**Results:** The treatment of tumour cells with the various EMILIN2 deletion mutants led to the identification of the pro-apoptotic region of the molecule. This N-terminal fragment binds to death receptors, induces apoptosis and reduces the clonogenic potential of tumour cells. EMILIN2 and its deletion mutant displayed also an *in vivo* antitumourigenic effect which correlated with a higher activity of both caspase-8 and -3. Unexpectedly, tumours treated with EMILIN2 or the deletion mutant displayed a significant increase of tumour angiogenesis. In view of these findings the co-treatment of the growing tumours with an antiangiogenic drug, resulted in most cases in a complete regression of tumour growth.
**Conclusions:** Taken together these results unravel the possibility to employ EMILIN2 fragments or peptides in combination with angiogenesis inhibitors as potent antineoplastic tools for cancer treatment.

### 484 Rapid adherence to collagen IV enriches for tumour initiating cells

X. Liang[1], F.H. Labeed[2], R. Abdallat[2], A.C. Johannessen[1], O. Tsinkalovsky[1], J. Wang[3], D.E. Costea[1]. [1]*Gades Institute, Department of Pathology, Bergen, Norway*, [2]*Centre for Biomedical Engineering, School of Engineering, Guildford, United Kingdom,* [3]*Translational Cancer Research, Department of Biomedicine, Bergen, Norway*

**Background:** There is now evidence for the existence of cancer stem cells in many solid tumours, including oral squamous cell carcinoma. However, there is still a need to develop robust methods to enrich for cancer stem cells for studying their biological properties. Data from normal epithelia indicate that the rapid adherence to collagen IV is an efficient method to enrich for normal epithelial stem cells.
**Objective:** To investigate the rapid adherence to collagen IV as a method for enrichment for cancer stemm cells in human oral carcinomas and to characterize the cell populations obtained using this method in terms of their self renewal potential and electrophysiological properties.
**Methods:** Rapid adherent cells (RAC) and middle adherent cells (MAC) were isolated after 10 and respectively 60 minutes incubation on collagen IV-coated dishes in a panel of oral carcinoma cell lines (H357, DOK and CaLH3). The non-attached cells were designed as late adherent cells (LAC). Their clonogenic ability was investigated in vitro (single cell colony forming assay and 3D organotypic model).and their ability to initiate tumours was investigated in vivo (tongue xenograft NOD/SCID mouse model). The electrophysiological parameters of cells were determined non-invasively, using dielectrophoresis (DEP)-an electrostatic phenomenon defined as the motion of particles resulting from polarisation forces. Parameters such as cytoplasmic conductivity (which relates to the cytoplasm ionic strength), membrane conductance (indicates how well ions are transported across the membrane), and specific membrane capacitance (relates to membrane morphology) were extracted using the single-shell model.
**Results:** Significantly higher number of cells were found to initiate colonies ($p < 0.05$) and form spheres in vitro ($p < 0.01$) in both RAC and MAC when compared to LAC. MAC tumour formation was the fastest to occur, but both RAC and MAC induced tumour formation at earlier time points and at lower cell numbers than LAC after tongue xenotransplantation in NOD/SCID mice. No difference was observed in 3D cultures in terms of biomatrix invasion, but RAC and Mac gave rise to thicker cultures when compared to LAC. DEP analysis revealed that RAC and MAC exhibited a significantly higher membrane capacitance relative to LAC ($p < 0.001$), indicating a difference in the membrane morphology between these subpopulations of cells.
**Conclusion:** This study brings evidence for the use of rapid adherence to collagen IV for enriching in cells with increased clonogencity and tumour formation ability in oral cancer cell lines, and indicates that these properties are associated to differences in electrophysiological properties.

