%. *Conclusion:* This study demonstrated for the first time that famitinib had a powerful antitumour activity against human gastric cancer *in vitro* and *in vivo*, which provided solid evidence for future clinical trials.

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P06-S-GC
CHANGES OF CIRCULATING TUMOUR CELLS BEFORE AND AFTER CURATIVE SURGERY IN CHINESE RESECTABLE GASTRIC CANCER

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Aim: To assess the changes of circulating tumour cells (CTCs) and clinical significance before and after curative surgery in patients with resectable gastric cancer. *Patients and Methods:* A total of 100 patients with resectable gastric cancer were prospectively collected from July 2013 to September 2014 in Peking University Cancer Hospital; the CTCs before and after curative surgery were detected using CellSearch System. *Results:* All patients had pre-operative CTCs and 65 patients had paired pre- and post-operative CTCs. Thirty-four (34%, 34/100) patients had ≥1 CTCs/7.5 ml in pre-operative samples and 21 (32.3%, 21/65) patients had ≥1 CTCs/7.5 ml in post-operative samples. No significant differences were found between CTCs in most characteristics except for depth of penetration either in pre- or in post-operative samples. Based on the associations between CTCs and characteristics, the threshold of ≥3 CTCs/7.5 ml was defined as positive in this study. Among 65 patients with paired pre- and post-operative CTCs, 4 out of 7 patients with positive CTCs in pre-operative samples became negative in post-operative samples. Seven out of 58 patients with negative CTCs in pre-operative samples became positive in post-operative samples. *Conclusion:* Compared to CTCs in pre-operative samples, CTCs in post-operative samples could be increased or decreased. The association between changes of CTCs and recurrence will be elucidated in the future.

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P07-S-GC
THE DUAL PI3K/mTOR INHIBITOR BEZ235 EXERTS PROMINENT ANTITUMOUR ACTIVITY IN HER2-POSITIVE GASTRIC CANCER AND HAS SYNERGY WITH TRASTUZUMAB

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Aim: To investigate the antitumour activity of the dual PI3K/mammalian target of rapamycin (mTOR) inhibitor BEZ235 in human epidermal growth factor receptor 2 (HER2)-positive gastric cancer *in vitro* and *in vivo* and the synergism between BEZ235 and trastuzumab. *Materials and Methods:* HER2-positive gastric cancer cells NCI-N87 and SNU216 were used in this study *in vitro*. Cell viability, cell cycle and HER2 downstream pathway were measured using MTS assay, flow cytometry and Western blot, respectively. *In vivo*, HER2-positive gastric cancer patient-derived xenografts (PDX) were established and treated with BEZ235 or trastuzumab alone or combined to assess the antitumour activity. *Results:* Both BEZ235 and trastuzumab inhibit the growth of NCI-N87 and SNU216 cells in a dose-dependent manner *in vitro* by inducing cell cycle arrest at G1 phase.

BEZ235 alone has superior inhibitory effect than trastuzumab alone *in vitro* cells and *in vivo* patient-derived xenografts (PDX). Also, BEZ235 had synergistic inhibitory effect with trastuzumab against gastric cancer *in vitro* and *in vivo* through inhibiting HER2 downstream important pathways as shown by repression of phosphorylated AKT, S6 and ERK. *Conclusion:* BEZ235 was demonstrated for the first time to exert powerful activity against HER2-positive gastric cancer in PDX and had synergy with trastuzumab, which provided solid evidence for future clinical trials.

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P09-S-GC
KINASE D-INTERACTING SUBSTRATE OF 220 KDa IS OVER-EXPRESSED IN GASTRIC CANCER AND ASSOCIATED WITH LOCAL INVASION

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Background/Aim: Kinase D-interacting substrate of 220 kDa (KIDINS220), also known as ankyrin repeat-rich membrane spanning protein (ARMS), is a transmembrane scaffold protein. KIDINS220/ARMS was initially identified as a substrate for protein kinase D (PKD) in neural cells and independently characterised as a downstream target of the signalling induced by neurotrophins and ephrins. It has been indicated in various malignancies, including melanoma, glioma and neuroblastoma. However, the role played by this protein remains unknown in gastric cancer. The present study aims to investigate the expression of KIDINS220 in gastric cancer and its association with pathological and clinical features of the disease. *Materials and Methods:* Gastric tumours (n=324) together with paired adjacent background tissues were collected immediately after surgery and stored at -80oC until use, with written consent from the patients at the Peking University Cancer Hospital. All protocols and procedures of the tissue collection were approved by Peking University Cancer Hospital Research Ethics Committee. RNA was extracted from the frozen tissues followed by reverse transcription. *KIDINS220* mRNA was determined using real time PCR. Association between *KIDINS220* transcript levels and pathological/clinical features was analysed using SPSS statistics. Anti- KIDINS220 ribozyme was designed based on the secondary structure of *KIDINS220* mRNA. The ribozymes were synthesised using touch-down PCR and subsequently cloned into a pEF/V5 HIS TOPO TA plasmid vector. Knockdown of *KIDINS220* was carried out in a gastric cancer cell line (HGC27). The effect on cell invasion was determined using a transwell invasion assay. *Results:* Over-expression of KIDINS220 is seen in the gastric tumours, p=0.015, compared with the paired background tissues. Local advanced tumours (T3 and T4, invasion into subserosal connective tissue, visceral peritoneum and adjacent structures) express higher levels of *KIDINS220*, being 964,567±386,918 copies, p=0.02, compared with 55,020±23,983 copies expressed by tumours at earlier stages (T1 and T2). Higher expression of KIDINS220 is more frequently seen in poorly differentiated tumours, p=0.008, in comparison with well differentiated and moderately differentiated tumours. An interesting observation has also been shown in the cohort: KIDINS220 was expressed at lower levels in tumours of female patients compared to that of male patients (p=0.023). Knockdown of *KIDINS220* resulted in decreased invasiveness of HGC27 cells. *Conclusion:* KIDINS220 is over-expressed in gastric cancer, which is associated with poorer differentiation and local invasion. Knockdown of *KIDINS220* in HGC27 cells exhibited reduced invasion. It suggests that KIDINS220 plays a crucial role in local invasive growth of gastric tumours.

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P10-S-GC
RAN BINDING PROTEIN M IS ASSOCIATED WITH DISTANT METASTASIS OF GASTRIC CANCER

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Background/Aim: Ran binding protein M (RanBPM), belonging to the RAS superfamily, is involved in nuclear translocation of RNA and shows pro-apoptotic function. It has been a candidate for research of target therapy in cancer. However, the role played by this molecule in gastric cancer remains unknown. This study is designed to evaluate the expression of RanBPM in gastric cancer and its association with pathological/clinical nature of the disease. Additionally, we also examined its impact on functions of gastric cancer cells. *Materials and Methods:* RanBPM expression in human gastric cancer (n=324) with paired adjacent background gastric tissues was analysed using real time PCR (Q-PCR). RanBPM expression in gastric cancer cells was examined using RT-PCR. Ribozyme transgenes were designed based on the secondary structure of *RanBPM* mRNA and subsequently cloned into a

mammalian expression vector for knockdown of the gene. Knockdown of *RanBPM* in transfected gastric cancer cells was verified using RT-PCR, Q-PCR and Western Blotting. Functional tests, including growth, adhesion, invasion and migration, were carried out to determine the impact of *RanBPM* knockdown on the gastric cancer cells. *Results:* The expression of RanBPM tended to be higher in the gastric tumours in comparison with its expression in the paired adjacent background tissues. Local advanced tumours (T3 and T4) appeared to express relatively higher levels of *RanBPM* compared with the tumours at earlier stages (T1 and T2). Tumours with distant metastasis express lower levels of RanBPM transcripts, p=0.036, compared to that of tumours without detectable metastasis. No association was observed in its expression with lymphatic metastasis. RanBPM knockdown in AGS cells has been verified at both mRNA and protein levels. The knockdown of RanBPM resulted in increased invasiveness of AGS cells, which was approximately 25% higher compared with the control cells. The reduced expression of RanBPM had little effect on the *in vitro* growth of AGS cells. *Conclusion:* RanBPM was reduced in the tumours, which developed distant metastases, though it tends to be highly expressed in gastric tumours in comparison with paired background tissues. It suggests that RanBPM may play different roles during tumour development and disease progression. Further investigation will elucidate its implication in gastric cancer.

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P11-S-GC
THE CLINICOPATHOLOGICAL SIGNIFICANCE OF NUCLEOPHOSMIN/B23 AND CRM1 EXPRESSION IN GASTRIC CANCERS

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Background/Aim: Gastric cancer (GC) is a highly aggressive malignant tumour with a poor outcome in patients with advanced stage. Its high mortality rate prompts the urgent need for novel therapeutic agents. This study investigated the prognostic significance of nucleophosmin/B23 and CRM1 in GC. *Materials and Methods:* Nucleophosmin/B23 and CRM1 protein expression in GC and adjacent non-cancerous tissues (ANCT) of gastrectomy specimens from 120 GC patients was measured by immunohistochemistry. *Results:* Positive expression rates of nucleophosmin/B23 and CRM1 were significantly higher in GC tissues than in ANCT. The positive expression rates of nucleophosmin/B23 and CRM1 were significantly higher in patients with more advanced tumour stages, positive human epidermal growth factor receptor 2 (HER2) status and distant metastasis (all p<0.05). Nucleophosmin/B23expression was positively correlated with CRM1expression in GC tissues (p<0.01). Univariate analysis showed that TNM stage (p=0.0001), metastasis (p=0.027), nucleophosmin/B23 (p=0.0111) and CRM1 expression (p=0.0019) were significant risk factors affecting overall survival of GC patients. Cox multivariate analysis showed that positive nucleophosmin/B23 (p=0.0231) and CRM1 (p=0.0048) expression were both independent prognostic factors that negatively correlated with survival. *Conclusion:* The current results indicated that high expressions of nucleophosmin/B23 or CRM1 could be used as indicators of poor prognosis and may represent novel therapeutic targets in GC. The combined clinical significance of nucleophosmin/ B23 and CRM1 remains to be further investigated.

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P12-S-GC
INTERLEUKIN 17B IS ABERRANTLY EXPRESSED IN GASTRIC CANCER

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Background/Aim: Interleukin 17B (IL-17B) is one of the interleukin 17 family of cytokines. It was discovered as being distinct from the founder member (now termed interleukin 17A (IL-17A)). Both have been found to have pro-inflammatory effects, for example in animal models of arthritis. While IL-17A is expressed mainly in lymphocytes, IL-17B has a broader distribution, including pancreas, small intestine and stomach. IL-17B binds *via* its receptor IL-17BR. IL-17B has been implicated in tumourigenesis and angiogenesis. IL-17A has been implicated in gastric cancer. This study investigates the expression of IL-17B and its receptor IL-17BR in a gastric cancer clinical cohort. *Patients and Methods:* IL-17B and IL-17BR expression was examined in a clinical cohort of gastric cancer (tumour=324, normal=189) using quantitative polymerase chain reaction (qPCR). Transcript expression of *IL-17B* and *IL-17BR* was subsequently analysed in comparison to the clinical pathological patient data over a 60 month follow-up period. *Results:* Median IL-17B expression levels were

found to be significantly elevated in normal gastric samples compared to tumour samples (p=0.01). An association was also seen between IL-17B expression and patient survival rates. Patients who expressed higher levels of IL-17B were generally seen to have longer overall survival (p=0.09) and disease-free survival rates (p=0.121) than those patients who displayed a lower expression of IL-17B. However, median transcript expression levels of *IL-17BR* were found to be significantly elevated in gastric cancer samples compared to normal samples (p<0.001). *Conclusion:* IL-17B and IL-17BR expression was found to be aberrantly expressed in our clinical cohort. Our data suggests that lower levels of IL-17B may be associated with a poorer patient prognosis and 5-year survival rates. In contrast to this, higher levels of IL-17BR were also observed in tumour tissues.

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P02-S-G
THE DEVELOPMENT OF CUSTOMIZED PDMS APPARATUS AND DYNAMIC ASSAYS FOR CANCER CELL MIGRATORY INVASION

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Background/Aim: Cell migration is an essential process of biological phenomena and cell behaviours, including embryogenesis, tissue regeneration and cancer metastasis. The tumour cell populations are homogeneous in nature and exert great divergence during the migratory process. The laboratory assays for analyzing the migratory dynamics of cultured cells are rather limited and not sufficient to describe and reveal the complexity of the cell migration processes. In this study, we aimed to develop an assay to dynamically monitor and analyse the migratory invasion behaviour of tumour cells using a bio-compatible material of polydimethylsiloxane (PDMS) to build tissue culture apparatus with the capability to simultaneously obtain both morphological and biochemical measurements. *Materials and Methods:* Rational design of a customized PDMS mini-culture apparatus with a separation blade was facilitated with the AutoCAD software. U87MG glioblastoma cells pre-transfected with reporter plasmids were seeded and analysed for the time-dependent migratory patterns in correlation with the transforming growth factor beta (TGF-β) responsive luciferase expression. The cells' invasive and migratory behaviours following hypoxia treatments were investigated and compared with the results from transwell assays. By bi-directional cross-migration assays with two cell types or conditions preset in different chambers with one of which were labelled by live fluorescent staining; the time course dynamics were directly visualized and compared. *Results:* The customized PDMS apparatus was manufactured with a standard modal. The U87MG cells were transfected with a TGF-β/SMAD driven luciferase reporter. The sensitivity and variation for detections were determined. The optimization of the sampling time points for the time course evaluations was performed. By separation and collection of the sub-populations of high-mobility cells within the PDMS chambers, significant (p<0.05) elevated reporter activities were observed in the retrieved cells. In cells treated under hypoxia conditions, the luciferase expression was also increased significantly, to similar levels as compared with the results from classical transwell assays. In the cross-migration assays, using non-invasive fluorescent labelled U87MG cells, asymmetrical patterns of cell distribution were observed at the assay's end points, which appeared to be sensitive and convenient to correlate with the treatment conditions. *Conclusion:* The dynamic characterization and analyses, using customized PDMS-based cell culture devices, can be an attractive approach to investigate the process and underlying mechanisms of cancer cell migration. The exemplary model of the apparatus of such designs demonstrated in this study showed promising potentials for the convenient and comprehensive investigation from both morphological and biochemical measurements during the process of tumour cell migration.

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P03-S-G
ENHANCEMENT OF ANTI-TUMOUR ACTIVITY BY COMBINATION OF TUMOUR LYSATE-PULSED DENDRITIC CELLS AND CELECOXIB IN A RAT GLIOMA MODEL

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Background/Aim: The use of dendritic cell (DC)-based vaccines for treatment of gliomas has emerged as a meaningful and feasible treatment approach for inducing long-term survival; however, thus far, this approach has failed to generate significant clinical responses. In the present study, we proposed a novel immunotherapy for gliomas using tumour lysate-pulsed DCs in

combination with celecoxib, a selective COX-2 inhibitor. The anti-tumour effects of this combined immunotherapy were evaluated in a rat brain glioma model. *Materials and Methods:* Rat models of brain glioma (C6) were vaccinated subcutaneously with C6 lysate-pulsed DCs combined with celecoxib (25 mg/kg/day) *p.o*. daily. The survival of mice bearing C6 glioma and the tumour volume were observed. Tumour vessel density was evaluated by counting the number of micro-vessels on the sections stained with anti-CD31 antibody and tumour apoptosis was determined by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. Enzyme-linked immuno-sorbent assay was used to determine the systemic prostaglandin E2 (PGE2), interleukin (IL)-12 and IL-10 levels of tumour-bearing animals. Cytolytic T lymphocyte (CTL) response was measured by a cytotoxic assay *in vitro*. *Results:* The combination therapy groups showed more significantly enhanced anti-tumour activity, apoptosis of tumour cells, reduced neovascularization, while it developed a strong CTL response in these mice. Celecoxib may reduce production of PGE2 and modulation of balance between Th1 and Th2 cytokines by increasing the pivotal Th1 cytokine IL-12 and reducing Th2 cytokine IL-10. *Conclusion:* Our results demonstrated that selective inhibition of COX-2, using celecoxib combined with DCs-based immunotherapy, could act as an important novel strategy for improved future treatment of malignant gliomas.

