Results and Conclusions: In contrast to HCT-116, $CD133^{-/low}$ HT29 cells showed a lower clonogenic survival and reduced spheroid formation capacity than their $CD133^{+}$ counterparts. HT29 cell survival decreased in a lactate-enriched milieu, an effect that was more pronounced in the $CD133^{-/low}$ population indicating that $CD133^{+}$ cells may better survive in a pathophysiological environment. All differences were significant but not as pronounced as expected. Also, no difference in response to treatment was observed for the different populations, and tumour formation capacity was 100% for as low as 500 cells injected s.c. per animal. We therefore analyzed CD133 expression after sorting and found a clear, yet unexpected rapid increase of the $CD133^{+}$ fraction in the $CD133^{-/low}$ sorted HT29 population in 2-D and 3-D culture under serum-supplemented conditions. The mechanisms of CD133 expression control have to be elucidated to verify if CD133 in CRC cell lines and tissue may be an epiphenomenon of environmental conditions.
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448 Targeting the p53 tumour suppressor activity in Glioblastomas using small molecule MDM2-inhibitor

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Introduction: Targeted therapies that inhibit the MDM2-p53 interaction and the downstream Rb-E2F signalling pathway have shown promising anticancer activity, but their efficacies in human glioma have not been investigated. Recently, small-molecule antagonists of MDM2, the MDM2-inhibitiors, have been developed to inhibit the MDM2-p53 interaction and to activate p53 signalling serving possible anti-cancer activity.
Aim: To investigate the therapeutic potential of disrupting the MDM2-p53 interaction in human glioma cells with various p53 status. We particularly followed whether MDM2-inhibition would sensitize gliomas to additional chemotherapy.
Methods: We investigated the activity of MDM2-inhibitor alone and in combination with chemotherapy on cell cycle regulating proteins by Western blot and *in vivo* by employing imaging sensing vectors.
Results: MDM2-inhibior alone and in combination with BCNU results in a dose- and time-dependent reduction in cell viability and proliferation. Western blot studies showed that MDM2-inhibition modifies expression of several genes and results in cell cycle arrest and induction of apoptosis. Moreover, we found consistent and robust accumulation of p53 protein and downregulation of E2F-1 protein triggered by MDM2-inhibition alone and in combination with BCNU in all glioma cells as well as primary glioma samples. The MDM2-inhibitor and BCNU mediated alteration of p53 and E2F1 activities could be quantified in vivo by bioluminescence imaging and correlated to our results in culture.
Conclusions: Our results demonstrate that MDM2 inhibition elicits a dose- and time-dependent antiproliferative effect of glioma growth and potentiates the effects of BCNU via p53-dependent and p53-independent mechanisms and multiple genes seem to be involved in this process. MDM2 inhibitors with broad spectrum of antitumour activities in human cancers regardless of p53 status, may provide novel approaches to the therapy of malignant brain tumours.
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449 Inhibition of vascular-like network formation of highly aggressive melanoma

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Melanoma cells display substantial plasticity, demonstrated by directly forming tube-like structures composed of tumour cells but not of endothelial cells, which conduct blood cells and fluid. This phenomenon was termed Vasculogenic Mimicry (VM). Recently, it was shown that the presence of VM in melanoma masses predicts poor prognosis. Noteworthy, several anti-angiogenic lines of therapy seem ineffective against melanoma. It could be speculated that this alternative vascularization pathway might be of importance for advancement of melanoma.
We examined the ability of two agents to abrogate VM: IFNalpha, an immunomodulator with an antiangiogenic effect, and nicotinamide, the amide form of vitamin B3 (niacin).
The in vitro effects of the agents were examined using the highly aggressive melanoma cells (C8161). VM was tested as formation of tubular networks when grown in three-dimensional (3D) culture. In addition, cell proliferation (measured with XTT), cell cycle analysis (DNA content) and invasion capacity through matrigel were tested concomitantly.
IFNalpha affected *in vitro* VM formation in a dose-depended manner (at concentrations of 5×10^4 and 5×10^5 IU). Further, IFNalpha significantly inhibited the proliferation of C8161 cells. Cell cycle analysis reveled a significantly increased proportion of apoptotic cells. Moreover, the invasion ability was decreased in the treated cells. Nicotinamide (at concentrations of 1 and 5 mM) significantly inhibited the proliferation of the melanoma cells, but had no effect on their invasion capacity. According to cell cycle analysis, nicotinamide treated cells showed no significant changes in their respective apoptotic indices. Nicotinamide inhibited VM formation, but the effect was inconsistent. All effects were compared to control treatments with carrier only.
Due to the fact that both IFNalpha and nicotinamide hold a wide range of biological activity, the dose for optimal results may differ greatly as different effects are mediated by different concentrations. Nevertheless, both demonstrated anti-melanoma properties, including an effect on VM formation. Targeting VM could be of great importance, especially in combination with anti-angiogenic strategies. This combination is expected to be synergistic and yield substantial anti neoplastic effect.