### 485 Checkpoint kinase 1 modulates sensitivity to chemotherapy in aneuploid cell lines

I. Sanchez-Pérez[1], A. Peralta-Sastre[2], C. Manguan-Garcia[1], A. Gonzalez-Gutierrez[2], C. Belda-Iniesta[3], R. Perona[1]. [1]*Instituto de Investigaciones Biomedicas Madrid. CIBERER, Modelos Experimentales de Enfermedades Humanas, Madrid, Spain,* [2]*Instituto de Investigaciones Biomedicas Madrid, Modelos Experimentales de Enfermedades Humanas, Madrid, Spain,* [3]*Hospital Universitario La Paz, Servicio de Oncologia Médica, Madrid, Spain*

Gastric cancer (GC) is one of the most frequent causes of death worldwide. Despite many advances in surgery and the diagnosis or the development of new regimens of chemotherapy (QT), after surgery most patients die of recurrent disease due to the presence of disseminated disease at the time of surgery. The main treatment of disseminated disease is chemotherapy, which only benefits a few and cause toxicity in the majority of patients. Therefore, it is necessary to improve the capability of selecting those patients most likely to have clinical benefit with a determined treatment. Our previous studies showed that SW620 cells showed higher BubR1 and Chk1 mRNA levels than control cells under normal conditions. These studies showed that these cells undergo synergistic cell death after spindle checkpoint activation (taxol treatment) followed by cisplatin treatment, suggesting a role of Chk1 in this checkpoint, very likely dependent on BubR1 protein. Importantly, Chk1-depleted SW620 cells lost this synergistic effect. In summary, we proposed that Chk1 could be used as a biomarker predictive of the efficacy of sequential chemotherapy across different types of tumours with aneuploidy. These results encouraged us to deeply study the role of Chk1 protein as a predictive factor of response to this combined chemotherapy in GC. A panel of cell lines derived from GC with and without aneuploidy, will be selected and treated with a combination of 5-Fluorouracil, cisplatin and taxane derivatives, in order to study the viability and the cross-talk between the activation of the checkpoint protein Chk1 and the spindle assembly checkpoint, as these are the main signaling pathways activated by these agents. The results of these studies will be reported at the Meeting.
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### 486 The sphingosine kinase-1 survival pathway is a molecular target for the tumour-suppressive tea and wine polyphenols in prostate cancer

L. Brizuela Madrid[1], A. Dayon[1], N. Doumerc[2], I. Ader[1], J.C. Izard[3], B. Malavaud[2], O. Cuvillier[1]. [1]*Institute of Pharmacology and Structural Biology, Cancer, Toulouse, France,* [2]*Hôpital Rangueil, Toulouse, France,* [3]*Actichem, Montauban, France*

*In vitro* and *in vivo* studies have reported that dietary polyphenols can affect a wide array of signaling and molecular pathways resulting in cancer cell growth inhibition, apoptosis and inhibition of invasion, angiogenesis and metastasis. Here we provide the first evidence that dietary agents, namely epigallocatechin gallate (EGCg), trans-resveratrol (RV) or a mixture of polyphenols from green tea (Polyphenon E, PPE) or red wine (vineatrol) impede prostate cancer cell growth in vitro and in vivo by inhibiting the SphK1/S1P pathway, which is up-regulated in prostate cancer patients. Our results establish that SphK1 is a downtream effector of the ERK/Phospholipase D (PLD) signaling pathway inhibited by green tea and red wine polyphenols. Enforced expression of SphK1 in both PC-3 and C4−2B prostate cancer cells markedky impaired the efficacy of green tea and red wine polyphenols, as well as pharmacological inhibitors of PLD- and ERK, to induce apoptosis. The inhibitory effects of green tea and red wine polyphenols on tumour growth and the SphK1/S1P pathway were confirmed in an heterotopic PC-3 tumour in place model established in nude mice. SphK1-overexpressing PC-3 cells implanted in animals developed remarkably larger tumours and resistance to treatment with polyphenols. Furthermore, in an orthotopic PC-3/green fluorescent protein model, EGCg and PPE diet induced a marked SphK1 inhibition associated with a pronounced decrease in primary tumour volume and occurrence and number of metastases. These results provide the first demonstration that the SphK1/S1P pathway is a molecular target of dietary polyphenols in prostate cancer.