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P01-S-CC
EXPRESSION OF CD44 AND CD15 AS THE TUMOUR STEM CELL MARKERS IN CERVICAL CARCINOMA CELLS

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Background/Aim: The existence of cancer stem cells (CSCs) is a subject of debate; however, in recent years, research has indicated the existence of CSCs in various solid tumours, including cervical cancer. For isolating CSCs from solid tumours, specific CSC markers have been widely used. CD133, CD44, CD24, CD90, EpCAM are the most widely used CSC makers in breast, brain, colon cancer and prostate cancer research. Nevertheless, cervical cancer stem cells and their specific cell surface markers have not yet been reported. Therefore, we focused on the expressions of CD44 and CD15 as candidate CSC makers in cervical carcinoma, analysed the specific markers of cervical cancer stem cell and tried to isolate and purify cancer stem cells from cervical carcinoma. *Materials and Methods:* The HeLa and SiHa cell lines were treated, respectively, with the two known chemotherapeutic drugs cisplatin (5 μM) or mitomycin (0.75 μM) for 72 hours. After treatment, more than 90% of the cells were killed and only less than 10 % of cells survived. The survived cells were collected and cultured in RPMI 1640 medium (10% FCS) for 48 hours and their mRNA expression level was detected by gene microarray. At the same time, we verified changes in mRNA expression patterns by RT-PCR. At last, CD44 and CD15 were selected to study in-depth. $CD44^+$ and/or $CD15^+$ cells were isolated from HeLa or SiHa cell lines using specific and flow cytometry, These cells were injected subcutaneously into BALB/c nude mice using the $CD44^-$ or $CD15^-$ cells as controls. Immunohistochemistry was used to detected CD44 and CD15 protein levels in tumour xenografts of nude mice after 2 months of treatment. *Results: CD44* mRNA in SiHa and HeLa cells, as well as *CD15* mRNA in SiHa cells was found to be expressed in higher levels in cisplatin- or mitomycin-treated cells than in the untreated cells. The number of $CD15^+$ cells isolated from 1×10^4 cells of SiHa cells was 9.1%, which is higher than the number of CD15+ cells isolated from HeLa (1.5%) cells and the control of normal cervical immortalized cells H8 (1.9%). Immunohistochemical staining (IHC) identifies scattered and clustered distribution of the $CD15^+$ or $CD44^+$ cells in tumour xenografts from SiHa or HeLa cell lines; $CD15^+$ cells (2×10^4 cells) isolated from SiHa cell lines regenerated tumour nodules in nude mice in 2 months. In contrast, the CD15- cells from all cell lines did not induce tumour formation in nude mice. *Conclusion:* Over-expression of CD44 and CD15 proteins may play an important role in the development of cervical cancer. $CD15^+$ might be one of CSC makers in cervical cancer but further experiments are needed to confirm these results.

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P01-S-OV
MIR-197 INDUCES TAXOL RESISTANCE IN HUMAN OVARIAN CANCER CELLS BY REGULATING NLK

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Background/Aim: Chemotherapy is the preferred therapeutic approach for the therapy of advanced ovarian cancer but a successful long-term treatment is prevented by the development of drug resistance. Increasing evidences have documented that microRNAs (miRNAs) play important roles in drug resistance in a variety types of cancer. However, the roles of miRNA, in regulating Taxol-resistance in ovarian cancer, and the detailed mechanism are less reported. *Materials and Methods:* We used Taqman probe stem-loop real-time PCR to accurately measure the levels of miR-197 in normal ovarian cells, ovarian cancer cells and Taxol-resistant ovarian cancer cells. miR-197 mimics or inhibitors were introduced into ovarian cancer cells to investigate its role on regulating drug-resistance, cell

proliferation and cell invasion. The target of miR-197 was identified by luciferase reporter assay and Western blot. A "rescue" experiment with NLK over-expression plasmid and miR-197 mimics was employed to demonstrate whether miR-197 regulation of drug resistance is dependent on NLK targeting. *Results:* miR-197 significantly increased in Taxol-resistant ovarian cancer cells. Enforced expression of miR-197 can promote Taxol-resistance, cell proliferation and invasion of ovarian cancer cells. Meanwhile, repression of miR-197 in ovarian cancer cells can sensitize its response to Taxol and also induced attenuated cell proliferation and invasion ability. Furthermore, investigation of the detailed mechanism showed that the promotion of miR-197 on drug resistance in ovarian cancer cells was partially mediated by down-regulating NLK, a negative regulator of Wnt signalling pathway. *Conclusion:* Our work demonstrated for the first time that miR-197 can confer drug resistance to Taxol, by regulating tumour suppressor, and NLK expression in ovarian cancer cells.

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P02-S-OV
METASTASIS SUPPRESSOR 1 (MTSS1) EXPRESSION IN HUMAN OVARIAN CANCER; THE IMPACT ON CELLULAR MIGRATION AND METASTASIS

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Background/Aim: Metastasis suppressor 1 (MTSS1) is a tumour suppressor protein involved in the regulation of cytoskeleton dynamics and cell motility and is recognised as a potential suppressor of cancer cell metastasis. MTSS1 expression is frequently reduced in a variety of cancer cells and tissues and this loss may account for increased invasive traits. The current study aims to assess the role of MTSS1 in the growth and invasive capability of epithelial ovarian cancer (EOC) cells where peritoneal or distant metastases are the major cause of high mortality. *Materials and Methods:* Expression of MTSS1 in clinical epithelial ovarian serous carcinoma tissue (n=10 non-metastatic; n=7 metastatic) was assessed by immunohistochemistry. MTSS1 expression was also investigated in ovarian cell lines (Cov504, Cov644 and SKOV-3) at both the mRNA and protein levels using reverse transcription-PCR (RT-PCR) and Western blotting, respectively. Full-length *MTSS1* cDNA expression vector was used. The effect of MTSS1 protein over-expression on EOC cell growth, adhesion and migratory/invasive capability was assessed using a variety of *in vitro* assays. *Results:* MTSS1 protein was detected in epithelial cell cytoplasm in both metastatic and non-metastatic ovarian carcinoma tissue but, typically, weaker/absent staining in the metastatic samples suggested some reduction in MTSS1 expression was associated with cancer spread. Cov504, Cov644 and SKOV-3 cells expressed relatively low levels of *MTSS1* mRNA. Over-expression of MTSS1 protein reduced the growth, invasion, adhesion and migration of EOC cell lines *in vitro*. *Conclusion:* This study revealed that MTSS1 plays an essential inhibitory role in the development and progression of ovarian cancers and may be a potential prognostic marker. MTSS1 over-expression is intimately related to migration and metastasis indicating that this protein can suppress the aggressiveness of human ovarian cancer. Our work suggests that preventing MTSS1 degradation, or partially restoring MTSS1 expression, could be a possible novel strategy to treat aggressive ovarian cancer growth and metastasis.

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P03-S-OV
ULTRASTRUCTURE ALTERATIONS IN ADVANCED OVARIAN SEROUS CYSTADENOCARCINOMA CELLS FOLLOWING DIFFERENTIATION-INDUCING THERAPY BY ALL-TRANS RETINOIC ACID WITH CONVENTIONAL SURGERY AND CHEMOTHERAPY

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Background/Aim: Ovarian cancer has the highest mortality rate among malignancies of the female genital tract. Retinoids can enhance the effect of cytotoxic drugs, such as cisplatin and docetaxel, in ovarian cancer cell lines but their clinical application for the therapy of ovarian cancer is still experimental. Our study aimed to observe the ultrastructure alterations in advanced ovarian serous cystadenocarcinoma cells following differentiation-inducing therapy by all-trans retinoic acid (ATRA) with conventional surgery and chemotherapy. *Patients and Methods:* Thirty-seven cases of advanced ovarian serous cystadenocarcinomas were randomly divided into two groups in the Gynecology Department of Yuhuang Ding Hospital of Yantai between 2000 and 2005 to observe the ultrastructure alterations in advanced ovarian serous cystadenocarcinoma cell following differentiation-inducing therapy by ATRA with conventional surgery and chemotherapy. *Results:* The tumour cells in the group of differentiation-inducing therapy were altered to differentiation of maturity: karyoplasmic ratio was diminished, cytoplasm was abundant, intercelluar desmosome conjunction was developed well and tight, mitochondria increased in numbers and morphous was regular, electron density of ground

substance increased, glycogen granule of cytoplasm was abundant, quantity of rough endoplasmic reticulum and regular morphous of Golgi complex were observed in some cells. *Conclusion:* Our study not only demonstrates the differentiation-inducing function of ATRA in ovarian serous cystadenocarcinoma cells but also provides effective adjunctive therapy for advanced ovarian cancer patients.

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P04-S-OV
THE ESTABLISHMENT AND PRELIMINARY ANALYSIS OF THE GENETIC DIFFERENCE EXPRESSION PROFILE BETWEEN OVARIAN CANCER TISSUE AND NORMAL TISSUE

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Background/Aim: The ovarian cancer ranks third in morbidity and first in mortality in gynaecologic malignant tumours. Tumour origination and development is a multi-genetic, multi-step and complex process influenced by many factors, which involve the abnormal activation of a number of oncogenes and the inactivation (mutation or deletion) of tumour suppressor genes, and complex interactions among multiple genes. Therefore, studying differences in tumour tissue at different stages of gene expression and exploring tumour molecular mechanisms from the overall perspective of functional genomics become today's research frontier. In this research, restriction fragment differential display-polymerase chained reaction (RFDD-PCR) technology is used, while ovarian cancer tissue and normal tissue of one person are taken as the research object to establish their genetic difference expression profile. Through bioinformatics, a large-scale research on functional genes related to tumour provides clues for further research. *Materials and Methods:* Collected ovarian cancer tissue and normal tissue of a patient with epithelial ovarian cancer were used to establish genetic difference expression profile with RFDD-PCR technology and bioinformatics analysis. *Results:* With RFDD-PCR technology, the genetic difference expression profile of ovarian cancer tissue and normal tissue can be established. The expression difference of thirty-six genes and factors closely related to the origination and development of tumour, such as proto-oncogenes and anti-oncogenes, cell cycle-related genes, the proliferation- and apoptosis-related genes, angiogenesis factors and metabolic enzymes, can be found and so can the change of the content and function of all members of ubiquitin-proteasomes system, closely related to the pathogenesis of tumour, all of which lay a solid foundation for further research of the pathogenesis and treatment of ovarian cancer. *Conclusion:* With RFDD-PCR technology, the genetic difference expression profile of ovarian cancer tissue and normal ovary tissue can be established, which is an effective way to investigate the pathogenesis of a tumour and the finding of therapeutic targets.

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P01-C-HCC
P53 GENE THERAPY-BASED TRANSARTERIAL CHEMOEMBOLIZATION FOR UNRESECTABLE HEPATOCELLUAR CARCINOMA: A PROSPECTIVE COHORT STUDY

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Background/Aim: Trans-arterial chemo-embolization (TACE) is used in the treatment of unresectable hepatocellular carcinoma (HCC); however, its efficacy still needs to be improved. Recombinant adenovirus p53 (rAd-p53) is a gene therapeutic agent, which could improve the prognosis of HCC patients. This study aimed to evaluate the efficacy and safety of rAd-p53-based TACE in the treatment of unresectable HCC. *Patients and Methods:* The study was conducted on a prospective cohort of patients who received the rAd-p53-based TACE or TACE monotherapy in the Chongqing Cancer Institute from January 1th, 2011 to December 31th, 2012. The primary endpoint was overall survival (OS). The secondary endpoints were progression-free survival (PFS), response rate (RR) and safety. *Results:* A total of 102 patients were enrolled in this study. Forty-nine patients received the rAd-p53-based TACE and fifty-three patients received TACE alone. The rAd-p53-based TACE treatment strategy improved OS (hazard ratio (HR)=0.58, 95% confidence interval (CI)=0.35-0.96, p=0.035), PFS (HR=0.60, 95%CI=0.37-0.97, p=0.037) and RR (p=0.047) compared with TACE monotherapy. Additionally, the rAd-p53-based TACE treatment caused more fever than TACE alone (p=0.01). However, symptomatic treatment may solve this problem. *Conclusion:* rAd-p53-based TACE treatment strategy is effective and safe in the treatment of unresectable HCC. Large scale randomized clinical trials are needed to evaluate these results.

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P02-C-HCC
EFFICACY EVALUATION OF TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION COMBINED WITH CT GUIDED WATER CIRCULATORY-COOLING RADIOFREQUENCY ABLATION AS A TREATMENT OF PRIMARY HEPATOCELLULAR CARCINOMA

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Background/Aim: Recent evidence shows that there are 740,000 new cases and 690,000 deaths related to liver cancer around the world each year, among them more than 50% are in China. Transcatheter chemoembolization (TACE) has emerged as the first choice in treating unresectable liver cancer, although its tumour necrosis rate is low and the curative effect is unsatisfactory. Radiofrequency ablation (RFA) has been extensively used in China and abroad and has proven to be effective in treating liver cancer. This study aimed to analyze the clinical value of TACE combined with computed tomography (CT)-guided water circulatory-cooling RFS in the treatment of primary hepatocellular carcinoma. *Patients and Methods:* The retrospective analysis was undertaken in 32 patients with 41 nodules from October 2011 to August 2014. Seven nodules were less than 3.0 cm in diameter, six nodules' diameter was between 3.0 cm~4.0 cm, nine were between 4.0 cm~5.0 cm, nineteen were longer than 5.0 cm; the average diameter was 4.9 cm±0.8 cm. All the patients were treated by TACE combined with CT-guided water circulatory-cooling RFA. All patients underwent enhanced CT scan after 1 and 3 months later. *Results:* According to the results of the CT scan, there were 11 complete remissions (CR), 24 partial remissions (PR), 5 stable diseases (SD) and 1 progressive disease (PD) in all of the nodules. Thirty of the 32 patients are alive now and 2 patients died of upper gastrointestinal bleeding. The longest followed span was 22 months and the shortest was 10 months; the average followed span was 15 months. *Conclusion:* TACE combined with CT-guided water circulatory-cooling RFA is a safe and effective method in the treatment of primary hepatocellular carcinomas with few complications.

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P01-C-LV
COMPARISON BETWEEN BILIARY CIRRHOSIS AND NORMAL PORCINE LIVER TREATED WITH MICROWAVE ABLATION USING A COOLED-TIP ELECTRODE

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Background/Aim: Percutaneous ablation therapies are novel, minimally invasive tumour therapies, which have become increasingly popular in the past decade. To date, the overwhelming majority of animal studies on microwave ablation have been conducted in normal healthy animal liver models. There are only few articles reporting microwave ablation using an animal model of liver cirrhosis. This study aimed to elucidate the difference in both *in vivo* and *ex vivo* microwave ablation in a biliary cirrhotic porcine liver model using a cooled-tip electrode. *Materials and Methods:* Two months after biliary ductal ligation, liver biopsy was performed to confirm the establishment of biliary cirrhosis in 4 Tibetan miniature pigs. Microwave ablation with cooled-tip electrode was conducted under laparotomy using 70 W for five minutes in the experimental group (4 pigs). The control group (two pigs) also received microwave ablation using the same settings but no surgery. Both *in vivo* and *ex vivo* ablations were performed in the two groups. Morphological and pathological characteristics of the ablation areas were compared. *Results:* In the cirrhotic liver group, *in vivo* ablation area was smaller than *ex vivo* ablation area in terms of short and long axes and volume (p=0.028, p=0.026 and p=0.008, respectively). With the same ablation settings, both *in vivo* and *ex vivo* ablation areas in normal pig liver were larger than their counterparts in cirrhotic liver in terms of the short and long axes and volume (p=0.019, p=0.00; p=0.024, p=0.036, respectively); however, the differences in the short axes of *in vivo* and *ex vivo* ablation areas failed to reach significance. *Conclusion:* Both *in vivo* and *ex vivo* ablation areas in biliary cirrhotic pig liver were smaller than their counterparts in normal pig liver suggesting that the ablation time or power should be relatively prolonged to enlarge the ablation zone in order to prevent incomplete ablation with viable residual tumour.