450 Leptin and estrogen receptor expression in breast cancer patients with different clinical characteristics

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Introduction: Leptin is a multifunctional hormone produced by adipocytes. It plays important role in angiogenesis. Induction of cell proliferation, survival and anchorage-independent growth.
These leptin activities are mediated through leptin receptor (ObR) that binds leptin molecule and stimulates Jak/STAT 3, ERK 1/2, cyclin D1 expression and other signal pathways. A recent data show that targeting leptin signaling may reduce mammary carcinogenesis and breast cancer (BC) progression. However, the link between obesity and leptin expression in serum/breast tumour as well as its role in modulation of estrogen receptors (EsR) and HER2/neu expression is not clear.
Material and Methods: We studied leptin, ObR, EsR, HER2/neu expression in patients with sporadic, familial and pregnancy-associated BC by RT PCR using BC fresh tissue and primers for genes encoding leptin, ObR, EsR-α, β. Leptin level in the patient sera was estimated also by ELISA (Leptin Sandwich DRG, DRG Diagnostics, Germany) followed by comassie staining. The data on routine immunohistochemical staining of BC paraffin embeded section for HER2/neu, EsR and PrR were also obtained. In control group were patients with benign fibroadenoma (BFa) and healthy women of comparable age.
Results: RT PCR results and immunohistochemistry method are mainly concordant: only 5% (5/29) of data were different. In triplonegative tumours (n = 40) leptin overexpression was significantly higher than in other tumour types. Blood sera leptin level was correlates positively with ObR expression. Leptin expression in tumour tissue also correlates with HER2/neu over expression in all BC groups except triplonegative ones: Esr (−), PrR(−), HER2/neu(−). Serum leptin levels in BFa patients was higher (100–300 ng/ml) than in healthy women of comparable age and body weight. Moreover, leptin serum level was positively correlates with ObR expression in tumours on I-III BC stages and with high body weight index (obesity).
Conclusions: The data obtained indicate that leptin/ObR may involved in BC progression. It indicates that ObR suppression is the possible way for target BC therapy, especially of triplonegative tumours which do not express HER2/neu, so hormone therapy is not effective for these neoplasia.

451 Progesterone regulation of breast cancer cell coagulative and invasive potential is dependent on the distinct membrane localization of tissue factor

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Background: The oncogene Tissue Factor (TF) is over-expressed in breast cancers and is correlated to metastasis and thus poor prognosis. The usage of exogenous progestins is associated with increased breast cancer incidence. We previously reported that TF is transiently regulated by progesterone at the level of transcription and that the blocking of TF activity by antibodies eliminates the progesterone-mediated coagulative and invasive potential of the breast cancer cell lines ZR-75 and T47D.
Material and Methods: Coagulation was measured in whole cells by the generation of FXa in the presence of FX and FVIIa. Invasion was measured