### 487 Prostaglandin E2 upregulates ErbB2 and enhances EGF-stimulated DNA synthesis in hepatocytes

J.O. Odegard[1], M.A.A. Monica Aasrum[1], S.P.B. Suman P. Bharath[1], I.H.T. Ingun H. Tveteraas[1], D.S. Dagny Sandnes[1], T.C. Thoralf Christoffersen[1]. [1]*University of Oslo, Department of Pharmacology, Oslo, Norway*

**Background:** Several G protein-coupled receptor (GPCR) agonists, including prostaglandin $E_2$ ($PGE_2$), act as comitogens in hepatocytes, by synergistically enhancing EGF-stimulated DNA synthesis. The underlying mechanism is not clear. In MH1C1 hepatoma cells $PGE_2$ transactivates the EGF receptor (EGFR), but in normal hepatocytes $PGE_2$ induces an upregulation of EGF-mediated phosphorylation of Erk and Akt independently of EGFR transactivation. EGFR belongs to the ErbB family, and one factor that may contribute to the diversity of EGFR signaling is the availability of other ErbB members that can engage in heterodimerization with EGFR. In this study we examined the role of $PGE_2$ on the expression of ErbB2 and ErbB3, and their role in the comitogenic effect.
**Methods:** Rat hepatocytes were cultured as primary monolayers in a defined medium. Expression and phosphorylation of signalling proteins, including EGFR, ErbB2, ErbB3, Erk, Akt, and cyclin D1, were assessed by Western blotting. ErbB2 and ErbB3 mRNA was measured by quantitative real time PCR. DNA synthesis was determined by incorporation of $^3$H-thymidine. Transfection with small interfering RNA (siRNA) was used to block the expression of ErbB2.
**Results:** At plating, the cells expressed EGFR (ErbB1) and ErbB3, but not ErbB2. As they were cultured, traversing G1 with relatively high synchrony, ErbB3 expression decreased, while ErbB2 expression, in contrast, appeared and then increased up to a point in mid/late G1 where the cells are optimally sensitive to EGF. Pretreatment with $PGE_2$ increased ErbB2 expression and reduced ErbB3 expression. $PGE_2$ also enhanced and hastened EGF-stimulated cyclin D1 expression and DNA synthesis. Also, blocking of the

ErbB2 expression with specific siRNA blocked the $PGE_2$-induced amplification of cyclin D1 expression and DNA synthesis in response to EGF.
**Conclusion:** The results suggest that the upregulation by $PGE_2$ of the mitogenic response of hepatocytes to EGF may at least in part be mediated by increased expression of ErbB2.

### 488 Quantitative proteomics reveals secreted factors governing enhanced motility in rat C6 glioma cells expressing connexin43

M. Mesnil[1], V.C. Chen[2], P.O. Strale[1], N. Stoynov[3], L.J. Foster[3], C.C. Naus[4]. [1]*Institut de Physiologie et Biologie Cellulaires UMR 6187, Université de Poitiers, Poitiers, France,* [2]*Life Sciences Institute University of British Columbia, Department of Cellular and Physiological Sciences, Vancouver, Canada,* [3]*Centre for High-Throughput Biology University of British Columbia, University of British Columbia, Vancouver, Canada,* [4]*Life Sciences Institute University of British Columbia, University of British Columbia, Vancouver, Canada*