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P02-C-LV
CLINICAL STUDY ON USING ^{125}I SEEDS COMBINED WITH BILIARY STENT IMPLANTATION IN THE TREATMENT OF MALIGNANT OBSTRUCTIVE JAUNDICE

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Aim: To study the feasibility and curative effect on using ^{125}I seeds combined with biliary stent implantation in the

treatment of malignant obstructive jaundice. *Patients and Methods:* Fifty malignant obstructive jaundice patients during the September 2010 to February 2013 period, including 29 male and 21 female, aged 41~80 years old (average=57.3), participated in the present study. The patients were divided into two groups, one of which where the participants were treated with biliary stent implantation and the other where the patients were treated with biliary stent implantation combined with intraluminal brachytherapy using ^{125}I seeds. The patients were followed up at the end of September 2013 unless they had died after treatment. To evaluate the curative effect, we analysed, preoperatively and postoperatively, total bilirubin, direct bilirubin, related tumour markers (CA-199, CA-242, CEA) and the biliary stent patency status by cholangiopancreatography. *Results:* Twenty-four patients from the second group had significantly decreased tumour markers compared to the control (first) group. The average patency rate of biliary stents in patients of the treatment group was 83.3%, which is significantly higher than the antitheses group. *Conclusion:* ^{125}I seeds combined with biliary stent implantation in the treatment of malignant obstructive jaundice is a safe and effective method, which should be studied further.

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P02-S-HCC
APOLIPOPROTEIN A-I SUPPRESSES COX-2 EXPRESSION BY REDUCING REACTIVE OXYGEN SPECIES IN HEPATOCYTES

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Background/Aim: Abnormal lipid metabolism may contribute to the increase of reactive oxygen species (ROS) and inflammation in the pathogenesis of non-alcoholic steatohepatitis (NASH), which may, eventually, develop into hepatocellular carcinoma. Apolipoprotein A-I (apoA-I) accepts cellular cholesterol and phospholipids transported by ATP-binding cassette transporter A1 to generate nascent high-density lipoprotein particles. Previous studies revealed that the over-expression of ABCA1 or apoA-I alleviated hepatic lipid levels by modifying lipid transport. Here, we examined the effect of apoA-I over-expression on ROS and genes involved in inflammation in both BEL-7402 hepatoma cells and mice. *Results:* Human apoA-I was over-expressed by transfection in BEL-7402 hepatocytes and by an adenoviral vector in C57BL/6J mice fed a methionine choline-deficient diet. The over-expression of apoA-I in both models resulted in decreased ROS and lipid peroxidation levels, as well as a reduced MAPK phosphorylation and decreased expression levels of c-Fos and COX-2. *Conclusion:* These results suggest that apoA-I over-expression can reduce steatosis by decreasing ROS levels and suppressing COX-2-induced inflammation in hepatocytes. MAPK and c-Fos are involved in this regulatory process.

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P03-S-HCC
UROTENSIN II-MEDIATED ROS GENERATION PROMOTES OVAL CELL PROLIFERATION *VIA* NADPH OXIDASE PATHWAY

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Background/Aim: Urotensin II (UII) is a somatostatin-like cyclic peptide, which influences several human diseases due to its mitogenic effect. Our previous study had proved that UII and its receptor (UTR) were up-regulated in diethylnitrosamine (DEN)-induced precancerous rat liver lesions and human hepatocellular carcinoma (HCC). *In vitro*, UII could mediate WB-F344 hepatic oval cell and BEL-7402 human hepatoma cell proliferation *via* PKC/MAPKs signalling pathways. Oxidative stress plays an important role in tumour progression. In this study, we aimed to clarify that UII-induced ROS generation *via* activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase promotes oval cell proliferation. *Materials and Methods:* The fluoroprobe dihydroethidium was used to determine activation of NADPH oxidase in human hepatocellular carcinoma samples. In addition, malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were also measured. WB-F344 oval cell line was used for further research. Protein levels were evaluated by Western blotting *in vivo* and *in vitro*. ROS generation and cell cycle were determined by flow cytometry. Bromodeoxyuridine (BrdU) incorporation was used to detect proliferating cells. *Results: In vivo*, the activity of NADPH oxidase was up-regulated in liver tumour tissue. The expression of gp91, p40phox, p47phox and p67phox were also elevated. MDA content was higher than in non-HCC; the activity of SOD was lower than non-HCC. *In vitro*, administration of UII increased ROS generation and gp91, p40phox, p47phox, p67phox protein level in oval cells. Pre-treatment with Apocynin decreased UII-induced phosphorylation of PKC and ERK. Cell cycle analysis showed that in Apocynin pre-treated group, cells were arrested in G1 phase. UII-promoted cell proliferation was also abolished by Apocynin treatment. *Conclusion:* UII may play an important role in mediating oxidative stress in liver tumour tissue. *In vitro*

experiments proved that UII-induced ROS generation *via* NADPH oxidase promotes oval cell proliferation through activating PKC/ERK signalling pathways and accelerating cell cycle from G1 to S phase.

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P05-S-HCC
STEROL REGULATORY ELEMENT BINDING PROTEIN 1C PROBABALY REGULATES HEPATOMA CELLS AUTOPHAGY

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Background/Aim: Sterol regulatory element binding protein-1c (SREBP-1c) is a key transcriptional regulator of lipid metabolism. SREBP-1c activation plays a crucial role in the progression of non-alcoholic fatty liver disease (NAFLD). Recent studies indicate that autophagy regulates lipid metabolism and steatosis develops because lipophagy is inhibited. However, the mechanism remains unclear. This study aims to investigate the relationship between SREBP-1c and hepatoma cells autophagy in oleic acid (OA)-induced lipotoxicity. *Materials and Methods:* H4IIE cells were treated with OA (800 μm) for 24 hours and cell autophagy was analyzed using Western blotting and transmission electron microscopy. Lipid accumulation in cells was tested by measurement of the triglyceride (TG) content and Nile red stain. Apoptosis level was detected by flow cytometry and Western blotting. *Results:* Overload oleic acid reduced *SREBP-1c* mRNA and mature protein levels in H4IIE cells. Meanwhile, the autophagy function is improved as reflected by elevated microtubule-associated protein 1A/1B-light chain 3 (LC3) and reduced p62 in OA-induced H4IIE cells. Rapamycin inhibits *SREBP-1c* mRNA and SREBP-1c protein ($p<0.01$) and elevate LC3 ($p<0.01$) further at the basement of OA. Elevated SREBP-1c by insulin accompanies with reduced autophagic level; the effect was more notable when OA was added ($p<0.001$). Meanwhile, TO901317, the liver x receptor (LXR) agonist, also lowered autophagic function ($p<0.01$). When endogenous *SREBP-1c* was knockdown by siRNA, the autophagy function was enhanced, as LC3 level was increased and p62 decreased. The effect was significant when chloroquine (CQ) was added. As expected, TG content decreased compared with control ($p<0.001$ in OA group). Interestingly, apoptosis ratio increased in OA-induced *SREBP-1c* knockdown cells ($p<0.01$); this effect was enhanced when CQ was added ($p<0001$). An *in vivo* study revealed that autophagy function, demonstrated by elevated LC3 and reduced p62 levels, was impaired in rats fed with high-fat diet for 8 weeks, 12 weeks and 16 weeks, respectively. *Conclusion:* SREBP-1c may inhibit cell autophagy function and apoptosis as a result of fatty acid injury. Our results show that SREBP-1c promotes steatosis development not only by promoting lipid synthesis but also by probably being involved in the inhibition of lipophagy and apoptosis.

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P06-S-HCC
IDENTIFICATION OF MIR-744 TARGETS IN HCC AND SYSTEMS BIOLOGICAL ANALYSIS OF ITS FUNCTIONAL IMPACT

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Background/Aim: MicroRNAs (miRNAs) are small regulatory RNAs targeting multiple effectors of cell homeostasis and development whose malfunctions are associated with major pathologies, such as cancer. We previously reported under-expression of the miR-744 in the hepatocellular carcinoma (HCC), as well as associated decrease of miR-744 expression with HCC recurrence and prognosis. The finding clearly suggested important roles of miR-744's low expression in HCC; however, its function in HCC and direct targets remains unclear. *Materials and Methods:* MiR-744 expression was further detected using real time RT-qPCR in formalin-fixed, paraffin-embedded HCC and normal liver tissues. Furthermore, the effect of miR-744 on HCC growth, cell cycle, invasiveness, as well as migration, was studied *in vitro* and *in vivo*. *ZNF512B* was identified as a novel direct target for miR-744 through use of the isobaric tagging reagent iTRAQ and the QSTAR Elite Hybrid LC-MS/MS system, combined with bio-informatic target prediction. *Results:* MiR-744 expression was significantly down-regulated in most of HCC tissues and all cell lines. Ectopic over-expression of miR-744 dramatically repressed proliferation, invasion, migration, as well as cell cycle progression *in vitro*, and suppressed tumourigenicity *in vivo*. Luciferase assay results confirmed *ZNF512B* as a direct target gene of miR-744, which negatively regulates *ZNF512B* expression in HCC. *Conclusion:* Tumour suppressor miR-744 represses HCC progression through directly targeting *ZNF512B* oncogene. Our findings suggest that miR-744 may represent a novel potential therapeutic target and biomarker for survival of HCC patients.

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P01-S-LC
OVER-EXPRESSION OF KEAP1 INHIBITS PROLIFERATION AND MOVEMENT OF NSCLC CELLS

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Background/Aim: The tumour suppressor Kelch-like ECH-associated protein1 (KEAP1), an actin-binding protein, has significantly low expression in non-small cell lung cancer (NSCLC). However, little is known about how this affects changes in cell behaviour and the mechanism for KEAP1 involvement in inhibiting cancer remains unclear. This study aimed to explore the role of KEAP1 in proliferation, invasion and metastasis of NSCLC. *Materials and Methods:* KEAP1 expression in NSCLC and cell lines was analysed using RT-PCR and Western blot. Over-expressing cells were created. Functional testing included cell growth, motility and invasion. Tumour-xenografts models were established to explore the growth and metastasis *in vivo*. *Results:* There was evident reduced expression of KEAP1 in NSCLC and cell lines. KEAP1 over-expressing cells (A549, H460 and H1299) showed inhibited proliferation, retarded motility and invasion ($p<0.001$). Immunocytochemistry results showed much stress fibres in over-expressing cells, while not in the control group. Evident large focal adhesion complexes were found in the cytoplasm of KEAP1 expressing cells, while fine focal adhesions were observed in the membrane of control cells. RhoA activity increased significantly in KEAP1 expressing cells than in the control group ($p<0.001$). An *in vivo* tumour model showed similar regulation, *i.e.* the tumour-xenograft had significantly smaller size and less metastasis in KEAP1 over-expressing mice than in the control group ($p<0.005$). *Conclusion:* Loss of KEAP1 was found in NSCLC. Over-expression of KEAP1 could stabilise filamentous actin (F-actin) cytoskeleton and regulate focal adhesion turnover by enhancing RhoA activity, thereby restraining the motility and invasion of NSCLC. The above *in vivo* and *in vitro* studies both demonstrated that KEAP1 can suppress tumour development and metastasis, which could provide a new explanation for the mechanism of tumour metastasis in NSCLC.

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P02-S-LC
THREE SPLICE VARIANTS OF OSTEOPONTIN IN HUMAN LUNG CANCER, EXPRESSION PATTERN AND CLINICAL/PROGNOSTIC RELEVANCE

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Background/Aim: Osteopontin (OPN) is associated with prognosis of patients with non-small-cell lung cancer (NSCLC). However, little work has been carried out in discovering the expression and function of three osteopontin splice variants (OPN a, b and c) in lung cancer patients. The aim of the current study was to identify the role of three splice variants of osteopontin in lung cancer and to establish if a prognostic relevance existed. *Materials and Methods:* Fresh frozen human lung cancer tissues (n=92) and normal background lung tissue (n=89) were used. The transcript levels of *OPNs* (OPN a, b, and c) were determined using quantitative real-time PCR. The results were analyzed against the clinical and pathological data. Statistical analysis was by two-sample *t*-test and Kaplan-Meier method. *Results:* Quantitative analysis of the *OPNc* transcript revealed a higher level of expression in tumours compared with normal tissues ($p=0.0071$). *OPNa* and *OPNb* transcripts showed little change in expression between tumour and background. Over-expressed OPNc was more frequently found in adenocarcinomas than in squamous cell carcinomas (SCCs) and other pathologic types ($p=0.071$, $p=0.028$, respectively). OPNc expression was significantly increased in patients with higher TNM staging compared to those in lower TNM staging (TNM 2, 3 and 4 compared to TNM 2; $p=0.096$). The OPNc expression in samples from patients with poor clinical outcome was higher than that in those from patients who remained disease-free. This was reflected by a shorter overall survival for patients with high *OPNc* compared with low *OPNc* transcripts ($p=0.003$). There were no significant differences in overall survival based on high levels of OPNa and OPNb ($p=0.146$, $p=0.151$, respectively). *Conclusion:* Our study shows that OPN variants OPNa, b and c have an aberrant pattern of expression and that high levels of the OPNc are linked to a poor prognosis. This suggests that *OPNc* may mediate an oncogenic effect on NSCLC cells and indicates that OPNc may be a potential therapeutic target.

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P03-S-LC
THE CLINICAL SIGNIFICANCE OF DOK-7 V2 FOR LUNG CANCER PATIENTS AND ITS BIOLOGICAL IMPACT ON LUNG CANCER CELL FUNCTIONS

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Background/Aim: Downstream of tyrosine kinase 7 (DOK-7) is a member of the DOK family, which has been associated with congenital myasthenic syndrome. It was also reported that DOK-7 was associated with the development and progression of human breast cancer. However, little work has been carried out in discovering how this effect changes cell behaviour. *DOK-7* gene can generate three transcript variants by alternative splicing. This study aimed to examine the expression of DOK-7 variant 2 (DOK-7 V2) and its roles in human lung cancer. *Materials and Methods:* Transcript levels of *DOK-7 V2* in a cohort of lung specimens (normal, n =89; cancer, n=85) were analysed using quantitative real time PCR. *DOK-7 V2* knockdown cells were created using a hammerhead ribozyme transgene. The effect on growth and motility following knockdown of *DOK-7 V2* was then investigated using *in vitro* models. *Results:* The transcript levels of *DOK-7 V2* in lung cancer tissues were significantly lower than that in normal tissues (p=0.042), although no correlation with the progression and metastasis of lung cancer had been found. The *DOK-7 V2* mRNA levels in lung cancer cells were a little low. After knockdown of *DOK-7 V2*, lung cancer cells had significantly enhanced cell growth (p<0.05) but had no obvious change in cell migration. *Conclusion:* DOK-7 V2 expression is decreased in lung cancer. It may play a crucial role in regulating the growth of lung cancer cells.

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P04-S-LC
SODIUM TANSHINONE IIA SULFONATE SUPPRESSES PULMONARY FIBROBLAST PROLIFERATION AND ACTIVATION INDUCED BY SILICA: ROLE OF THE NRF2/TRX PATHWAY

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Background: Silicosis, a disease caused by chronic inhalation of crystalline silica, is characterized by inflammation and scarring in the form of nodular lesions in the lungs. The presence of silicosis has been known for centuries and industrial dust has led to the increase of silicosis worldwide, especially in developing countries. However, the pathological mechanism of silicosis is unclear. Alveolar macrophages are believed to induce oxidative stress *via* reactive oxygen species (ROS) when silica particles are inhaled. This process can contribute to the pathogenesis of silicosis; however, the mechanism is unclear. A traditional Chinese herbal derivative, sodium tanshinone IIA sulfonate (STS), displays significant antioxidant effects. *Materials and Methods:* We determined whether STS can attenuate the oxidative stress induced by silica. Traditionally, studies on the toxic effects of silica have focused on monocultures of macrophages or fibroblasts. A co-culture model of macrophages (Raw 264.7) and pulmonary fibroblasts (MRC-5) was used in this study to mimic a more *in vivo*-like environment. We investigated the protective effects of STS on the abnormal proliferation of MRC-5 fibroblasts in an *in vitro* model. *Results:* The results showed that fibroblast viability increased with the accumulation of intracellular ROS induced by co-cultured Raw 264.7 cells after silica exposure. Treatment with STS markedly ameliorated the silica-induced cell proliferation and oxidative stress. Western blotting and immunofluorescence analysis of the Nrf2 and thio-redoxin (Trx) system were conducted and the results confirmed that treatment with STS enhanced nuclear Nrf2 accumulation and mediated antioxidant Trx system expression. *Conclusion:* Our results suggest that STS likely inhibited silica-induced MRC-5 proliferation and activation by inducing Nrf2 expression and up-regulation of the oxidative response genes *TRX* and *TRXR*. This finding may form the basis of a new strategy for treating silicosis.

