Material and Methods: The individual PI3K isoforms were targeted by either siRNA or isoform-specific pharmacological inhibitors. Additionally, combinational treatments with isoform-specific inhibitors and chemotherapeutic agents were performed. Cell proliferation (MTS assay), colony formation (soft agar assay), and induction of apoptosis (caspase activity by Caspase Glo 3/7 assay and PARP cleavage by WB) were measured after treatment. Activation of the PI3K/Akt signaling pathway was analyzed by using phosphorylation site-specific antibodies of downstream elements (western blot).
Results: Concentration dependent decrease in GBM cell proliferation was observed when using two distinct p110α-specific inhibitors. Targeting p110α additionally impaired anchorage-independent growth and down-regulation of the isoforms p110α or p110β by siRNA induced apoptosis in GBM cells. In agreement with these observations, treatment of GBM cells with p110α-specific inhibitors led to decreased activation of Akt and phosphorylation of the ribosomal protein S6. Combinational treatment of p110α inhibitors with chemotherapeutic agents led to a sensitization of GBM cells to chemotherapy.
Conclusions: Targeting individual PI3K isoforms has an impact on cellular responses, though it might be necessary to target more than one isoform simultaneously. This study will lead to a better understanding of the specific functions of class I_A PI3K isoforms in human GBM cell biology and will thus help in developing new targeted therapies to cure this common type of malignant brain tumour.

360 Role of DLL4 and JAG1 in tumour angiogenesis

C. Oon[1], J. Li[1], R. Sainson[1], H. Sheldon[1], H. Turley[1], R. Leek[1], A. Harris[1]. [1]*Weatherall Institute of Molecular Medicine, Department of Medical Oncology, Oxford, United Kingdom*

Background: The notch signalling pathway plays a crucial role in vascular development and physiology. Delta-like 4 (DLL4) and Jagged1 (JAG1) are two key notch ligands that are implicated in angiogenesis. The aim of this work is to study the functional difference between these two ligands *in vitro* and *in vivo*.
Material and Methods: HUVECs were seeded on DLL4 or JAG1 coated plates in a time series experiment and subjected to quantitative PCR to study the regulation of notch target genes such as HEY1, HEY2, EFNB2 and DLL4. Two U87 cell lines were generated which over-expressed murine DLL4 or murine JAG1. Growth of these cell lines *in vitro* or as subcutaneous tumours *in vivo* was compared to that of control wild-type U87 cell line. Subsequent changes in vascular phenotype, markers of hypoxia (CA9), proliferation (MIB-1) or apoptosis (TUNEL), and notch downstream target genes as mentioned above were then assessed.
Results: Preliminary quantitative PCR results showed that JAG1 is less potent than DLL4 in stimulation of notch target genes in HUVECs. The growth of U87 cell lines which over-expressed murine DLL4 or murine JAG1 was slower compared to wild-type U87 cell line *in vitro*. This inhibitory effect was not observed *in vivo*, where the cell lines over-expressing mDLL4 or mJAG1 grew faster than the wild type control cell line as subcutaneous tumours ($P < 0.01$). The two over-expressing notch ligand cell lines displayed the phenotype of lower number of apoptotic and proliferative cells compared to control tumours ($P < 0.05$) while U87 mDLL4 displayed a lower magnitude of hypoxia compared to control tumours ($P < 0.01$). Interestingly, tumours over-expressing mDLL4 had larger vessels compared to control, whereas mJAG1 produced more yet functional vessels ($P < 0.01$); both cell lines significantly reduced pericyte coverage ($P < 0.01$).
Conclusions: In conclusion, JAG1- and DLL4-notch signalling have different effects on vessel formation, which impacted on the growth of the tumours *in vivo.* The mechanism behind the differential responsiveness of the notch receptors to DLL4 or JAG1 ligands could either reflect modulation by fringes, a family of glycosyltransferases that regulate notch signalling or by a positive feedback loop present for DLL4-notch signalling only. This work has highlighted some key novel differences between the two notch ligands, which require further investigation and is highly relevant for anti-angiogenic therapy.