**Background:** Glioblastoma multiforme is a devastating tumour of the brain demonstrating higher rates of motility and invasion potential. Recent evidence has implicated the gap junction protein connexin43 (Cx43) in the motility of brain tumour cells. Supporting these findings, we also observed a correlated increase in motility of C6 glioma cells over-expressing Cx43 (C6−13 cells) compared to their wild-type counterparts (C6 cells). Since migration of tumour cells involves the secretion of proteolytic enzymes and cytokines, we tested the effect of the conditioned medium from C6−13 cells and observed that it increased the migration capacity of C6 cells up to the C6−13 cell level. In order to understand the molecular pathways associated with such a process, we have undertaken a proteomic approach to identify and quantify proteins secreted within the conditioned media of wild-type C6 cells and C6−13 cells.
**Materials and Methods:** Proteins isolated from media of 80% confluent C6 or C6−13 cell cultures were isotopically labeled at the peptide-level by reductive dimethylation and analyzed on a high performance liquid chromatograph hyphenated to a high-resolution linear trapping quadrupole-Orbitrap mass spectrometer by using Xcalibur software. Fragments spectra were identified using Mascot (v.2.2, Matrix Science) and quantitative ratios were extracted using MSQuant (http://msquant.sourceforge.net/).
**Results:** Differential analysis revealed, within the conditioned media of C6−13 cells, a significant up-regulation of secreted proteins involved in cell migration and known as markers of glioma aggressiveness. Such proteins were either cytokines (small inducible cytokine A2, osteopontin, latent TGF-B binding protein-1, lectin galactoside-binding soluble 3 binding protein), proteolytic enzymes (MMP3, cathepsins B and L1) and extracellular matrix compounds (collagen alpha-1 (VI), SPARC, tenascin-C and fibronectin). However, some extracellular matrix compounds were found to be decreased in the C6−13 culture medium (elastin microfibril interface 1, various procollagens) as a possible direct consequence of the action of the oversecreted proteolytic enzymes.
**Conclusion:** Findings presented in this study provide insights into enhanced cell motility linked to Cx43 expression and the molecular cues associated with the migration of tumour cells. Determining how Cx43 triggers the secretion of such diffusible factors involved in glioma cell invasion may lead to new therapeutics considerations.
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### 489 New animal model in colorectal cancer

D. Priolli[1], A.M. Abrantes[2], M. Dourado[3], A.B. Sarmento[4], L. Carvalho[3], C. Gonçalves[4], S. Neves[4], M.F. Botelho[2]. [1]*Universidade São Francisco, Cirurgia, Bragança Paulista, Brazil,* [2]*Universidade Coimbra, Biofisica, Coimbra, Portugal,* [3]*Universidade Coimbra, Patologia, Coimbra, Portugal,* [4]*Universidade Coimbra, Bioquimica, Coimbra, Portugal*

**Background:** To gain confidence in the validity of animal models research is essential to unequivocal quality and convincing data. Colon cancer is one of the most prevalent tumours in the world. Despite this, only in 2007 was presented a colon adenocarcinoma model in null mice. In this model, cancer cells were inoculated in animal cecum. A few considerations about this model should be made. Firstly, colorectal cancer is less usual in cecum, actually for this tumour type the most prevalent localization is distal colon. Secondly, inoculation in serosa layer in detriment of colonic mucosa where these tumours originate and, finally, maintenance of impossibility of monitoring tumour growth over time as an additional disadvantage. The aim of this study is to present new adenocarcinoma animal model in left colon that allows us monitoring tumour growth.
**Material and Methods:** Colon exclusion was made and distal fisula was kept open. Adenocarcinoma cells (WiDR) was inoculated in mucosa fistula after normal bowel function return. Neoplastic growing was monitored daily. Scintigraphic method was performed to tumour detection.
**Results:** After 4 days tumour growing was observed. Fifteen days after cells inoculation, tumour detection was possible to use molecular imaging, ten minutes after 99mTc-MIBI administration. Macroscopy demonstrated tumour invasion in proximal colon and it partial lumen occlusion. Microscopy demonstrated undifferentiated tumour with infiltration in all colon layers.
**In conclusion** this new colorectal cancer animal model is feasible and allows measuring it external growth and monitoring by $^{99m}$Tc-MIBI scintigraphy.

### 490 Changes in expression profiles of apoptosis, invasion, metastasis, angiogenesis, transcription factors, cell cycle control and tumour supressor genes in nilotinib treated chronic myeloid leukemia cells

Y. Baran[1], A. Camgoz[1], G. Can[1]. [1]*Izmir Institute of Technology, Department of Molecular Biology and Genetics, Izmir, Turkey*