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P05-S-LC
INVESTIGATING THE EFFECT OF NWASP (NEURAL WISKOTT-ALDRICH SYNDROME PROTEIN) INHIBITORS ON HUMAN LUNG CANCER CELL BEHAVIOUR

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Background/Aim: Neuronal Wiskott-Aldrich syndrome protein (nWASP) is involved in the regulation of cell motility through its role in the reorganisation of the actin cytoskeleton. Previous reports have highlighted aberrant nWASP activity in non-healing human wounds and recognised nWASP inhibition as a promising therapeutic approach for the treatment of chronic wounds. The use of nWASP inhibitor treatments and the corresponding effect on cell behaviour has, therefore, been considered with a focus towards skin cells and wound

models. However, the effects of using nWASP inhibitors have not been fully explored with respect to other cell types, in particular cancer cell behaviour. The aim of this study is to investigate the effect of nWASP inhibition on lung cancer cell behaviour with a particular focus towards examining adhesive and migratory changes. *Materials and Methods:* The effect on the adhesion, migration and motility of SK-MES-1 and A549 lung carcinoma cells, following treatments with the nWASP inhibitor wiskostatin, was observed in functional assays, such as electrical cell-substrate impedance sensing (ECIS), cytodex-2-bead motility assays and scratch assays. *Results:* The resistance of cells measured during ECIS wounding assays (n=3) is significantly lower in SK-MES-1 cells treated with 10 μM wiskostatin in a 5-hour period following electrical wounding, suggesting that the rate of migration of the cells closing the wound is slower following the inhibitor treatment. However, over a longer time period, the resistance is significantly increased, suggesting more adhesive properties in SK-MES-1 and A549 cells treated with 10 μM wiskostatin compared with the control. SK-MES-1 and A549 cells showed a significantly decreased rate of migration during scratch wounding assays (n=1, $p<0.0001$ and n=2, $p<0.001$, respectively) when treated with 10 μM wiskostatin, which agrees with the outcome from the ECIS experiments. Furthermore, a significant decrease in motility and increased adhesion, following the same level of inhibitor treatment, was inferred in cytodex-2-bead motility assays (n=3) in both A549 ($p<0.01$) and SK-MES-1 cells ($p<0.001$). *Conclusion:* It is clear that treatment with the nWASP inhibitor wiskostatin can influence the adhesive and migratory behaviour of lung cancer cells. The full effect of this inhibitor on the properties of lung cancer cells still needs to be fully explored with further functional assays and the mechanisms behind its action is yet to be elucidated. However, these initial studies propose a novel method through which the migratory behaviour of human lung cancer cells can be influenced, which is an important consideration with respect to the metastatic behaviour of cancer cells and potential treatments.

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P06-S-LC
miRNA STRAND SELECTION PROCESS IN LUNG CANCER

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Background/Aim: Altered miRNA expression profiles are associated with a variety of diseases, including cancer. In the last step of miRNA biogenesis, the precursor molecule (pre-miRNA) duplex is formed by two strands called -5p and -3p, one of which is then cleaved and removed by Argonaute2. This process of miRNA strand selection is finely regulated and may vary among tissues and between normal and malignant cells. We aimed to evaluate the expression of the two forms of miRNAs 181, 221, 339 and 660 in human non-small cell lung cancer and investigate the effect of miRNA strand selection on behaviours of cancer cells. *Materials and Methods:* miRNA expression profiling in human non-small cell lung cancer cells and control cells was analysed using miRNA-specific-RT-qPCR. Motility and wound healing of the cancer cells was monitored using the electric cell-substrate impedance sensing (ECIS) system after treatment with miRNA mimics, inhibitors and scramble controls. Tumour cell invasion was assessed using Matrigel invasion chambers. Proliferation was determined using the alamarBlue® assay. *Results:* All the selected miRNAs were found to be down-regulated in cancer cells compared to the control. The predominant form for miRNAs 181, 339 and 660 in the lung cancer cells is the -5p, whereas the expression of 221-3p is higher than 221-5p. Data from ECIS indicated that miR339-3p promotes the migration of the lung cancer cells. *Conclusion:* A change in the miRNA strand selection process may represent one of the mechanisms used by the tumour for its survival and spread. In fact, the two forms of the same miRNA are associated to different sets of target genes, so an inversion in the expression of the two forms may result in a loss of regulation in favour of the malignant state. However, a proper control to verify this hypothesis is needed. We will further screen miRNA expression in tumour tissue compared to tumour adjacent normal tissue in lung cancer so as to better understand the association of miRNA strand selection with stage, prognosis and survival of the lung cancer.

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P07-S-LC
EFFECT OF WARMING YIN-COLD OR CLEARING YANG-HEAT TCM PRESCRIPTIONS ON GEFITINIB IN NSCLC

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Background/Aim: The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are used as first-line treatment in advanced non-small cell lung cancer (NSCLC) with sensitive *EGFR* gene mutations. In our clinical study, we found that patients with Yin-cold syndrome type in Traditional Chinese Medicine (TCM) theory have a higher chance of sensitive *EGFR* gene mutation. Thus, different TCM therapeutic principles treating Yang-heat or Yin-cold syndrome may have different effect on EGFR-TKIs in NSCLC. The purpose of this study was to identify which therapeutic principle of TCM,

clearing Yang-heat or Warming Yin-cold, will improve the efficacy of gefitinib on NSCLC *in vitro*. *Materials and Methods:* We used two TCM patent prescriptions, the Qingkailing Injection (QI) and the Shenfu Injection (SI), which do not have anticancer effects but with opposite therapeutic principles in TCM theory. The QI is with clearing Yang-heat effect, while SI with warming Yin-cold effect. The lung cancer cell lines A549 and H1650, with resistance to EGFR-TKIs, were treated *in vitro* with QI, SI, gefitinib (G), QI combined with gefitinib (QI+G) or SI combined with gefitinib (SI+G). Cell proliferation and apoptosis were observed. *Results:* Using the MTT assay, a significant inhibition of cell proliferation was observed in both A549 and H1650 when treaeted with QI+G, compared with gefitinib alone ($p<0.05$). The Q value for QI and gefitinib was between 0.85 to 1.15 in A549 and Q>1.15 in H1650. Significant differences in cell proliferation were observed in neither A549 nor H1650 after treatment with SI+G compared with gefitinib alone ($p>0.05$). The Q value for SI and gefitinib was between 0.85 to 1.15 in A549, and Q<0.85 in H1650. In A549, the apoptosis rate was significantly increased after treatment with QI+G, compared with gefitinib alone (23.2±6.5% *vs*. 8.3±2.2%, $p=0.047$). No significant differences in apoptosis were observed between SI+G and G in A549. The effect of the different medicines on apoptosis was not significantly different in H1650. *Conclusion:* Clearing Yang-heat therapeutic principle of TCM may reduce the resistance of NSCLC to gefitinib *in vitro*.

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P01-C-M
A RANDOMIZED PHASE II STUDY EVALUATING THE ACTIVITY OF BEVACIZUMAB IN COMBINATION WITH CARBOPLATIN PLUS PACLITAXEL IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED MUCOSAL MELANOMA (NCT02023710)

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Background/Aim: Mucosal melanoma is rare in Caucasian population but accounts for 25% in Asian population. It is usually associated with extremely poor prognosis. No standard treatment for advanced mucosal melanoma has been established. The BEAM study has shown the potential effectiveness of bevacizumab combined with carboplatin plus paclitaxel in patients with previously untreated advanced cutaneous melanoma. This study aimed to evaluate the activity of bevacizumab combined with carboplatin plus paclitaxel in patients with previously untreated advanced mucosal melanoma. *Materials and Methods:* This study is an open-label, multi-centre, randomized phase II trial. Eligible patients had metastatic, recurrent or unresectable mucosal melanoma without previous systemic therapy. Additional inclusion criteria included: ≥1 measurable disease, Eastern Cooperative Oncology Group Performance Status (ECOG PS) 0/1 and adequate haematological, renal, and hepatic functions. Patients with mutations in *C-KIT* or *BRAF* (treated with targeted therapy) will be excluded from this study. Patients were randomly allocated in a 1:1 ratio to receive intravenous infusion of bevacizumab (CPB arm, 5 mg/kg every two weeks) or placebo (CP arm) in combination with carboplatin (area under the curve, 5) plus paclitaxel (175 mg/m^2). Treatment was continued for both groups until occurrence of disease progression, unacceptable toxicity, death or withdrawal of consent. The primary study endpoint is progress-free survival (PFS). Overall survival, disease control rate and safety will also be assessed. *Results:* The first patient visit was in December 1, 2013. Fifty-seven of a planned 182 evaluable patients have been enrolled: 63.2% female, median age=58. They were randomly assigned to the CBP (n=29) or CP arm (n=28). The median follow-up time was 11 months and the median progression-free survival was 6.3 months and 3.1 months, respectively. There was a significant difference in mPFS between CPB and CP arm ($p=0.023$). The objective response rate was 10.3% and 7.4%, respectively. The median overall survival has not been reached. Treatment emergent grade 3 or 4 adverse events were neutropenia (13.8%), hypertension (3.4%), proteinuria (3.4%) in the PCB arm and neutropenia (7.4%) in the PC arm. *Conclusion:* The primary data show that bevacizumab combined with carboplatin plus paclitaxel might benefit patients with advanced mucosal melanoma. More advanced mucosal melanoma patients will be enrolled in this study.

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P02-C-M
RESULTS OF A PHASE II TRIAL WITH CONTINUOUS INTRAVENOUS INFUSION OF RH-ENDOSTATIN IN COMBINATION WITH DACARBAZINE AS THE FIRST-LINE THERAPY FOR METASTATIC ACRAL MELANOMA

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Background/Aim: A phase II study has revealed that rh-endostatin (Endostar) was effective when combined with dacarbazine (DTIC) for metastatic melanoma patients (pts), especially for pts with acral melanoma. Preclinical data suggest that continuous infusion of endostatin is more potent than intermittent dosing. A phase II study was performed to evaluate the efficacy and safety of continuous intravenous infusion (CIV) of Endostar in combination with DTIC as first-line therapy for pts with metastatic acral melanoma. *Patients and Methods:* Twenty treatment-naive pts with metastatic acral melanoma and without *C-KIT/BRAF* mutation were enrolled in this study.The efficacy and safety of DTIC (250 mg/m^2, d1-5) plus Endostar CIV (Baxter infusion pump 2 ml/h; 7.5 mg/m^2/d or 15 mg/m^2/d, d1-14) were evaluated. The regimens continued in a 28-day cycle until disease progression (PD) or intolerable toxicity occurred. The primary endpoint was progression-free survival (PFS), while the secondary endpoints included disease control rate (DCR) and safety. *Results:* From December 2013 to October 2014, 10 pts were enrolled in the 7.5 mg/m^2/d Endostar group (group A) and another 10 pts were enrolled in the 15 mg/m^2/d group (group B). In group A, M1a disease was 30%, M1b 50% and M1c 20%; in group B, M1a 20%, M1b 30% and M1c 50%. Mean treatment cycle was 3.1 (range=1-6) in group A and 2.6 (range=1-6) in group B. Until last follow-up in Januaty 2015, 9 pts achieved PD. The estimated mPFS was 3.0 months (95% confidence interval (CI)=0.86~5.14) in group A and 6.0 months in group B (95%CI=1.69~10.31, p=0.178, log-rank test). DCR in group B (80%; 8 SD and 2 PD) was higher than that in group A (50%; 5 SD and 5 PD) without statistical significance. This combination was well tolerated without grade 3-4 toxicity; the most common side effect was intermittent palpitation in group B (2/10) with sinus tachycardia but without ST-T change by ECG monitoring. *Conclusion:* Endostar administered by continuous infusion was safe and 15 mg/m^2/d Endostar combined with DTIC might cause better estimated PFS and DCR than 7.5 mg/m^2/d Endostar plus DTIC in pts with metastatic acral melanoma. A controlled study with a larger sample size is still needed. Also, trials with longer follow-up times should be encouraged.

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P01-S-M
EFFECT OF NANOSECOND PULSED ELECTRIC FIELDS IN COMBINATION WITH EVEROLIMUS ON MELANOMA

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Background/Aim: Nanosecond pulsed electric field (nsPEF) is a bioelectrical technology generateing high voltage electric field in nanosecond duration to destroy tumour cells with non-thermal effect. It is a potential technique for cancer therapy. The PI3K/mTOR/AKT pathway is activated in most melanomas but single agent activity of mTOR inhibitors is limited in advanced melanomas. The aim of the study was to evaluate the anti-growth efficacy of nsPEF in combination with everolimus in melanoma. *Materials and Methods:* Human A375 melanoma cells were exposed to everolimus for 48 hours, followed by 25 pulses of nsPEF with 100 ns duration at the electric field strength of 15 -20kV/cm. Cell viability was measured by the CellTilter-Glo luminescent cell viability assay 24 hours later. Cell apoptosis and cell cycle were analysed by flow cytometry. BALB/c mice were inoculated subcutaneously with A375 cells and randomly divided into four groups: group I as control, group II treated with nsPEF in a single treatment (30 kV/cm, 100 ns, 100 pulses), group III given everolimus (4 mg/kg) by oral gavage daily for 2 weeks and group IV treated with nsPEF plus everolimus. *Results:* With the combination of nsPEF, cell growth was decreased gradually according to the nsPEF intensity but the synergistic effects of nsPEF were saturated when everolimus concentration reached 1 μM. nsPEF could induce melanoma cell apoptosis and the ratio of apoptotic cells was correlated with nsPEF intensity. Cell cycle analysis showed that both everolimus and nsPEF alone could induce G_2/M phase arrest and combination of everolimus with nsPEF could further increase the percentages of G_2/M-arrested cells. *In vivo* experiments showed that the skin lesions caused by nsPEF all healed in one week and no adverse effect were observed. When compared to control group, the everolimus group, the nsPEF group and the combination group suppressed tumour growth to 32%, 44% and 10%, respectively. *Conclusion:* nsPEF and everolimus have a synergistic anti-tumour effect on melanoma cells through induction of G_2/M phase arrest and apoptosis. Our findings indicate that nsPEF in combination with mTOR inhibitor could be used as a potential treatment for advanced melanoma.

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P02-S-M
EFFICACY OF HIGH-DOSE ADJUVANT INTERFERON THERAPY IN HIGH-RISK MELANOMA HARBORING GENE MUTATIONS

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Background/Aim: BRAF/NRAS mutations are predictors of poor prognosis in melanoma. High-dose interferon (HDI) is the only drug approved by FDA as adjuvant therapy in high-risk resected melanomas. However, efficacy of HDI in high-risk melanomas harbouring *BRAF/NRAS* mutations has not been evaluated systemically. This study aimed to clarify whether there is a beneficial effect of HDI in these patients. *Patients and Methods:* Melanoma patients, after melanoma resection, with *BRAF* mutation (Exon 15) or *NRAS* mutation (Exon 2) in melanoma of high risk (Stage IIB to Stage IIIC) were enrolled in this study. Patients were randomized (ratio of 2:1) into 1-year adjuvant HDI therapy group (n=88) and observation group (n=52). The endpoint was disease-free survival (DFS). The median follow-up is 16 months till December 2014. Somatic mutations were detected by DNA sequencing. All the statistical analyses were performed using the SPSS 16.0 software. *Results:* The proportion of stage IIB/IIC and III disease was 55.0% and 45.0%, respectively. 53.6% patients had acral melanomas. Primary ulceration was found in 52.9% lesions. Of the 140 patients, 108 cases harboured *BRAF* mutation and 32 cases with *NRAS* mutations. At the end of follow-up, 55% patients had metastatic or local recurrence. The overall median DFS was 19.0 months. DFS in the HDI group was significantly longer than in the observation group (21.0 months *vs*. 10.0 months, p=0.002). DFS of HDI *vs*. observation group was 19.0 *vs*. 9.0 months (p=0.021) in *BRAF*-mutated patients. However, stratified analysis did not show significantly DFS improvement of HDI in acral or stage IIB/IIC subgroups in *BRAF*-mutated patients. *NRAS*-mutated patients did not get a significant benefit from HDI therapy (DFS=24.0 months *vs*. 20.0 months, p=0.21). Stratified analysis did not show significant difference in DFS between the two groups either in different stages (stage IIB/IIC *vs*. III) or in different subtypes (non-CSD/CSD *vs*. acral). *Conclusion:* In the *BRAF*-mutated high-risk melanoma, HDI may provide beneficial effects in the resected patients. However, more powerful adjuvant therapy should be explored for *NRAS*-mutated melanoma patients.