by the ability of cells to cross porous inserts covered in matrigel. Membrane localization was determined by sucrose gradients and immunocytochemistry.
Results: Herein, we report the surprising observation that the TF protein increased by progesterone localizes to the heavy portion of the plasma membrane and does not contribute to coagulation. Instead progesterone-increased coagulative activity is localized to lipid rafts. This activity of progesterone is dependent on transcription, the progesterone receptor and is independent of caveolin-1 presence. In the presence or absence of progesterone, TF cannot be detected in lipid rafts by western blotting, but blocking antibodies against this protein eliminate coagulation. These results suggest that progesterone increases the capacity of basal levels of TF located in the lipid raft region to cleave coagulation factor X in the presence of its ligand Factor VIIa. In confirmation of this theory, the use of either 2-methoxyestradiol or inhibitors of the c-src pathway, which we have to shown to eliminate the increase in TF by progesterone, do not inhibit the capacity of progesterone to increase coagulation. Interestingly both these inhibitors eliminate the ability of progesterone to increase breast cancer cell invasion, shown previously by us to be dependent on TF.
Conclusion: We demonstrate that TF levels do not correlate to coagulative ability in breast cancer cells and show that progesterone can modulate coagulation without increasing TF levels.
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452 A novel method to enrich for glioma stem cells from glioma cell lines

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Background: Glioma stem cells (GSC) are inherently similar to stem cells except they can transform into tumours reminiscent of the pathological features of the originated tumour mass. GSCs serve as an excellent pre-clinical model to comprehend tumour re-growth and treatment resistance. Several approaches were previously described to purify GSCs, but seemingly appeared to be laborious, costly and sometimes with poor yield. Our objective was to investigate alternative strategies to cost-effectively and efficiently enrich for GSCs.
Methods-Results: We grew 3 glioma cell lines in a modified serum free media that promotes the growth of stem cells over a 10 day period and with ease of harvesting from the supernatant. The tumour spheres had cell line specific morphologies. For instance, those from U87 and DB54MG were significantly larger with tightly associated spheres, in comparison to those from U251. The tumour spheres expressed stem cell markers and in fact were 80−96% rich in CD133+ve cells. Upon growth in DMEM/10% FCS tumoursphere diffentiation occured. In addition, the tumour spheres can transform in in-vitro and with the ability to grow into tumours having similar pathological hallmarks but faster growth in comparison to xenograft tumours derived from the growth of glioma cell lines. These findings were overall similar with passages 1, 10 and 30 GSCs examined.
Conclusions: We have discovered an alternative strategy to enrich for glioma stem cells from glioma cell lines in a cost-effective, easy and efficient manner. Current efforts are undertaken to utilize our protocol to enrich for glioma stem cells from surgical tissues.

453 The effectiveness of Fas apoptosis signalling pathway determined by the combined action of functional polymorphisms at Fas, FasL and Fadd

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Background: In previous reports we described germ-line functional polymorphisms that differentiate *Fas* and *FasL* genes in two mouse strains (SEG/Pas and C57BL/6J) exhibiting extreme differences in susceptibility to gamma-radiation induced T-cell lymphomas. In this study we provide new data about the functional significance of the intra-cellular and extra-cellular polymorphisms of *Fas* and *FasL*, and report new polymorphisms in the coding sequence of *Fadd*, another key element of this pathway.
Material and Methods: Chimerical Fas and FasL proteins were constructed, combining the intra- and extra-cellular regions derived from C57BL/6J and SEG/Pas. TUNEL apoptosis assay was used to evaluate the induction of apoptosis in cells bearing wild-type/chimerical Fas and FasL molecules. Caspases cleavage assessment through Western Blot served to confirm the TUNEL assay results. C57BL/6J and SEG/Pas-*Fadd* cDNAs were genotyped and sequenced. Fadd-FLAG and Fas-HA constructs were used in co-immunoprecipitation assays.
Results: When assaying TUNEL in the chimerical systems, we found significant reductions in the levels of apoptosis they induce, as compared with those of the SEG/Pas system. This suggests evidence that the polymorphic residues we identified at the intra- and extra-cellular regions of both the Fas receptor and its ligand exhibit a different functionality. As expected, the accumulation of polymorphisms, represented in the double-chimerical systems, produces the highest differences of apoptosis. Strikingly significant seems as well the functionality of those polymorphic amino acids located on the intracellular region of Fas, through which it interacts with Fadd. These might determine different affinities of interaction between Fas and Fadd, given that none of the polymorphic residues found at *Fadd* cDNA between C57BL/6J and SEG/Pas lie in its death domain. Co-immunoprecipitation experiments show that the interaction Fas-Fadd is stronger when Fas derives from SEG/Pas, confirming the different functionality of the polymorphic residues at the intracellular region of Fas.
Conclusions: Our results support the functionality of polymorphisms located at the intra- or extra-cellular regions of Fas and FasL, but prompt us to consider that the functional consequences of any of those changes should be assessed within the general context of the system.