361 Targeting phosphoinositide 3-kinase signalling in small cell lung cancer

A. Wojtalla[1], B. Fischer[2], F.A. Mauri[3], J. Sobek[4], S. Zoller[4], M.J. Seckl[3], A. Arcaro[1]. [1]*University of Bern, Department of Clinical Research, Bern, Switzerland,* [2]*University Children's Hospital Zurich, Division of Clinical Chemistry and Biochemistry, Zurich, Switzerland,* [3]*Imperial College Faculty of Medicine, Lung Cancer Biology Group Division of Medicine, London, United Kingdom,* [4]*ETH and University of Zurich, Functional Genomics Center Zurich, Zurich, Switzerland*

The phosphoinositide 3-kinase (PI3K) pathway, fundamental for cell proliferation, survival, and motility, is known to be frequently altered and activated in neoplasia, including carcinomas of the lung. Based on the high frequency of alterations, targeting components of the PI3K signalling pathway is considered to be a promising therapeutic approach in cancer treatment. In this study we investigated the potential of targeting PI3K signalling in small cell lung cancer (SCLC), which is the most aggressive of all lung cancer types and almost entirely related to smoking. SCLC accounts approximately 15−20% of all lung cancer cases. Although most patients initially respond to chemotherapy, the overall 5-year survival is less than 5%. In order to control metastasis and chemoresistance, new targets for development of drug therapies are urgently required. An immunohistochemistry analysis in primary SCLC tissue samples showed over-expression of the PI3K isoforms p110α and p110β, compared to normal lung tissue. By targeting the PI3K isoforms p110α or p110β with specific pharmacological inhibitors or small interfering RNA (siRNA), we could observe strongly affected cell viability in SCLC cells. Expression and phosphorylation state of PI3Ks and signalling molecules were studied by Western blot and Taqman analysis. Downstream signalling molecules like vascular endothelial growth factor A (VEGFA) showed a downregulated gene expression in response to PI3K inhibition. We could also observe decreased phosphorylation levels in PI3K pathway components, such as Akt, ribosomal S6 protein, and 4E-BP1, and reduced expression of cell cycle-related molecules, like p21, Cyclin D_1, and Cyclin E. A DNA microarray analysis was performed in H69 SCLC cells treated with the inhibitors PIK75 (p110α) or TGX221 (p110β). In particular, PIK75 strongly affected gene expression in widespread functional categories. As targets for further investigation we chose genes related to cell growth, proliferation, cell cycle, and cell survival. A phosphorylation antibody array performed in H69 cells showed that targeting p110α with PIK75 led to a decreased activation state of PI3K/Akt/mTOR pathway components. Together, our studies will lead to a better understanding of the biological function of PI3K isoforms in controlling cell responses, such as proliferation, apoptosis, and metastasis in SCLC. Furthermore, this knowledge could point out novel targets and contribute to the development of urgently required new therapies

362 Targeting the glycolytic cancer cell − cancer-specific modulation of NAD+ and NADH and implications for anti-cancer therapy

S.J. Allison[1], J.R.P. Knight[1], J. Milner[1], R.M. Phillips[2]. [1]*University of York, Biology, York, United Kingdom,* [2]*University of Bradford, Institute of Cancer Therapeutics, Bradford, United Kingdom*

Cancer and non-cancer cells differ fundamentally in their metabolism and this may be exploited therapeutically. Cancer cells are metabolically reprogrammed to utilise glycolysis as their primary source of energy even in the presence of oxygen. To fuel glycolysis cancer cells avidly consume NAD^+. Modulation of NAD^+ levels by inhibiting enzymes involved in NAD^+ biosynthesis is a recognised strategy for targeting cancer's "sweet tooth". However clinical trials indicate damage to normal tissues by this approach consistent with non-cancer cells requiring a basal level of NAD^+ for normal cell functions. We demonstrate by RNAi that by targeting a key enzyme involved in the glycolytic switch (lactate dehydrogenase A) it is possible to selectively modulate the NADH/NAD^+ redox status of cancer cells and not that of non-cancer cells. We also show that: (i) this may enable cancer-selective targeting of NAD(H)-dependent enzymes with critical roles in cancer survival but for which direct targeting is problematic because of functions in non-cancer cells (eg. SIRT1); and (ii) this can increase the efficacy of redox-sensitive anti-cancer drugs (eg. EO9) in a cancer-selective manner. These findings identify a unique strategy for selectively targeting cancer and increasing the effectiveness of certain current anti-cancer drugs (via combinatorial therapy) based upon exploiting a fundamental metabolic difference between cancer and non-cancer cells.