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P03-S-M
ANALYSIS OF *MTOR* MUTATIONS IN CHINESE MELANOMA PATIENTS AND EVALUATION OF THEIR SENSITIVITY TO PI3K-AKT-mTOR PATHWAY INHIBITORS

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Background/Aim: Mammalian target of rapamycin (mTOR) is a validated target in cancer treatment. *MTOR* mutations are estimated to be 2.46% in all cancers and 5.76% in cutaneous melanoma. However, *MTOR* mutations in other types of melanoma have not been reported. *Materials and Methods:* This study involved tumour samples from 412 Chinese melanoma patients, including 210 acral melanomas, 105 mucosal melanomas, 30 melanomas on skin with chronic sun-induced damage and 58 melanomas on skin without chronic sun-induced damage. Tissue samples were analyzed for mutations in all exons of *MTOR* gene in genomic DNA by Sanger sequencing. Mutation status was confirmed using the Agilent's SureSelect Target Enrichment system. HEK293T cells stably expressing mutant *MTOR* were constructed by transcription activator-like effector nucleases (TALEN). Function of *MTOR* mutants in these cells and *in vitro* sensitivity of *MTOR* gain-of-function mutations to PI3K-AKT-mTOR pathway inhibitors were analyzed. *Results:* The overall incidence of somatic mutations within the *MTOR* gene was 10.4% (43/412). Increased *MTOR* mutations were relatively more frequent in acral (11.0%) and mucosal (14.4%) melanomas than in CSD (6.7%) and non-CSD (3.4%) melanomas. Of the 43 cases with *MTOR* mutations, 41 different mutations were detected, affecting 25 different exons. Point mutations resulting in single amino acid substitutions (totalling 40 mutations detected in 43 patients) were the most common type of MTOR mutation. The median survival time for melanoma patients with MTOR mutation was significantly shorter than that for patients without *MTOR* mutation (p=0.028). Transient expression of several *MTOR* mutants in HEK293 cells strongly activated the mTOR/p70S6K pathway. In HEK293 cells with stable expression of H1968Y or P2213S *MTOR* mutants, LY294002 and AZD5363 showed higher potency than temsirolimus or BYL719 in inhibiting the mTOR/p70S6K pathway. *Conclusion:* These data demonstrate that *MTOR* mutations are more frequent in Chinese melanoma patients than in Caucasian melanoma patients. *MTOR* mutation is a worse prognostic factor in Chinese melanoma patients.

Clinical trials with PI3K-AKT-mTOR pathway inhibitors may be beneficial for melanoma patients with *MTOR* mutations.

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P04-S-M
COMPARISON OF CLINICAL PRESENTATION AND PROGNOSES BETWEEN ACRAL CUTANEOUS MELANOMA AND NON-ACRAL CUTANEOUS MELANOMA

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Background/Aim: Acral cutaneous melanoma (ACM) is rare in Caucasian populations and a long controversy has been apparent as to whether this lesion carries a worse prognosis than non-acral cutaneous melanoma (NACM). The purpose of this observational study was to bolster the limited literature on the difference between ACM and NACM. *Materials and Methods:* A database was prospectively established. Medical records of all patients with pathologically diagnosed cutaneous melanoma in our centre since 2006 were retrieved and reviewed. Statistical analyses, including survival and multivariate analyses of factors associated with survival, were respectively performed by Kaplan-Meier method and Cox proportional hazard model. *Results:* There were 658 cases (61.1%) of ACM and 419 cases (38.9%) of NACM evaluated. The proportion of patients with clinical stage I, II, III and IV diseases were 10.3%, 50.8%, 24.5%, 14.4% in ACM and 6.9%, 40.8%, 33.2%, 19.1% in NACM. The 5-year overall survival rate of all the patients was 44.7% in ACM and 52.6% in NACM; the median survival time (OS) was 55.0 months (95% confidence interval (CI)=48.9 to 61.1) and 68.0 months (95%CI=56.4 to 79.6), respectively (p=0.027). Five-year survival rates of patients with stage I, II, III and IV diseases were 89.1%, 53.8%, 24.5%, 8.0% in ACM and 96.8%, 63.2%, 35.2%, 9.6% in NACM, respectively. The median OS for patients with ACM and NACM were 65 months *vs*. 87.0 months in the stage II group (hazard ratio (HR)=1.630, p=0.023), 30 months *vs*. 36 months in the stage III group (HR=1.371, p=0.043), 12 months *vs*. 13 months in the stage IV group (HR=1.466, p=0.048). The median disease-free survival (DFS) was shorter in ACM than in NACM, *i.e*. 31 months *vs*. 37 months (HR=1.213, p=0.042) in all the patients, 35 months *vs*. 47 months (HR=1.340, P=0.015) in stage II subgroup, 14 months *vs*. 23 months (HR=1.498, p=0.000) in stage III subgroup. The median OS and DFS for stage I patients was not reached in both groups. Multivariate analysis revealed that the type of the disease (ACM *vs*. NACM) was significant prognosticator for OS (HR=1.80, 95% 1.429 to 2.269, p=0.000) and DFS (HR 1.725, 95% 1.187 to 2.508, p=0.004). *Conclusion:* In China, ACM is the most commonly diagnosed subtype of cutaneous melanoma. The type of ACM was significantly associated with clinical outcome in terms of OS and DFS. Patients with ACM were more susceptible to recurrence and associated with worse prognoses than that of non-acral cutaneous melanoma.

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P05-S-M
A PHASE II STUDY OF EVEROLIMUS FOR ADVANCED MELANOMA PATIENTS WITH *MTOR* MUTATIONS

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Background/Aim: Everolimus, a rapamycin (mTOR) inhibitor, has shown minimal activity as a single agent in patients with metastatic melanoma, unselected by potential predictive biomarkers (J Clin Oncol 24:463s, 2006). Selection of patients with melanoma harbouring *MTOR* mutations may improve the efficiency of mTOR inhibitor in patients with melanoma (J Clin Oncol. 2012, 30(4):e37-40). This study (NCT01960829) aims to observe the efficacy of mTOR inhibitor in melanoma patients with *MTOR* mutations. *Materials and Methods:* Patients (pts) who had un-resectable melanoma harbouring mutation in *MTOR* who had measureable disease by Response Evaluation Criteria in Solid Tumours (RECIST) and who failed to systemic treatment (at least 1) were enrolled. Pts received everolimus 10 mg daily continually until disease progression or unacceptable toxicity occurred. Response assessments were performed every 8 weeks. The endpoints were progression-free survival (PFS) and overall survival. *Results:* Of 396 pts screened, 10.3% had a *MTOR* mutation: 22/207(10.6%) acral, 15/104 (14.4%) mucosal, 2/28(7.1%) CSD and 3/57(5.2%) non-CSD. Thus far, 8 pts have been treated, with 6 pts currently evaluable for response. The median age was 54 years (range= 38-65); the male:female ratio was 3:5; the median number of prior therapies was 2 (range=1-4). Six pts achieved stable diseases. The PFS were: 26 weeks+ (Exon 41: G5741C; acral melanoma; failed to DTIC, DDP and PTX), 18 weeks+ (Exon 41: A5794G; mucosal melanoma; failed to TMZ, bevacizumab, DDP and nab-PTX), 17 weeks+ (Exon 41: G1914R; acral melanoma; failed to DTIC), 12 weeks+ (Exon 46: C6520T; acral melanoma; failed to TMZ, carboplatin, bevacizumab, PTX and

fotemustine), 11 weeks+ (Exon 47: C6637T; mucosal melanoma; failed to nab-PTX, DDP and TMZ) and 10 weeks+ (Exon 43: C6026T; CSD melanoma; failed to DTIC, DDP, bevacizumab and PTX). All 6 pts showed median tumour shrinkage (12%, 10%, 9%, 23%, 1% and 5%, respectively). *Conclusion:* While mTOR inhibitors show limited activity in a non-selected melanoma patient population, a substantial proportion of melanoma pts harbouring *MTOR* mutation appear to respond. It may be expected to identify appropriate pts prospectively for treatment with mTOR inhibitors. (Supported by the National Natural Science Foundation of China (81301984), the Beijing Nova Program (2013027) and Novartis Oncology in China).

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P06-S-M
CLINICAL PRESENTATION, SYSTEMIC THERAPY AND PROGNOSIS OF MUCOSAL MELANOMA, A STUDY OF 463 CONSECUTIVE CASES

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Background/Aim: Mucosal melanoma (MM) is the second common subtype in Asians with an incidence of 22-25%; however, there have only been few epidemiologic studies. Thus, we performed a retrospective analysis of MM about its clinical presentation, gene mutation, systemic therapy and prognosis. *Materials and Methods:* Using institutional databases, we identified patients with basic demographics and clinical outcomes of MM who were treated in Chinese melanoma centres between 2006 and 2014. *Results:* 463 MM patients (pts) were included. Median age at diagnosis was 56 years (range=17-86); 61% female; primary site: 174 head/neck, 125 genitourinary, 119 anal-rectal, 30 oesophageal and 15 others. Mutation status: 14.1% (45/319) KIT mutation (mut); 9.7% (31/319) BRAF mut; 10.3% (33/319) NRAS mut. Stage I-II/III/IV at referring – 53%/24%/23%, M1a/b/c – 17%/18%/65%. The median overall survival (mOS) was 28.0 months (95% confidence interval (CI)=25.1-30.9 months). Multivariate analysis showed that clinical stage and lactate dehydrogenase (LDH) level were significant prognostic factors for OS. The application of adjuvant therapy and LDH level were significant prognostic factors for disease-free survival. For 293 pts who developed stage IV, mOS was 14.0 months, for M1a, M1b, M1c, 20.2, 16.0, 11.1 months, respectively (p=0.004). In 1st-line setting, the objective response rate (RR), disease control rate (DCR) and median progress-free time (PFS) was 11.8%, 54.8% and 3.1 months (95% CI=2.6-3.8 months); most of the patients using dacarbazine/temozolomide, cisplatin plus rhEndostatin had RR, DCR and PFS, 12.3%, 64.6% and 4.5 months, respectively. In 2nd-line setting, paclitaxel plus carboplatin was the common regimen and RR, DCR and PFS was 8.3% 61.1% and 3.3 months (95% CI=2.0-4.4 months). The mPFS of 3rd-line therapy was 1.3 months without complete regression and partial regression. Eight out of 20 pts harbouring *C-KIT* mutation used imatinib, 3/8 pts had SD for a median PFS of 6.7 months. Five out of 15 pts harbouring *BRAF* mutation used vemurafenib. Of those, 3/5 pts obtained response; mPFS could not be evaluated. *Conclusion:* The prognosis for MM is still poor, while clinical stage has its significance for OS. For pts with stage IV, the efficacy of systemic therapy, including chemotherapy and targeted therapy, is modest. Further investigation into the biology and treatment of MM is needed.

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P07-S-M
THE EFFICACY AND SAFETY ANALYSIS OF SUNITINIB PLUS TEMOZOLOMIDE THERAPY IN PATIENTS WITH METASTATIC MUCOSAL MELANOMA

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Background/Aim: Mucosal melanoma (MM) is a special subtype melanoma with poor prognosis and no standard therapeutic options. Temozolomide (TMZ) is still the first-line choice. As MM has more abundant blood supply than cutaneous melanoma, we wanted to know if anti-vascular endothelial growth factor / platelet-derived growth factor (VEGF/PDGF) tyrosine kinase inhibitors (TKI) could demonstrate activity when combined with TMZ in metastatic MM. *Patients and Methods:* Patients (pts) with metastatic MM, harbouring no *BRAF*/*NRAS*/*C-KIT* mutation, were enrolled. The combination regimen sunitinib (SUN, 37.5 mg, d1-28) and TMZ (200 mg/m^2, d1-5) continued in a 28-days cycle until disease progression or intolerable toxicity occurred. The primary endpoints were objective response rate (ORR) and safety, which were evaluated according to Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria for every cycle and National Cancer Institute/Common Toxicity Criteria (NCI/CTC) 4.0, respectively. The secondary endpoints included progression-free survival (PFS) and overall survival (OS). *Results:* From August 2008 to December 2014, 26 pts (4 rectum, 8 vagina, 5 sinonasal cavity, 6 oral cavity, 1 urinary tract, 2 oesophagus) were enrolled. The combination therapy was well tolerated and only 2 pts required a dose reduction of

SUN to 25 mg due to grade 4 thrombocytopenia. Grade 3-4 toxicities mainly included thrombocytopenia (19.2%), leucopoenia (19.2%) and hepatic injury (3.9%). No treatment-related death occurred. Eighteen of 26 pts had been previously treated. The median treatment cycle was 3.0 months (range=1-9). The ORR was 19.2% (five PR and 16 SD) with a disease control rate of 80.8%. The median PFS and OS were 3.0 months (95% confidence interval (CI)=1.0~5.0) and 7.0 months (95% CI=5.0~9.2), respectively. *Conclusion:* For metastatic mucosal melanoma, SUN+TMZ might be an alternative choice. The regimen is well-tolerated and worthy of further study.

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P01-C-LYM
THE CLINICAL VALUE OF ^{18}F-FDG PET/CT IMAGING FOR DIAGNOSIS, STAGING AND TREATMENT EVALUATION IN LYMPHOMA

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Background/Aim: Lymphoma is a malignant tumour, which can be cured by chemotherapy and auxiliary therapy. Lymphoma's correct diagnosis and staging is the foundation for the appropriate treatment plan and infer to prognosis. Positron emission tomography/computed tomography (PET/CT) is one kind of imaging technology characterized by whole-body functional scanning; by analyzing the ratio of contrast medium that disease focus had taken, PET/CT could determine the pathologic and metabolic feature of disease. This work investigated the clinical value of PET/CT imaging for the diagnosis staging and treatment evaluation in lymphoma, expecting to find a new way to diagnose the lymphoma as quickly as possible. *Materials and Methods:* Thirty pathological result-proved lymphoma patients were studied retrospectively; the imaging results before and after the treatment were compared and analysed. *Results:* Before the treatment, 27 patients' PET/CT imaging was nearly the same with CT imaging, the clinical stages were unchanged; 3 patients' PET/CT imaging was different from CT imaging (the stages were ascended from 2'2'1 to 4'4'2). There was no obvious statistical difference between the efficacy group and the inefficacy group of Hodgkin lymphoma (HL) patients but there was obvious statistical difference between the efficacy group and the inefficacy group of non-Hodgkin's lymphoma (NHL) patients and there was obvious statistical difference between the maximum standardized uptake value (SUVmax) of the same region before and after the treatment. *Conclusion:* ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT imaging has significant value on the diagnosis staging and treatment evaluation of lymphoma.

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P02-C-LYM
ANALYSIS OF THE RELATIONSHIP BETWEEN MICROSATELLITE INSTABILITY AND THYMIC LYMPHOMA INDUCED BY N-METHYL-N-NITROSOUREA IN C57BL/6J MICE

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Background/Aim: Microsatellite instability (MSI) has been found closely associated with many types of human tumours and often shows strong correlations with specific tumour features. However, the relationship between MSI and tumours are still unclear. Exploring the relationships between MSI, tumour formation and underlying mechanism of tumourigenesis is very important. *Materials and Methods:* Mice were administered with either N-methyl-N-nitrosourea (MNU) (90 mg/kg) or solvents (control) at the beginning of the experiment and were euthanized at 16 weeks after the initial injection. We detected MSI in the tissues of MNU-treated mice using a panel of 42 mutation-sensitive loci. Then, we examined the expression of tumour suppressor genes *P53, Pten* and mismatch repair gene *Msh2* by Western blot. *Results:* Of the 31 mice that survived at the end of experiment, 19 (61.3%) mice developed thymic lymphomas. In addition, 52.6% (10/19) of the tumours had metastasized to the liver. Nineteen loci (45.2%) in six organs showed 70 MSI events. Locus D8Mit14 showed enhanced MSI compared with the other examined loci. MSI frequency in thymus was higher than in other organs. Interestingly, there was no significant difference observed between the metastatic and non-metastatic livers. The MSI frequency in the MNU-treated thymus with non-tumour (4.6%, 23/(42×12)) was significantly higher than this in the thymic lymphomas (0.5%, 4/(42×19)) ($p<0.0001$). In thymus, the expression of P53 levels in MNU-treated group were significantly lower than that in control thymus tissues ($p=0.007$ and $p=0.031$, respectively), and Pten was down-regulated in MNU-induced thymic lymphoma relative to control and non-tumour group ($p=0.027$ and $p=0.01$, respectively). Conversely, the expression of Msh2 was elevated in tumour-bearing livers compared to control ($p=0.000$) and non-tumour group ($p=0.000$). *Conclusion:* These results indicate that, although thymic tumourigenesis is associated with MSI, it occurs with higher frequency in those that have not developed tumours upon the MNU-treatment, which provides additional insights into the relationship between MSI

occurrence and tumourigenesis. Furthermore, the distinctive expression features of P53, Pten and Msh2 may suggest that they are involved in tumour development through different pathways.