454 c-Met endosomal signalling and breast cancer cell migration

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Background: c-Met, the receptor of HGF (Hepatocyte Growth Factor), is a tyrosine kinase receptor overexpressed or mutated in various cancers. In breast cancer, c-Met has been associated with cancer progression and metastasis and is considered to be a marker for poor prognosis. Therefore specific targeted therapy against c-Met may provide a valuable therapy for patients.
The mechanisms of c-Met signalling that promote breast cancer progression are poorly understood. Recently, it has been shown that tyrosine kinase receptors, including c-Met, continue to signal from the endosome after internalisation. This endosomal signalling may have unique consequences on cellular outcome due to the spatial and temporal activation of downstream signalling pathways.
The objectives of this study were to investigate and compare the role of c-Met endosomal signalling in the migration of a range of human breast cancer cell lines, ranging from a pre-invasive to a highly invasive phenotype.
Material and Methods: c-Met internalisation, intracellular trafficking and downregulation were compared using FACS analysis and confocal microscopy. The relationship of c-Met trafficking to signalling, cell migration and invasion was determined using western blot analysis, transwell migration assays and 3D organotypic invasion assays.
Results: We find that the requirement of c-Met for endocytosis in the stimulation of several signalling pathways and in cell migration varies significantly between the cell lines. The more aggressive cell lines seem more reliant on c-Met endocytosis for the full activation of Gab1, ERK and AKT downstream of c-Met. Consequently, these cells require c-Met trafficking for their full migration and invasion.
Conclusions: Our results suggest that c-Met endosomal signalling might play a role in breast cancer progression.

455 Bcl-2 regulates HIF-1alpha protein stabilization in hypoxic melanoma cells via the molecular chaperone HSP90

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Background: Hypoxia-Inducible Factor 1 (HIF-1) is a transcription factor that is a critical mediator of the cellular response to hypoxia. Enhanced levels of HIF-1α, the oxygen-regulated subunit of HIF-1, is often associated with increased tumour metastasis, therapeutic resistance and poor prognosis. In this context that we previously demonstrated that the antiapoptotic protein bcl-2 cooperates with hypoxia topromote HIF-1/Vascular Endothelial Growth Factor (VEGF)-mediated tumour angiogenesis.
Material and Methods: Expression vectors encoding human bcl-2, *wild type* or hydroxylation resistant HIF-1α were used for stable and transient transfections of M14 human melanoma line. The effect of bcl-2 stable transfection will be evaluated in cells under hypoxic conditions in terms of bcl-2 and HIF-1α protein expression and localization (*western blot and confocal microscopy analyses*) HIF-1α protein stability and ubiquitination (*Western blot and immunoprecipitation analyses*) and HIF-1 transcriptional activity (*reporter assay*). The role of Heat Shock Proteins (HSPs) in the bcl-2-mediated regulation of HIF-1α expression and transcriptional activity (*Western blot analysis and reporter assay*) was evaluated by using chemical inhibitors. Immunoprecipitation experiments were also performed to investigate the possible effect of bcl-2 protein on the interaction of HIF-1α and HSPs.
Results: By using M14 human melanoma cell line and its derivative bcl-2 overexpressing clones, we demonstrated that bcl-2-induced accumulation of HIF-1α protein during hypoxia was not due to an increased gene transcription. In fact, it was related to a modulation of HIF-1α protein expression at a post-translational level, indeed its degradation rate was faster in the control lines than in bcl-2 transfectants. The bcl-2-induced HIF-1α stabilization in response