363 The secretory small GTPase Rab27B drives poor prognosis in ER-positive breast cancer

A. Hendrix[1], W. Westbroek[2], V. Cocquyt[3], M. Bracke[1], O. De Wever[1]. [1]*Ghent University Hospital, Laboratory of Experimental Cancer Research, Ghent, Belgium,* [2]*NIH, NHGRI, Bethesda, USA,* [3]*Ghent University Hospital, Medical Oncology, Ghent, Belgium*

Background: Vesicle exocytosis, controlled by secretory GTPases such as Rab27B, delivers critical pro-invasive growth regulators into the tumour microenvironment. The biological role and expression status of Rab27B in breast cancer was unknown.
Materials and Methods: Rab27B was studied in estrogen receptor (ER)-positive human breast cancer cell lines (MCF-7, T47D, ZR75.1) using GFP-fusion constructs, including wild type Rab3D, Rab27A, Rab27B and Rab27B point mutants defective in GTP-binding or geranylgeranylation, and transient siRNA targeting. In cell culture, cell-cycle progression was evaluated by flow cytometry, Western blotting and measurement of cell proliferation rates, invasion was assessed using Matrigel and native collagen type I substrates. Orthotopic tumour growth, local invasion and metastasis were analyzed in mouse xenograft models. Mass spectrometry was performed to identify Rab27B-secreted pro-invasive growth regulators. In clinical breast cancer, Rab3D, Rab27A and Rab27B mRNA levels were analyzed by quantitative RT-PCR ($n = 20$) and Rab27B protein level was evaluated by immunohistochemistry ($n = 60$). Statistical tests were two-sided.

Results: Rab27B-upregulation promoted G1/S phase cell cycle transition and increased proliferation, F-actin reorganization and invasion in cell culture, and invasive tumour growth and haemorrhagic ascites in a xenograft mouse model (at 10 weeks, survival of MCF-7 GFP vs GFP-Rab27B injected mice was 100% vs 62.5%, P = 0.0307). Proteomic analysis of purified Rab27B-secretory vesicles and the secretome of exogenous Rab27B-expressing breast cancer cells identified heat shock protein (HSP)90α as key pro-invasive growth regulator. HSP90α secretion occurred in a Rab27B-dependent manner and was required for matrix metalloproteinase(MMP)-2 activation. All Rab27B-mediated functional responses were GTP- and geranylgeranyl-dependent. Endogenous Rab27B mRNA and protein, but not Rab3D and Rab27A mRNA, associated with lymph node metastasis (P = 0.0002) and differentiation grade (P = 0.0014) in ER-positive human breast tumours.
Conclusion: Rab27B regulates invasive growth and metastasis in ER-positive breast cancer.

364 JAM-A is over-expressed in aggressive Her2 positive breast cancers

K. Brennan[1], T. Roche[2], E.A. McSherry[1], T. O'Grady[2], E.W. Kay[2], A.M. Hopkins[1]. *[1]Royal College of Surgeons, Surgery, Dublin, Ireland, [2]Royal College of Surgeons, Pathology, Dublin, Ireland*