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P01-C-RDT
A COMPARISON OF RAPIDARC AND SLIDING-WINDOW INTENSITY-MODULATED RADIOTHERAPY (IMRT) IN THE TREATMENT OF STAGE I-II NASAL NATURAL KILLER/T-CELL LYMPHOMA

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Aim: The present study aimed to compare the dosimetric difference between RapidArc and sliding-window intensity-modulated radiotherapy (IMRT) for stage I-II nasal natural killer/T-cell lymphoma (NNKTL). *Patients and Methods:* Ten patients with stage I-II NNKTL treated with sliding-window IMRT were re-planned with RapidArc (two arcs) for dosimetric comparison. The prescribed dose of the planned target volume (PTV) was 50 Gy in 25 fractions. The Eclipse treatment planning system (Version 8.6) was used to design RapidArc plans with the Anisotropic Analytical Algorithm (Version 8.6.15). The monitor units (MU) and treatment time (T) were scored to measure the expected treatment efficiency. *Results:* All treatment plans administered the prescribed doses. Patients were compared with regard to the quality of target coverage, efficiency of delivery and the exposure of normal adjacent organs at risk (OARs). RapidArc was associated with a better conformal index (CI) and better homogeneity index (HI) (both $p<0.05$) than IMRT in regard to PTV and tended to provide equivalent or slightly worse OAR avoidance. The MU with RapidArc (650.80±24.59) were fewer than with IMRT (1,300.10±57.12) (relative reduction 49.94%, $p<0.05$) when using 2-Gy dose fractions. The treatment time with RapidArc (3.20±0.02 s) was shorter than with IMRT (7.38±0.18 s) (relative reduction 56.64%, $p<0.05$). *Conclusion:* Stage I-II NNKTL patients treated with RapidArc received equivalent or better dose distribution and fewer monitor units over a shorter time than patients treated with IMRT. The organ-at-risk dose was higher with RapidArc.

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P01-C-TEC
EFFICACY OF DIFFERENT METHODS OF ANAESTHESIA FOR LAPAROSCOPIC HYSTERECTOMY

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Background/Aim: To investigate the efficacy of different methods of anaesthesia for laparoscopic hysterectomy. *Materials and Methods:* Sixty patients with American Society of Anaesthesiology (ASA) I or II score, aged 45-60 years, weighing 55-65 kg, scheduled for laparoscopic hysterectomy were equally and randomly divided into two groups: combined intravenous-inhalational anaesthesia group (group A) and combined spinal-epidural anaesthesia (CSEA)+general anaesthesia group (group B). In group A, anaesthesia was maintained with inhalation of sevoflurane and infusion of remifentanil after induction of anaesthesia. In group B, CSEA was performed and once the upper level of sensory block was stable, general anaesthesia was induced and maintained with inhalation of sevoflurane; state entropy (SE) was maintained at 45~60. Arterial blood samples were taken to determine the plasma concentrations of adrenaline (E), norepinephrine (NE) and dopamine (DA) after admission to the operation room after completion of pneumoperitoneum, at 10 min after pneumopentaneum, during uterus traction, during removal of the laryngeal mask airway and at 10 min after removal of the laryngeal mask airway ($T_{0\sim5}$). The time for recovery of spontaneous breathing, extubation time and time of regaining consciousness were recorded at the end of the operation. The side-effects and number of patients requiring increments of analgesics were also recorded within 48 h after operation. Patient's satisfaction was also recorded at 48 h after operation. *Results:* Compared with group A, the plasma concentrations of E and NE at $T_{3\sim5}$ and the plasma concentrations of DA at $T_{3,5}$ were significantly decreased, the time for recovery of spontaneous breathing, extubation time and time of regaining consciousness were significantly shortened, while the incidence of agitation and the number of patients requiring increments of analgesics were significantly decreased in group A ($p<0.05$). There was no significant difference in the incidence of intra-operative awareness, nausea and vomiting after operation and the level of patient's satisfaction at 48h after the operation between the two groups ($p>0.05$). *Conclusion:* CSEA+general anaesthesia has better efficacy than combined intravenous-inhalational anaesthesia when used for laparoscopic hysterectomy.

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P02-C-TEC
DISCUSSION OF HYPOCALCAEMIA AFTER TOTAL KNEE REPLACEMENT AND THE CLINICAL SIGNIFICANCE

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Background/Aim: Transient hypocalcaemia is a frequent complication after total knee arthroplasty. In this study, we investigated the factors associated with development of hypocalcaemia after total knee arthroplasty, to explore its clinical significance and the treatment. *Materials and Methods:* A retrospective analysis of the change of serum calcium levels for 50 patients after total knee arthroplasty. We investigated the patients prospectively for age, gender, operative time, operating surgeon and amount of bleeding at the operation. Two days after operation, serum calcium was evaluated. The chi-square test was applied in the analysis of categorical variables. Linear correlation analysis was used to determine the risk of hypocalcaemia in the univariate analysis. *Results:* After knee arthroplasty, serum calcium is significantly lower than that before operation ($p<0.05$), the incidence of hypocalcaemia after operation is 90.23%, the decline is positively correlated with intraoperative blood loss. *Conclusion:* Hypocalcaemia occurs frequently after knee arthroplasty; however, the clinical symptoms associated with hypocalcaemia are rare but, as the important electrolyte, neurotransmitter and blood coagulation factor, it is suggested that one has to routinely monitor serum calcium levels during peri-operation and timely deal with hypocalcaemia.

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P03-C-TEC
ENDOSCOPIC REMOVAL OF ETHMOID OSTEOMAS UNDER NAVIGATION GUIDANCE

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Background/Aim: Osteoma is a benign tumour of paranasal sinuses tending to grow slowly. Surgery is a well-accepted treatment and, in recent years, endoscopic trans-nasal approach, as a better method, has been widely applied to complete ethmoid osteomas removal avoiding major complications. However, the tumours' proximity to critical structures in a narrow anatomical corridor increases the risk of inadvertent injury and image-guidance systems might help to avoid the disadvantages. *Materials and Methods:* A retrospective analysis was performed on 19 patients with osteomas of ethmoid sinuses who were hospitalized from April 2005 to October 2010. All patients had sixteen row computed tomography (CT) scans and three-dimensional reconstructions were done preoperatively. All patients underwent operations with the help of navigation system and nasal endoscope. *Results:* The osteomas of all 19 patients were removed completely with endoscope and navigation system. In 2 cases, open procedures were followed to remove the tumours, while, in 17 other patients, the neoplasms were removed under endoscopic and navigation guidance. As of five patients whose osteomas were localised or with a diameter of less than 2 cm, the osteomas were located with the help of navigation system and resected without ectorhinal incision. In 2 cases, the basilar parts of the osteomas were narrow and relatively dissociative and the tumours were removed out of the oral cavity. The basilar parts of twelve patients' osteomas connected to vital structures and, thus, the neoplasms were removed using electric drills. All patients are followed up for 8 to 64 months and are asymptomatic. Two patients underwent removal of crista galli and one of them developed postoperative cerebro-spinal rhinorrhea (recovered after repairing procedure) but both suffered from anosmia and never recovered. One patient with an enormous osteoma suffered from repeated crusting and abnormal odour; the patient recovered after nasal flushing. *Conclusion:* Endoscopic ablation of ethmoid osteomas with the guidance of navigation system is an accurate, secure, minimally-invasive and calleidic procedure and preoperative CT scan is a safeguard of an accurate approach. Osteomas on median line and localised in ethmoid sinus is an indication of this operation. If the lesion affects frontal sinus and maxillary sinus extensively, a combination of superciliary arch incision and labiogingival groove incision is an easy and slinky option. A limited exposure of orbital fasciae, endocranium, optic nerve sheaths on the tumour border is an effective way to determine whether the tumour is completely resected and to protect important structures.

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P01-S-BBB
COMPARISON OF BLOOD BRAIN BARRIER ENDOTHELIA REVEALS A DIFFERENTIAL EXPRESSION OF TIGHT JUNCTION PROTEINS

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Background/Aim: Tight Junctions (TJs), present in endothelia, impose a barrier to invasive metastatic cancer cells by physically occluding transmigration across blood vessel walls, thus preventing the development of secondary metastatic foci responsible for the majority of cancer deaths.

TJs are also a fundamental component of the blood-brain barrier (BBB), a highly selective barrier responsible for separating the vascular compartment from the central nervous system (CNS) and, thus, preventing the transmigration of invasive cancer cells. This study aimed to determine the differential expression of TJ component expression in endothelial cell from the BBB in comparison to vascular endothelial cells. *Materials and Methods:* Six endothelial cell lines were used for comparison of TJ component expression using RT-PCR. These were HECV (human endothelial cells from vein), HUVEC (human umbilical venous endothelial cells), hMVEC (endothelial cells of dermal origin), TY09 (brain endothelial cells), TY10 (brain endothelial cells) and hCMEC-D3 (immortalised human cerebral microvascular endothelial cells). Comparison was then made between all the cells for cell attachment, migration and barrier function using electrical cell impedance sensing (ECIS). *Results:* RT-PCR revealed a greatly differing expression pattern of TJ molecules between the cell lines; this was true of those usually associated with endothelia, such as claudin-5 and JAM-B and also plaque molecules and associated signalling molecules. From ECIS tests, it was revealed that HECV's showed significantly differenct barrier function than the three brain endothelial cell lines, which was not anticipated ($p<0.5$). All cells showed similar attachment rates but migration rates were significantly reduced in brain endothelial cells ($p<0.5$). *Conclusion:* The expression pattern of TJ molecules varies widely between endothelial cells of different origins. Moreover, they also differ functionally, which is particularly pertinent to barrier function. This has a direct bearing on the ability of cancer cells to migrate through the BBB and raises numerous questions as to how this can be controlled. Further work will elucidate how this control can be expedited.

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P02-S-BBB
THE EFFECTS OF IDH1 MUTATION ON GLIOBLASTOMA STEM CELLS

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Background: Neural stem and progenitor cells are a likely source of brain tumours, such as glioblastoma (GBM), the most malignant brain tumour according to WHO classification. Glioblastoma patients with isocitrate dehydrogenase 1 (IDH1) mutation have a longer survival time than their wild type counterparts. However, the mechanism of this phenomenon remains unclear. Here, we use mouse and human GBM stem cells to explore the role of IDH1 mutation during tumour development. *Methods:* IDH1 status in mouse and human neuronal stem cells (NSC) was verified by sequencing and western blot. Proliferation, migration rate and invasion capacity were compared between IDH1 mutant and wild type cells. The level of D-2-hydroxyglutarate (D2HG), a catalysate of mutant IDH1, was measured by an enzymatic assay. Differential expression map of microRNAs from human GBM stem cells was created by complete-linkage together with Euclidean distance measure. Results: IDH1 mutation reduces mouse and humsn GBM stem cell (mGSC and hGSC, respectively) proliferation, migration and invasion ability *in vitro* ($p<0.01$). Both mGSC and hGSC with IDH1 mutation produced high level of D2HG, which was undetectable in IDH1 wild type cells. In addition, both temozolomide and AGI-5198, an inhibitor of mutant IDH1 enzyme activity could reduce the amount of D2HG more than 5 fold ($p<0.001$). Differential expression analysis of hGBM miRNAs screened out 19 miRNAs with significant fold change ($p<0.05$; fold change cutoff of more than 1.5 or less than -1.5). *Conclusion:* IDH1 mutation slows down tumour growth, of which mechanism is involved in modifying GSC metabolism along with gene expression. IDH1 mutation slows down tumour growth, of which besides, identification of main targets of these miRNAs may also provide us new insight into targeted therapy.

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P01-S-ENDO
IMPACT OF PROTEIN TYROSINE PHOSPHATASE BETA (PTPRB) KNOCKDOWN ON THE HUMAN ENDOTHELIAL CELL

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Background/Aim: Protein tyrosine phosphatases (PTPs) are known as signalling molecules that regulate cell functions, including proliferation, differentiation and oncogenic transformation. PTPs have been indicated in anti-tumourigenesis and progression of various solid tumours; for example, receptor-type tyrosine-protein phosphatase gamma (PTPRG) plays as a negative regulator in breast and ovarian cancers. PTPRB, receptor-type protein tyrosine phosphatase beta, plays a crucial role in both vasculogenesis and angiogenesis. It has been shown that PTPRB-up-regulated Tie2 promotes proliferation of endothelial cell. PTPRB has also been shown to inhibit activation of vascular endothelial growth factor receptor 2 (VEGFR2) blocking this VEGF signalling pathway. However, the role played by PTPRB in angiogenesis remains unknown. In the present study, the effect of *PTPRB* knockdown on functions of HECV cells was

investigated. *Materials and Methods:* Ribozyme transgenes were constructed to knockdown *PTPRB* expression in human vascular endothelial cells. Verification of the knockdown was carried out using RT-PCR, real time q-PCR and Western blot. Resultant effect on cellular functions including *in vitro* growth, adhesion and migration were examined using a variety of functional assays. *Results:* Knockdown *PTPRB* in HECV cells ($HECV^{PTPRBkd}$) resulted in an increase of *in vitro* cell proliferation. The growth rate of $HECV^{PTPRBkd}$ after 96 hours of incubation was 499.55±33.86, $p<0.001$ compared with empty plasmid control ($HECV^{pEF}$, 395.30±27.10). Moreover, HECV-*PTPRB* knockdown cells exhibited increased cell motility. However, cells with reduced *PTPRB* expression showed decreased adhesion to extracellular matrix. The number of adhered cells for $HECV^{PTPRBkd}$ was 38.67±6.15, $p<0.001$, compared with that of $HECV^{pEF}$ control cells (67.75±9.96). Additionally, HECV cells with *PTPRB* knockdown showed differential responses to different growth factors. A remarked increase of cell migration was seen in the control cells in exposure to fibroblast growth factor (FGF), which was not observed in the *PTPRB* knockdown cells. *Conclusion:* Knockdown of *PTPRB* promoted growth and motility of vascular endothelial cells but inhibited the cell-matrix adhesion. Furthermore, PTPRB participates in the co-ordination of FGF-regulated cell motility. It suggests that PTPRB is a negative regulator for tha angiogenic process. This anti-angiogenic potential requires further investigation.

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P01-S-KDY
AUTOPHAGY-MEDIATED TRANSFORMING GROWTH FACTOR-B EXPRESSION IN RENAL CARCINOMA CELLS REQUIRES mTOR/STAT3 SIGNALLING

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Background/Aim: Renal carcinoma cells (RCC) undergo starvation and subsequent autophagy during the progression of tumour growth. During this process, transforming growth factor (TGF)-β is induced to promote the migration of cells to a nutrition-enriched environment and further protect cell survival from starvation. Mammalian target of rapamycin (mTOR) is the most important signalling molecule in induction of cell autophagy and Stat3 signalling that predominantly takes charge of protein synthesis. This study aims to elucidate the precise contribution of autophagy to TGF-β expression in RCC as the underlying mechanisms remain unclear. *Materials and Methods:* Autophagy was induced in ACHN and 769-P cells by starvation in Hanks' balanced salt solution. Production of TGF-β in cells and corresponding conditioned medium during autophagy was evaluated by real-time PCR, immunoprotein blotting and ELISA. Pre-treatment of ACHN and 769-P cells with pharmacological inhibitors of mTOR and Stat3, as well as construction and transfection lentiviral over-expressing vector of phosphorylated Stat3 and mTOR in ACHN and 769-P cells was used to identify the role of mTOR and Stat3 signalling in TGF-β production during autophagy. *Results:* Induction of autophagy induced the expression of TGF-β and activated mTOR and Stat3 in RCC. Pre-treatment of RCC with rapamycin both inhibited activation of mTOR and Stat3 resulting in deceased TGF-β production during starvation-induced autophagy, while over-expression of phosphorylated Stat3 by lentivirus-mediated transfection rescued the rapamycin-inhibited TGF-β production. However, treatment with stattic, the specific inhibitor of Stat3 signalling significantly inhibited TGF-β expression without influence on activation of mTOR. The small interfering RNAs (siRNAs) for Atg3 or Atg7 and chloroquine inhibited autophagy of ACHN and 769-P cells during starvation leading to decreased production of TGF-β and inactivation of mTOR and Stat3, whereas over-expression of phosphorylated mTOR by lentivirus-mediated transfection reactivated Stat3 and inversed TGF-β production in RCC. Nevertheless, the over-expression of phosphorylated mTOR in autophagy-deficient RCC failed to reactivate Stat3 and inverse TGF-β expression in the presence of stattic. *Conclusion:* These findings suggest that mTOR and Stat3 constitute a regulatory pathway and play a critical role in autophagy-induced production of TGF-β. Inhibition of the mTOR/Stat3 pathways may represent novel therapeutic interventions for hepatic ischemia/reperfusion injury (IRI).