**Background:** Chronic myeloid leukemia (CML) is a hematological malignancy arising from a reciprocal translocation between long arms of chromosomes 9 and 22. The resulting BCR/ABL fusion protein is a strong oncogenic protein that regulates cell growth and proliferation, apoptosis and senescence, migration and adhesion. Imatinib was the first tyrosine kinase inhibitor for the treatment of CML. Although there were significant hematologic and cytogenetic responses to imatinib, resistance cases were observed in patients during treatments and this was the major drawback of imatinib treatment. After identification of the mechanisms of imatinib resistance, a more effective anticancer agent, nilotinib, was developed and started to be used for the treatment of Philadelphia chromosome positive hematological malignancies.
**Aims:** In this study, we aimed to examine the molecular mechanisms of nilotinib-induced cell death in addition to inhibition of BCR/ABL in K562 chronic myeloid leukemia cells.
**Materials and Methods:** Antiproliferative effects of nilotinib were determined by XTT cell proliferation assay. Increasing concentration of Nilotinib (20 and 50 nM) were applied to K562 cells. After 72 hours incubation, total RNAs were isolated and converted to cDNA. Changes in expression levels of 84 genes involved in apoptosis, cell cycle, senescence, adhesion, invasion, metastasis, angiogenesis, transcription factors, and signal transduction molecules were examined by PCR array.
**Results:** There were 40 and 55% decreases in proliferation of K562 cells in response to 20 and 50 nM nilotinib, respectively, as compared to untreated controls. Gene expression results revealed that 50 nM nilotinib application resulted in more than 4-fold increases/decreases in expression levels of 41/6 genes as compared to untreated controls and normalized to housekeeping genes. On the other hand, lower concentration of nilotinib, 20 nM, increased/inhibited expression levels of 2/22 genes more than 2-fold comparing to untreated controls and normalized to housekeeping genes.. Nilotinib induced expression levels of apoptotic (Bax, Serpin5B, GZMA, TNF, TNFRSF25, APAF1) cell cycle controlling (CDK2, CDKN2A, MDM2), and inhibitor of metastasis (TIMP1, TIMP3) genes and decreased expression levels of growth inducing (AKT1, IGF1, MYC, NFKB, MAP2K1, PLAU), antiapoptotic (SNCG, SYK), metastatic (MMP1, MMP2, ITGB5, ITGA3) and angiogenic (IL-8, ANGPT2) genes. The highest increases were observed in apoptotic TNF and GZMA genes while the highest decreases were observed in growth inducing MAP2K1 and PLAU genes.
**Conclusion:** In this study, we demonstrated the molecular mechanisms of nilotinib induced cell death in addition to inhibition of oncogenic BCR/ABL protein. More importantly, we have also showed for the first time that nilotinib also has the potential to inhibit metastasis and angiogenesis through manipulating metastatic and angiogenic genes.

### 491 J7, a methyl jasmonate analogue, enhances TRAIL-mediated apoptosis through reactive oxygen species generation

Y. Choi[1], C. Park[2], C. Jin[3], B. Kim[4], G. Kim[5], J. Jung[6], W. Kim[7], Y. Yoo[8]. [1]*Dongeui University College of Oriental Medicine, Department of Biochemistry, Busan, South Korea,* [2]*Dongeui University, Blue-Bio Industry Regional Innovation Center, Busan, South Korea,* [3]*Dongeui University Graduate School, Department of Biomaterial Control, Busan, South Korea,* [4]*Dongeui University, Department of Life Science and Biotechnology, Busan, South Korea,* [5]*Jeju National University, Department of Marine Life Sciences, Jeju, South Korea,* [6]*Busan National University, College of Pharmacy, Busan, South Korea,* [7]*Chungbuk National University College of Medicine, Department of Urology, Cheongju, South Korea,* [8]*Dong-A University College of Medicine, Department of Anatomy and Cell Biology and Mitochondria Hub Regulation Center, Busan, South Korea*

**Background:** The jasmonates are fatty-acid-derived cyclopentanones that occur ubiquitously in the plant kingdom and they serve as natural bioregulators and are involved in plant intracellular signaling and defense in response to injury. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is known to induce apoptosis in cancer cells but spare most normal cells. However, its effect (s) is limited in some types of cancer cells, including HepG2 human hepatocarcinoma cells. In the present study, we showed that treatment