Background: Each year breast cancer is diagnosed in an estimated 1 million women worldwide, and is the cause of death of over 400,000. Most breast cancers originate from previously normal breast epithelial cells. During their transformation into cancer cells, the epithelial cells often lose the expression of cell adhesion proteins, which may also confer a motile or migratory advantage to breast cancer cells. Among the cell adhesion proteins potentially affected by this transformation is Junctional Adhesion Molecule A (JAM-A) a membranous cell-cell adhesion protein involved in tight junction formation in epithelial cells. Previous data by McSherry et al. (2009) demonstrated a novel association between JAM-A gene and protein upregulation and aggressive tumours in breast cancer patients.
Objective: To investigate a potential association between JAM-A and aggressive breast cancers.
Methods: A breast cancer tissue microarray (TMA) consisting of benign and invasive cancers was analysed for JAM-A protein expression (n = 48). A second TMA in which Her2 staining had been performed was also analysed for JAM-A expression (n = 166). JAM-A and HER2 protein expression levels were also compared in a range of breast carcinoma cells by western blotting. The effects of functional antagonism of JAM-A (using an inhibitory antibody) on cell proliferation was examined using an MTT proliferation assay. Alterations in the phosphorylation of downstream PI3K and JNK pathway components were investigated by western blotting.
Results: We have observed a significant association between JAM-A protein upregulation and invasive cancers (p = 0.023), particularly those of the HER2 subtype (p = 0.023). In our second TMA, high JAM-A expression was significantly associated with HER2 protein expression (p = 0.019), lower patient age (p = 0.02) and poorly differentiated breast cancers (p = 0.001). Moderate to high JAM-A expression also significantly associated with the more aggressive luminal B, HER2 and basal subtypes of breast cancer (p = 0.005). Furthermore, high JAM-A expression was noted in invasive breast cancer cells which over-express HER2 (UACC-893). In contrast, JAM-A expression was low in invasive MDA-MB-231 cells which express very low levels of HER2. Additionally, functional antagonism of JAM-A reduced cell proliferation and altered the phosphorylation of PI3K and JNK pathway components in MCF-7 breast cancer cells.
Conclusion: Our data reveals that JAM-A is up-regulated in HER2-expressing breast cancers, associating with the more aggressive breast cancers affecting a younger portion of the population. Since JAM-A is highly expressed at the membrane we speculate it may offer promise as a future diagnostic and therapeutic target.

365 Cell delivery of the Met docking site peptide inhibits angiogenesis and vascular tumour growth

A.R. Cantelmo[1], R. Cammarota[1], D.M. Noonan[2], C. Focaccetti[1], P.M. Comoglio[3], M. Prat[4], A. Albini[1]. *[1]Casa di Cura Multimedica – IRCCS, Oncology Research, Milan, Italy, [2]University of Insubria, Department of Experimental Medicine, Varese, Italy, [3]Institute for Cancer Research and Treatment (IRCC), University of Turin Medical School, Candiolo, Italy, [4]Università del Piemonte Orientale "A. Avogadro", Department of Medical Sciences, Novara, Italy*

Background: Hepatocyte Growth Factor (HGF) and its receptor Met are responsible for different cell responses involved in developmental processes and pathological conditions, including cancer. In cancer, Met can act as an oncogene in tumour cells as well as a pro-angiogenic factor activating endothelial cells and inducing new vessels formation. Angiogenesis is a necessary step for tumour growth and metastatic dissemination. Current studies suggest that tumour growth would be hampered by blocking the neovascularisation process. Molecules interfering with the activity of Met could be valuable therapeutic agents that could interfere with the tumourigenic and metastatic processes triggered by Met in human cancer. Here we investigated the anti-angiogenic properties of a synthetic peptide mimicking the docking site of the Met C-terminal tail, which was delivered into cells by fusion with the internalization sequences of two cell penetrating peptides.
Material and Methods: *In vitro* we treated human endothelial cells with three different peptides containing Met docking site fused to the internalization sequences of Antennapedia homeodomain or of Tat peptides and we quantified proliferation. We then evaluated the ability of peptides to interfere with HGF-induced migration, invasion and morphogenesis of endothelial cells. By western blotting analysis we investigated whether these peptides affect Met activation and downstream signaling. *In vivo* we performed matrigel sponge assay and Kaposi's sarcoma xenograft to test the peptides efficacy on angiogenesis and tumour growth.
Results: We observed that in endothelial cells internalized peptides inhibited ligand-dependent cell proliferation, motility, invasiveness and morphogenesis *in vitro,* which correlated with interference of HGF-dependent downstream signaling, as shown by reduction in erk 1/2 activation. *In vivo,* the peptides inhibited HGF-induced angiogenesis when subcutaneously co-injected with HGF in mice in the matrigel sponge assay and significantly impaired Kaposi sarcoma xenograft tumour growth and vascularization.
Conclusions: these data show that the carboxyl-terminal sequence of Met receptor impaired angiogenesis triggered by HGF/Met interaction, suggesting the feasibility of using anti-docking site compounds as therapeutic agents to interfere with tumour progression and angiogenesis-impaired diseases.