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P01-S-LEU
MONOSOMAL KARYOTYPE IS AN INDEPENDENT PROGNOSTIC FACTOR IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background/Aim: Accurate evaluation of the prognosis of patients with acute myeloid leukaemia (AML) is of great

significance in establishing clinical therapeutic protocols. Fifty to 60% of the AML patients have accompanying cytogenetic abnormalities. Complex karyotype (CK) is an independent unfavourable prognostic factor in AML patients and recent studies have proved that patients with a monosomal karyotype (MK) have shorter survival than those with CK. At present, MK, as an unfavourable prognostic factor, has not been included in those traditional prognostic scoring systems based on cyto- and molecular genetics. This study focused on exploring the prognostic significance of MK and CK in a cohort of 498 patients with AML. *Patients and Methods:* From July 2001 to July 2013, 498 patients with AML were included in this study. The chromosome karyotype results of all subjects were analyzable. Bone marrow cells were collected from all patients and cultured for 24-48 h and, then, routinely prepared on slides with banding on G fragments. *Results:* AML patients with a MK had a higher age (median age being 62.5 *vs*. 52 years, p=0.003), lower haemoglobin levels (62.5 *vs*. 77 g/l, p=0.009) and lower white blood cell counts (7.0×10^9/l *vs*. 11.7×10^9/l, p=0.008). Univariate analysis showed that the prognosis was poorer in patients with a MK than those without (median survival time of 7.3 *vs*. 26.3 months, p<0.001) and in patients with a CK than those without (median survival time of 14.8 *vs*. 26.3 months, p<0.001). COX regression risk model suggested that MK was an independent risk factor of National Comprehensive Cancer Network (NCCN) prognostic group as CK (hazard ratio (HR)=2.610 (1.632-4.175), p<0.001). *Conclusion:* MK is an independent factor of poor prognosis in AML patients.

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P01-S-P
ASSOCIATION OF MTA-1 EXPRESSION IN PITUITARY TUMOURS WITH BONE INVASION

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Background/Aim: Metastasis associated gene-1 (*MTA-1*), discovered from aggressive human cancer cell lines, has been demonstrated to have an important role in regulating the metastatic potential of cancer cells and also to have clinical significance in certain solid tumours, including gastrointestinal-, lung- and endocrine-related cancers. The current study investigated the expression of *MTA-1* in pituitary tumours and the potential clinical implications. *Materials and Methods:* A cohort of human pituitary tumours (n=95) was examined for *MTA-1* transcript expression by quantitative gene transcript analyses. The relationship between *MTA-1* expression and the pathological, clinical and aggressiveness of the pituitary tumours was evaluated. *Results: MTA-1* was expressed at a significantly higher level in large tumours and in tumours with higher tumour grade. It was also observed that tumours, which had invaded the suprasellar bones, and tumours, which caused destruction of the sella, also had significantly higher levels than those without bone involvement (p<005). Although there did not appear to be a relationship between *MTA-1* and cystic lesions in the tumours, endocrine active tumours, namely those secreting prolactin, growth hormone, follicle stimulating hormone and luteinizing hormone had significantly lower levels than those tumours that were inactive. *Conclusion: MTA-1* expression in pituitary tumours is linked to the aggressive nature of the tumour type, in particular bone invasion, and may be a potential therapeutic target in this tumour type.

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P01-S-TCM
YANGZHENG XIAOJI SENSITISES THE RESPONSE TO SIS3 INHIBITOR IN HUMAN CANCER CELLS, *IN VITRO* AND *IN VIVO*; THERAPEUTIC IMPLICATIONS

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Background/Aim: The SMAD pathway, in particular SMAD-3, is a key intracellular signalling pathway for the transforming growth factor-beta (TGF-beta) superfamily involved in the aggressive behaviour of cancer cells. SMAD-3 has been found to be widely involved in tumourigenesis and cancer progression and indicated as a potential therapeutic

target. Here, we investigated the potential implication of a combinational approach of targeting SMAD3 by small inhibitor and traditional medicine. *Materials and Methods:* Human prostate cancer cells and osteosarcoma cells were used. Cell-matrix adhesion, cellular migration and cell growth in response to specific inhibitor of SMAD-3 (SIS3) inhibitors and YangZheng XiaoJi extract were investigated. An *in vivo* xenograft tumour model was used in which the effects of SIS3 inhibitor and YangZheng XiaoJi extract on tumour growth were evaluated. SMAD-3 protein and phospho-SMAD-3 in cancer cells and tumours were assessed by immunofluorescence. *Results:* YangZheng XiaoJi extract had a profound effect on the matrix adhesion of the cancer cells used. SIS3 inhibitor had a mild effect on the adhesion at non-toxic level. However, a combination of SIS3 inhibitor and YangZheng XiaoJi extract had a markedly significant inhibition on the adhesion. A similar effect was seen with the migration of the cancer cells. *In vivo*, low dosage of SIS3 inhibitor had little demonstrable effect on the tumour growth of osteosarcoma at concentrations without side effect(s). However, when SIS3 inhibitor was delivered together with YangZheng XiaoJi, either orally or by way of intraperitoneal route, there were significant results; the inhibitory effect on growth was markedly enhanced (tumour size 60.6±28.8 mm^3 for oral treatment, 36.6±27.5 for peritoneal treatment, $p<0.05$, compared with control (98.7±70.6)). YangZheng XiaoJi extract had a marked influence on the distribution and intensity of phospho-SMAD-3 in cancer cells. *Conclusion:* The present study has shown that SIS3, a SMAD-3 small inhibitor, has an inhibitory effect on the adhesion and migration of cancer cells. However, YangZheng XiaoJi, a traditional medicine used in the treatment of cancer, can sensitise cancer cells to SIS3, which results in SIS3 becoming a more potent anti-cancer and anti-growth agent.

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P01-S-TEC
ACTIVATED LEUKOCYTE CELL ADHESION MOLECULE (ALCAM) IN DISTANT METASTASIS OF CANCER

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Background/Aim: A number of cancers display a predisposition to metastasise to the bone environment, including prostate, lung and breast cancer. Metastatic spread to the bone has serious implications on patient prognosis and survival. Mechanisms accounting for the tendency of certain cancers to establish bone metastasis are currently poorly understood. The activated leukocyte cell adhesion molecule (ALCAM) has been identified as a molecule where expression is frequently altered in the progression of many cancer types and suggested as a molecule involved in the establishment of bone metastasis. *Materials and Methods:* Prostate cancer cell lines were used as a model system and transfected with ALCAM ribozyme transgenes to generate knockdown lines. ALCAM suppression was subsequently verified using qPCR and Western blot analysis. Functional assays were carried out to establish the cellular impact of ALCAM suppression both in the presence and absence of a bone matrix extract (BME) derived from femoral heads on traits, such as cell growth, invasion, matrix-adhesion and motility. *Results:* Targeting of ALCAM using ribozyme transgenes successfully suppressed the expression of this molecule in both PC-3 and LNCaP cell lines at the transcript and protein level. Knockdown of ALCAM resulted in a more aggressive phenotype in both cell lines, thus significantly enhancing cell motility and invasiveness. ALCAM-suppressed PC-3 cells displayed a greater response to BME treatment than control cells. In contrast, little difference in the responsiveness to BME was seen between ALCAM-suppressed and control LNCaP cells. *Conclusion:* Loss of ALCAM enhances the aggressive nature of PC-3 and LNCaP cells and appears to play key roles in cell motility and invasiveness. Initial data also suggest that ALCAM may be involved in regulating a subtype of prostate cancer cells to respond to certain bone proteins, present in our BME, but not other prostate cell subtypes. Our data suggest ALCAM may play a role in the responsiveness of certain prostate cancer cell types in the bone environment and that this may subsequently impact on the developing metastatic tumour.

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P02-S-LEU
IDENTIFICATION OF AN NF-κB-DEPENDENT CD38 POSITIVE FEEDBACK LOOP IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background/Aim: Chronic lymphocytic leukemia (CLL) is an incurable disease characterized by the clonal accumulation of malignant CD5+/CD19+ B-lymphocytes in peripheral blood, bone marrow, lymph nodes and spleen. High expression of CD38 is a marker of poor prognosis in CLL; however, the molecular mechanisms that regulate its expression are currently unknown. Previous studies have suggested that micro¬environmental stimuli, such as CD40L, may play a key role. Here we have investigated the hypothesis that CD40L-dependent expression of CD38 is regulated by nuclear factor-κB (NF-κB)-mediated transcription. *Materials and Methods:* Primary CLL cells were co-cultured with sub-confluent monolayers of irradiated CD40L-expressing mouse embryonic fibroblasts (CD40L), or their non-transfected counterparts (NTL), in the presence of interleukin-4 (IL-4). Changes in CD38 expression were subsequently measured by flow cytometry and qPCR, while NF-κB expression and activity were analyzed using Western blotting and ELISA. NF-κB activity was inhibited using the IKK inhibitor, BAY 11-7082. *Results:* A significant increase in CD38 expression was observed in primary CLL cells following 48 h co-culture with CD40L-expressing fibroblasts compared to NTL co-culture (mean fluorescence intensity (MFI)=901 *vs*. 1,598; p=0.03; n=40). The magnitude of CD38 induction in response to CD40L was highly variable between patients, with a strong positive correlation evident between basal CD38 expression and the percentage change in CD38 expression following CD40L stimulation (r=0.51; p=0.0009). Responding patients typically possessed basal CD38 levels greater than 50%; as such, patients were segregated into CD38 low expressers ($CD38^{Lo}$; expression <50%) and CD38 high expressers ($CD38^{Hi}$; expression ≥50%). The increase in CD38 expression following CD40L co-culture was far greater for $CD38^{Hi}$ patients (MFI=2,247 *vs*. 4,517; p=0.03; n=12) than $CD38^{Lo}$ patients (MFI=324 *vs*. 347; p=0.05; n=28), although both were significant. The variable responses observed between $CD38^{Lo}$ and $CD38^{Hi}$ patients were also seen in the sub-populations of patients bimodal for CD38 (n=7), with an increase in CD38 expression evident in the $CD38^{Hi}$ sub-population of cells only. Using BAY 11-7082 (2.5 μM), we were able to negate the induction of CD38 expression by CD40L (MFI=4,230 *vs*. 3,014; p=0.02; n=13), thus confirming a role for NF-κB in CD40L-dependent expression of CD38. *Conclusion:* CD38 expression was increased following CD40-ligation and this modulation occurred as a function of basal CD38 expression. Pharmacological inhibition of NF-κB negated the observed induction of CD38 expression. Together, the data suggest $CD38^{Hi}$ CLL cells are preferentially responsive to NF-κB-mediated *de novo* transcription of the CD38 gene, implying that a CD38 positive feedback loop may be operational in CLL.

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P01-S-PNC
CAPILLARY MORPHOGENESIS GENE 2 IS OVER-EXPRESSED BY LOCAL ADVANCED PANCREATIC TUMOURS AND ITS CORRESPONDING THERAPEUTIC POTENTIAL

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Background/Aim: Capillary morphogenesis gene 2 (*CMG2*), also known as anthrax toxin receptor 2, was identified as a gene being up-regulated in capillary morphogenesis. It has been shown to be involved in cell adhesion and motility, which are critical capacities of cancerous cells to disseminate. Our recent studies have shown that *CMG2* is deregulated in breast cancer and prostate cancer and its extracellular part is also a valuable target for suppressing tumour growth directly or indirectly *via* interfering angiogenesis. The present study aims to investigate its implication in pancreatic cancer. *Materials and Methods:* Pancreatic tumours (n=230) together with paired background pancreatic tissues were collected at Peking University Cancer Hospital immediately after surgery with written consent by the patients. All protocols and procedures of the tissue collection were approved by the Peking University Cancer Hospital Research Ethics Committee. Total RNA was isolated from the sectioned frozen tissues and converted to cDNA using a reverse transcription kit. *CMG2* transcripts were then determined using real time-PCR. The association with clinical outcomes and other clinicopathologic parameters will be analysed using SPSS statistics. The expression of *CMG2* in pancreatic cancer cells was also assessed using RT-PCR. Knockdown of *CMG2* was performed using anti-CMG2 ribozyme transgene and its effect on cell adhesion was determined using electric cell-substrate impedance sensing (ECIS) assay. *Results:* The expression of *CMG2* tends to be higher in pancreatic tumours. Higher transcript levels were seen in ductal carcinoma and adenocarcinoma compared to other histological types. Tumours at the tail and locations other than the pancreatic head and body express lower *CMG2* transcripts. Local advanced tumours (T3 and T4) exhibit higher levels of *CMG2* transcripts, p=0.0003, compared with tumours at earlier stages (T1 and T2). Knockdown of CMG2 resulted in an impaired adhesion of pancreatic cancer cells to extracellular matrix (ECM) and peritoneal mesothelium. *Conclusion:* Higher expression of *CMG2* is more frequently observed in the adenocarcinoma and ductal carcinoma,

which is associated with the local invasive progression. Targeting CMG2 expressed by pancreatic cancer cells reduced their adhesion to ECM and mesothelium. These experimental evidences suggest important roles played by *CMG2* in the disease progression of pancreatic cancer and warrant therapeutic potential by targeting this molecule.

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P02-S-PNC
COMPARISON OF THE COMPOSITION OF BILE ACID PROFILES IN PATIENTS WITH ADENOCARCINOMA OF THE PANCREAS AND BENIGN DISEASE

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Background/Aim: In the UK, 21 people are diagnosed with adenocarcinoma of the pancreas each day, with only 3% of people surviving 5 years after diagnosis. Currently, only surgery offers the possibility of cure. A better understanding of the pathogenesis of this disease is required in order that prevention, early detection and effective treatments are realised. Bile acids have been implicated in the development of gastrointestinal malignancies. Both the specific nature of individual bile acids and their concentration appear key factors in the carcinogenic potency of bile. Using liquid chromatography mass spectrometry (LC-MS), the aim of this study is to analyse bile acid profiles, by extracting bile directly from the common bile duct, of patients with pancreatic cancer and benign disease. *Patients and Methods:* The study was divided into two groups, each consisting of 15 patients. The first group had a diagnosis of biliary colic with patients who underwent cholecystectomy surgery. The second group of patients underwent pylorus preserving pancreatico-duodenectomy and diagnosed with adenocarcinoma of the pancreas. Bile acids were isolated and quantified using LC-MS. *Results:* A trend towards a higher concentration of unconjugated bile acids was seen in the malignant group compared to the benign group. A significant difference (p=0.018) was seen in the concentration of unconjugated cholic acid in the malignant group (0.350 mg/ml) compared to the benign group (0.012 mg/ml) with an overall significant difference (p=0.037) seen in the level of total unconjugated bile acids in the malignant group (1.025 mg/ml) compared to the benign group (0.039 mg/ml). *Conclusion:* We have demonstrated a significant difference in the bile acid profiles on comparing patients with adenocarcinoma of the pancreas and benign biliary disease. Further research is required to determine the mechanism underlying these changes seen in bile acid composition.