366 Screen for inhibitors of cell migration in cancer metastasis using adenoviral knock-down

R. de Pril[1], A. Lekkerkerker[1], E. Frische[1], D. van Steenhoven[1], I. Maghrani[1], T. Perera[2], J. Arts[2], M. Page[2], D. Fischer[1], R. Janssen[1]. *[1]Galapagos B.V., Target Discovery, Leiden, The Netherlands, [2]Janssen Pharmaceutica, Ortho Biotech Oncology R&D, Beerse, Belgium*

Enhanced cell migration is a hallmark of metastatic cancer cells. The propensity of cancer cells to close an open wound in a cell monolayer is thought to predict this ability. Using our adenoviral shRNA knock-down library we have established a high-throughput wound healing assay to identify novel genes involved in cell migration. Therefore, a 96-pin scratch tool was designed to apply a constant mechanical scratch-wound in the cellular monolayer. Cells were fixed at a preset time period after scratching and the plates were imaged on an InCell Analyzer1000. We used transmitted light imaging for segmentation and quantification of the scratch wound that remained open. Accordingly, we developed an algorithm to measure the open space, which comprises the area of the wound as well as open spaces between cells. Genes whose knock-down inhibit cell migration can be identified by their effect on the open wound. In addition, genes that affect either proliferation or viability can thereby be counter screened as these disrupt the monolayer. Using this approach we demonstrated that two knock-down constructs targeting a known player in motility, CXCR4, inhibit wound healing, validating our set-up.
Using this wound healing assay we have identified a number of novel genes associated with cancer cell motility. These targets are currently validated for their effect in 3D invasion using Boyden chambers. We use confocal imaging on a Pathway 435, of both the seeded cells in the top compartment and the invaded cells on the bottom of the filter. In this way, we have identified a number of control genes which inhibit invasion through matrigel. Using this assay we will validate the hits that inhibit migration, for their ability to additionally inhibit 3D invasion. As our adenoviral knock-down libraries focus on drugable targets, these validated targets can quickly be employed to generate small molecule compounds or antibody therapeutics targeting cancer metastasis.

367 Genomic profiles of single tumour cells in metastatic breast cancer patients

R.R. Mathiesen[1], R. Fjelldal[2], E.U. Due[1], J.B. Geigl[3], S. Riethdorf[4], A.L. Borresen-Dale[1], K. Pantel[4], M.R. Speicher[5], L.O. Baumbusch[1], B. Naume[6]. *[1]Institute for Cancer Research Oslo University Hospital Radiumhospitalet, Department of Genetics, Oslo, Norway, [2]Institute for Cancer Research Oslo University Hospital Radiumhospitalet, Department of Pathology, Oslo, Norway, [3]Institute of Human Genetics Medical University of Graz, Center for Applied Biomedicine, Graz, Austria, [4]Institute of Tumour Biology Center for Experimental Medicine University Medical Center Hamburg Eppendorf, Hamburg, Germany, [5]Institute of Human Genetics Medical University of Graz, Center for Applied Biomedicine, Graz, Austria, [6]Division of Surgery and Cancer Medicine Oslo University Hospital Radiumhospitalet, Department of Oncology, Oslo, Norway*

Background: In breast cancer progression the critical step is the dissemination of tumour cells to distant organs. An early marker for tumour dissemination is the presence of circulating tumour cells (CTC) in blood and disseminated tumour (DTC) in the bone marrow. DTC has been shown to be an independent prognostic factor for systemic relapse and breast cancer related