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P03-S-PNC
CLINICAL IMPLICATIONS OF EPITHELIAL PROTEIN LOST IN NEOPLASM (EPLIN) IN PANCREATIC CANCER

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Background/Aim: Pancreatic cancer has recently begun to increase in the general population by reasons that remain unclear, although it may be linked to exposure to potential risk factors and/or genetic determinants. Currently, the UK has screening programs for breast, cervical and bowel cancer; however, no screening program exists for pancreatic cancer as no suitable biomarker is currently available. Pancreatic cancer has a poor prognosis, with only 1% of people surviving greater than 10 years following diagnosis. Identification of a specific molecular target, which may elucidate the aggressiveness of pancreatic cancer, would, therefore, be crucial in understanding its pathophysiology and may aid development of potential therapies. Epithelial protein lost in neoplasm (EPLIN) is a cytoskeletal-associated molecule involved in actin organisation and regulation at epithelial cell junctions. Frequent loss or aberrant expression of EPLIN has been previously associated with progression of various cancer types, including breast, lung, oesophageal and prostate. This study aims to evaluate the expression levels of EPLIN-α in a pancreatic cancer cohort and its association with clinical pathological factors using quantitative polymerase chain reaction, in order to determine the functional role it may play in pancreatic cancer progression. *Materials and Methods:* Tissues were collected immediately after surgery and subsequently homogenised in an RNA extraction solution to extract total RNA following which cDNA was generated by reverse transcription (RT). Expression of the *EPLIN-α* transcript was determined in a

pancreatic cohort (n=223 paired tissue samples) using quantitative polymerase chain reaction (Q-PCR). Data was compared against clinical and pathological follow-up of the patients. *Results:* EPLIN levels were lower in cancerous tissues compared with normal tissue. Highly differentiated tissue had higher EPLIN expression compared with lower differentiated tissue. Lower EPLIN expression was also associated with lymph node involvement and tissue where metastases were present. Patients who died of pancreatic cancer had lower levels of EPLIN compared to patients who survived. *Conclusion:* This study provides a putative link between EPLIN and the development of pancreatic cancer. EPLIN levels are expressed at lower levels in pancreatic cancerous tissues compared to normal tissue and lower EPLIN levels are associated with more advanced cancerous traits. This down-regulation indicates prognostic value for EPLIN. These data, taken together with previous findings of EPLIN's role in breast, lung, oesophageal and prostate cancer, illustrate an intriguing role for EPLIN in cancer progression and metastasis.

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P01-S-PRC
PROHIBITIN CAUSES THE DOWN-REGULATION OF E2Fs AND MCMs LEADING TO CELL ARREST IN PROSTATE CANCER

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Background/Aim: Prostate cancer (PCa) is the most common cancer in Western males and, initially, is dependent upon androgen stimulation carried out by the androgen receptor (AR). Therapies, which inhibit this androgen stimulation, are successful until the tumour becomes non reliant upon androgen supply through aberrant AR signalling, thus relapse occurs. The transition of androgen dependence to androgen independence still needs to be elucidated. However, investigating the co-repressors of the AR may provide an alternative avenue for androgen independent prostate cancer treatment. One such repressor, namely prohibitin (PHB), has been previously identified to be down-regulated in response to androgen stimulation and is down-regulated in PCa. Low levels of PHB allow PCa cells to become sensitized to a low androgen environment and, thus, may contribute to the androgen independence mechanism. The aim of this project was to identify the mechanism of action of PHB and how it affects gene expression of cell cycle-associated genes, cell proliferation and cell migration. Also the same analysis will be carried out using a stably transfected cell line with complete or partial knockdown of *PHB*. *Materials and Methods:* RNA-Seq was used to assess genes that were modulated in response to PHB over-expression. These genes were then validated by SYBR® green real time Q-PCR. The crystal violet assay was used to monitor the proliferation of the cells. Electric cell impendence sensing (ECIS) was used to monitor migration after a wound was initiated. *Results:* Increased expression of PHB showed a decreased expression in family members of the MCM, E2Fs, cyclins and Rb (n=2). These results were further confirmed with real time SYBR® green Q-PCR. Over-expression of PHB also significantly reduced the cell proliferation in 24 and 48 hours. This was also supported by ECIS, which demonstrated that PHB over-expression not only reduced proliferation before a wound was initiated but also reduced the wound closure after a wound was initiated. *Conclusion:* Results collected so far indicate that PHB plays a crucial role in preventing cell proliferation by down-regulation of genes associated with the cell cycle. Moreover, PHB has preliminarily been shown to impact the migration of cells after a wound is initiated. To conclude, PHB could be a potential target to overcome androgen independence.

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P03-S-PRC
ASSESSMENT OF PI3-KINASE, MAP-KINASE AND WNT DEPENDENT PATHWAYS TO HELP RISK-STRATIFY PATIENTS WITH PROSTATE CANCER

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Background: The spectrum of genetic mutations in prostate cancer (PCa) is diverse, with tumour heterogeneity revealing a low rate of recurrent lesions. However, recurrent alterations in certain signalling pathways, such as PI3-Kinase, MAP-Kinase and Wnt do predominate. The precise roles of these pathways and the interplay between them remain poorly understood. *Materials and Methods:* Three hundred and seventeen prostate samples from 245 patients were used to construct a tissue-micro-array (TMA). Immuno-histochemistry was used to assess each pathway. To assess deregulation and interaction between these pathways, mice harbouring deletion of *Pten* and activated mutations in beta-catenin (*Ctnnb1*) and *K-Ras* were generated. *Results:* The expression levels of PI3-Kinase, MAP-Kinase and Wnt are increased in PCa and in higher-risk disease (GG>7). Activation of each pathway also predicts biochemical relapse (BCR) following prostatectomy. Furthermore, when a greater number of pathways are activated, the risk of early BCR is

greater. To support this interaction, mice harbouring deregulation of a greater number of pathways develop more aggressive tumours with a reduced survival. *Conclusion:* PI3-Kinase, MAP-Kinase and Wnt are important in PCa and can predict higher risk disease and risk of BCR. This work enables risk-stratification of patients based on pathway analysis at the time of prostatectomy, highlighting cases that could benefit from adjuvant or targeted systemic therapies.

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P04-S-PRC
EXPRESSION ANALYSIS OF PUTATIVE STEM CELL MARKERS IN HUMAN PROSTATE CANCER

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Background/Aim: The cell of origin or the cancer stem cell (CSC) has been extensively researched; however, a robust marker for the normal stem cell or CSC has yet to be found in prostate cancer. The CSC has the ability to self-renew and proliferate, mechanisms that are thought to be instrumental in the development of recurrence and metastasis. *Materials and Methods:* CD44, integrin B1, Foxa-1, Notch 1, Notch 4, Trop2, FMRD4a and CD49f expression was detected by immunohistochemical (IHC) staining using tissue microarray (TMA) assay. There were 244 prostate samples from 205 patients. The mean patient age was 65.5 years (range=40-86). There were 117 Gleason 6, 55 Gleason 7, 26 Gleason 8, 4 Gleason 9 and 3 Gleason 10 samples on the TMA. In addition, there were 72 normal samples, which were histologically normal prostate tissue adjacent to tumour. The scoring was performed based on proportion and intensity of staining. Proportion: 0, no staining; 1, 1/100; 2, 1/10; 3, 1/3; 4, 2/3; 5, 1. Intensity: 0, negative; 1, weak; 2, intermediate; 3, strong. An overall score was then calculated combining proportion and intensity staining. *Results:* We found that the mean expression levels of integrin B1, Foxa-1, Notch 1, Notch 4, Trop2 and CD49f were significantly increased in prostate cancer when compared to normal tissue adjacent to tumour ($p<0.001$). In addition, integrin B1, Foxa 1, Notch 1 and Notch 4 expression levels were higher in high-grade tumours ($p<0.01$). There was no significant difference in expression levels of CD44 and FMRD4a. *Conclusion:* Our results, using IHC, show increased staining patterns of many putative CSC markers in prostate cancer with some positively correlated to high-grade tumours (Foxa-1, Notch 1, Notch4, integrin B1). A larger number of cells were stained for these markers. This finding does not fit with the hypothesis that the CSC is a small fraction of the total cell population. We propose that these markers stain for cells that possess greater 'stemness': the ability to self-renew and proliferate. As the tumour progresses, the tumour cells become more 'stem-like' resulting in expansion of the tumour cell population. Targeting the CSC could be fundamental for future treatments to prevent progression and treatment failures.

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P05-S-PRC
A ROLE FOR RECEPTOR-ACTIVATED NUCLEAR FACTOR κB (RANK) IN PC-3 PROSTATE CANCER CELL PROLIFERATION AND MATRIX-ADHESION

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Background/Aim: Prostate cancer is the most commonly diagnosed cancer amongst men in the UK and, though treatment of localised disease has improved in the last decade, metastatic disease results in substantial morbidity and mortality. The role of receptor activator of nuclear factor κB (RANK) has been extensively studied in bone biology and links have been made to breast cancer; however, its role in prostate cancer cell biology is poorly understood. The aim of this study was to explore the functional role of RANK in PC-3 prostate cancer cells. *Materials and Methods:* RANK expression was targeted in PC-3 prostate cancer cells using anti-RANK targeted hammerhead ribozyme transgenes. Successfully targeting of RANK expression was verified by qPCR and Western blot. The function of targeting RANK was assessed using a variety of *in vitro* function assays, including cell proliferation and cell-matrix. *Results:* Successful transfection of PC-3 cells resulted in a significant reduction in transcript and protein levels. Suppression of RANK expression resulted in significantly increased cell proliferation (PC-3^{RANKKD}) over both 3- and 5-day incubation periods compared to the control cell line (PC-3^{pEF6}) ($p\leq0.01$). RANK suppression also significantly increased cell-matrix adhesion compared to the control PC-3^{pEF6} cells ($p<0.05$). *Conclusion:* Much of the previous work, characterising the role of RANK in prostate cancer, has focused on its interaction with its receptor RANKL in the bone environment. This study has shown that there may be potential for tumour-expressed RANK to affect prostate cancer development and progression. Further investigation into the signalling pathways affected by altered RANK expression may further elucidate the role RANK plays in prostate tumour cell behaviour.

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P06-S-PRC
THE ERM PROTEINS AND EMT

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Background/Aim: Epithelial-mesenchymal transition (EMT) is the process of disaggregating structured polarised epithelial cells into single motile fibroblast-type cells to enable cell movement and morphogenesis. EMT gained widespread recognition as a potential mechanism for the progression of malignancy, attributed to the loss of epithelial characteristics and the acquisition of a migratory and highly matrix-invasive phenotype. Members of the Ezrin, Radixin and Moesin (ERM) protein family act as membrane cytoskeleton linkers and have been shown to play a role in cell adhesion, motility and morphogenesis and participate in various signal transduction pathways. This study aimed to knockdown expression of the ERM proteins in prostate cancer cells (PC3) and to look at changes in transcription factors, as well as changes in cell migration after knockdown. *Materials and Methods:* Ribozyme transgenes for Ezrin, Radixin and Moesin were constructed and PC3 knockdown cells were created ($PC3^{Ezrin\ R1}$; $PC3^{Radixin\ R2}$; $PC3^{Moesin\ R2}$). PCR and Western Blot analysis was carried out to assess changes in transcription factors. Scratch assay, using the EVOS® system, was performed to look at any changes in cell migration after knockdown. *Results:* PCR analysis revealed a reduction in occludin (*OCLN*) and *ZO1* expression in the PC3 Ezrin-knockdown cells. These Ezrin knockdown cells also showed a significantly faster cell migration after wounding than the PC3 wild type and control plasmid cells ($p<0.001$). However, knockdown of Radixin in the PC3 cells appeared to slow down migration of these prostate cancer cells ($p<0.03$). *Conclusion:* Knockdown of Ezrin in the PC3 cells lead to a decrease in the tight junction proteins occludin and ZO1 suggesting a breakdown in cell-cell contacts, which was seen by the increased motile nature of these cells. Interestingly, knockdown of Radixin resulted in impaired migration of prostate cancer cells. These initial results will be further investigated by looking at the morphology of these cells in order to elucidate the links with migratory status.

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P07-S-PRC
STUDYING miRNA EXPRESSION PROFILE IN PROSTATE CANCER CELL LINES

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Background: Prostate cancer is the 6th cause of death in males worldwide and, for this reason, early detection of the disease and application of successful treatments is important. Knowing that prostate-specific antigen (PSA) screening is controversial, the need of identification of alternative biomarkers having the ability to minimise the over-diagnosis associated with PSA screening would be of benefit. Recently a great interest has been expressed on the investigation of small non-coding RNA molecules called microRNAs because they have been associated with the initiation and progression of many types of human cancer, including prostate cancer. *Materials and Methods:* In this study, the expression of miRNA27a, miRNA132 and miRNA155 has been validated through RT-qPCR in PC-3, DU146 wt and DU145Rab27aKD prostate cancer cell lines. In addition, we produced PC-3 conditioned media in which we analysed the released miRNAs and we studied their effects on MRC5 fibroblasts and HECV endothelial cells in an attempt to find possible phenotypic variations, such as changes in their growth rate, which potentially could be explained as result of miRNAs effect. We used size exclusion chromatography and nanosight technology as orthogonal approaches to characterize miRNA combinations derived from conditioned media of PC3 cells and DU145 wt cells. *Results:* The expression of the above studied miRNAs has been identified in the intracellular space of the prostate cancer cell lines and also in their conditioned media. The effect of conditioned media on the incubation of MRC5 and HECV cells stimulated their growth. We found that the above circulating miRNAs co-fractionated with protein complexes and also with nanovesicles. The assays were performed in triplicate and the results were evaluated with unpaired *t*-test of one-way ANOVA using the GraphPad Software 6.0. *Conclusion:* Our results reveal that our prostate cancer cell lines over-express internally miRNA27a, miRNA132 and miRNA155 but also release the above functional miRNAs in two populations of circulating molecules associated with nanovesicles and also as independent molecules associated with other compounds of conditioned media.

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P01-S-WH
EXPRESSION OF PIGMENT EPITHELIUM-DERIVED FACTOR IN WOUND HEALING CELLS

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Background/Aim: Pigment epithelium-derived factor (PEDF) is a 50kDa secreted glycoprotein that has been identified as a member of the *SERPIN* gene family and has been shown to exhibit neurotrophic, neuroprotective, anti-angiogenic and anti-tumourigenic effects. The aim of our study was to determine the expression profile of PEDF in human dermal fibroblast, human HaCaT keratinocyte, human HECV endothelial cell lines and human adipose-derived stem cells and chronic wound tissue samples. *Materials and Methods:* Four human cell lines (dermal fibroblasts, HaCaT, HECV and human adipose-derived stem cells) were analysed using polymerase chain reaction (PCR) and quantitative transcript analysis (qPCR). Tissue from chronic wounds (healing and non-healing) were collected and analysed with immunohistochemistry. *Results: PEDF* transcript was highly positive in human adipose stem cells. On qPCR, *PEDF* was more highly expressed in human adipose stem cells and human dermal fibroblasts when compared to human HaCaT keratinocyte and human HECV endothelial cell lines ($p<0.05$). Immunohistochemistry staining showed PEDF expression in all layers of the epidermis in the majority of chronic wound tissue samples studied. There was also evidence of high PEDF expression at the dermal/epidermal junction, as well as blood vessel and fibroblast staining in most of the chronic wounds. There was a tendency to have decreased PEDF expression at the leading wound edge in healing wounds. *Conclusion:* PEDF expression was high in human adipose stem cells, as well as dermal fibroblasts. Further work is proposed to investigate the effect of PEDF expression on cell function and potentially provide evidence to develop PEDF or its fragments as therapeutics for wound healing.

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P02-S-WH
THE ROLE OF CLAUDIN-5 IN THE CELL-CELL ADHESION OF HUMAN KERATINOCYTES

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Background/Aim: Claudins are a family of proteins that are the most important components of the functioning adherens and tight junctions (TJ) in the intercellular space between the cells of an epithelium. Aberrant expression is associated with wound healing but little work has been carried out in discovering how this affects a change in cell behaviour. This study aimed to ascertain the distribution and the effect of Claudin-5 in human keratinocytes. *Materials and Methods:* Claudin-5 expression in human tissue was analysed using Q-PCR and immunohistochemistry. Over-expressiong/knockdown cells were created and functional testing included cell growth, adhesion, invasion and scratch assays. *Results:* The wild type HaCaT cells expressed Claudin-5 and so were chosen to create a Claudin-5-knockdown. Claudin-5 knockdown cells has reduced growth ($p<0.05$). In adhesion experiments, the results showed that Claudin-5-knockdown cells (HaCatΔCLD5) were significantly less adhesive ($p<0.005$). The invasion experiment did not reveal differences between the wild type, plasmid control or Claudin-5-knockdown cells. Scratch assay revealed that the Claudin-5-knockdown cells have significantly less motility ($p<0.001$). *Conclusion:* This aberrant expression of HaCat-knockdown cells indicates that Claudin-5 may be an important factor in the formation of cell junctions.

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Amso Nazar **FBO3B03**
An Wei **SBO5C02**
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Chen Zhenwen **FBO2C05**
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McGuigan Christopher **SBO4C01**
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