no G3580). In each experiment 6 replicate wells were used for each drug concentration and the experiment was repeated 3 times. The average of the three experiments was taken, plotted onto a graph and IC50 values calculated.
Results: Rapamycin showed significant growth inhibition in the NCI-H2052, NCIH2452 and A549 cell lines with IC50 values of 675pM, 565pM and 620pM respectively, but not in the MSTO-211H cell line up to a maximum concentration of 1μM. Similarly, Ku0063794 demonstrated significant growth inhibition in the NCI-H2052, NCIH2452 and A549 cell lines with IC50 values of 10 nM, 135 nM and 100 nM respectively, but not in the MSTO-211H cell line up to a maximum concentration of 1 μM.
Conclusions: This study demonstrates that inhibition of MTORC1 alone or combined inhibition of MTORC1 and MTORC2 may be an important therapeutic strategy in patients with MPM.

1158 POSTER

Inhibition of Epidermal Growth Factor Receptor in Malignant Pleural Mesothelioma

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Background: Advanced malignant pleural mesothelioma (MPM) is associated with poor prognosis with median survival of approximately 12 months, despite recent advances in chemotherapy. The incidence of MPM varies from country to country, but is on the rise in most parts of the world. Great Britain, Australia and Belgium have the highest annual crude incidence rates of 30 cases per million population. Immunohistochemical studies have shown that Epidermal Growth Factor Receptor (EGFR) is over expressed in 44 to 97% of MPM tissue samples. In this study we aimed to determine the cytotoxic effect of EGFR inhibition in MPM cell lines using the MTS cell proliferation assay.
Materials and Methods: The MPM cell lines MSTO-211H, NCI-H2052 and NCIH2452 and the lung cancer cell line A549 were incubated with the anti-EGFR monoclonal antibody, Cetuximab (provided by Merck KGaA, Germany), and EGFR tyrosine kinase inhibitor, Gefitinib (Tocris, cat no 3000), at various dilutions for 72 hrs in a 96 well plate. At the end of 72 hrs the 96 well plate was analysed for cell viability using MTS assay (Promega, cat no G3580). In each experiment 6 replicate wells were used for each drug concentration and the experiment was repeated 3 times. The average of the three experiments was taken, plotted onto a graph and IC50 values were calculated.
Results: Cetuximab demonstrated significant growth inhibition in the MSTO-211H cell line with an IC50 value of 1.6 μM. No significant growth inhibition was seen in the NCI-H2052, NCI-H2452 and A549 cell lines at the maximum concentration of 1.75 μM, which was more than the maximum achievable serum concentration (1.57 μM) in Phase 1 studies. Similarly Gefitinib demonstrated significant growth inhibition in the MSTO-211H cell line with an IC50 value of 1.6 μM. The NCI-H2052, NCI-H2452 and A549 cell lines showed growth inhibition at much higher concentration with IC50 values of 3.7 μM, 6 μM and 13 μM respectively, which were more than the maximum achievable serum concentration (3.1 μM) in Phase 1 studies.
Conclusions: Our study suggests that anti-EGFR therapy may be effective in a select subset of patients with MPM. Despite there being significant over expression of EGFR receptors in MPM, various resistance mechanisms may exist resulting in resistance to anti-EGFR therapy.

1159 POSTER

Modulating Effect of Microenvironment Factors on Hormone Therapy of Breast Cancer

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Background: The most effective drugs for breast cancer are antiestrogen supplements such as tamoxifen (TAM). Loss of cell sensitivity to TAM may be associated with decreasing of number of steroid receptors in breast tumours. Indeed, estradiol, pro-inflamatory cytokines and IFN-γ may modulate ER expression of, but they are activated in different stages of malignization process, that's why its influence on receptor status will also have differences. Thus it will be important to investigate the impact of factors of cell microenvironment on ER expression, proliferation, apoptosis and cell cycle in MCF-7 cells on models of different breast cancer stages.
Materials and Methods: MCF-7 cells were cultured under standard conditions. For cocultivation a cell line MT-4 (human cell chronic lymphocytic leukemia) were used. Recombinant IFN-γ was added at a concentration of 10 U/ml, TAM − 100 nM, E2 − 10 nM, condition medium (C-medium) from T-lymphocytes − 1:1 with culture medium. Cell survival was determined by MTT test. The distribution of the cell population between cell cycle stages was measured using flow cytometry. Expression of ER and EGF-R was visualised by immunocytochemistry (DAKO, USA).
Results: Our results have indicated that recombinant IFN-γ has a cytostatic effect in comparison with a cytotoxic effect of TAM and a proliferative effect of estradiol. Increasing of cell number was shown for C-medium with E2, IFN-γ with E2, TAM with E2 and IFN-γ with TAM in suspension fraction. Decreasing of the cell number was demonstrated for IFN-γ, TAM, C-medium with TAM in suspension fraction. In adhesion fraction TAM, TAM with E2 and TAM with C-medium decreased the number of alive cells. IFN-γ, C-medium, IFN-γ with TAM and TAM with C-medium decreased cell number in S phase. IFN-γ and TAM increased cell number in G2/M phase, C-medium from T-lymphocytes and IFN-γ with TAM increased cell number in G0/G1 phase. In adhesion fraction apoptosis was stimulated by IFN-γ with E2, TAM, C-medium with E2. IFN-γ and C-medium from T-lymphocytes stimulated ER expression in MCF-7 cells.
Conclusion: Perhaps TAM has become a first agent for target therapy. Thus, our data demonstrated that cell microenvironmental conditions (hormonal and humoral) have a strong influence on ER expression in breast cancer cell and as a result modulate sensitiveness to antiestrogen therapy. Combination antiestrogen therapy with balanced approach IFN-γ, activated T-cells and level of E2/Pr may has commulative effect in antitumour treatment.

1160 POSTER

Induction of Hypoxia by Vascular Disrupting Agents and the Significance for Their Combination With Radiation Therapy

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Background: Targeting tumour vasculature is an increasingly popular therapeutic approach. The resulting vascular changes may also alter the tumour microenvironment and could influence conventional therapies given in combination. We investigated this issue using various vascular disrupting agents (VDAs) currently in clinical evaluation.
Materials and Methods: Restrained non-anaesthetised mice with 200 cubic mm foot implanted C3H mammary carcinomas were used. The VDAs were combretastatin A-4 phosphate (CA4P), its analog Oxi4503, and 5.6-dimethylxanthenone-4-acetic acid (DMXAA); they were dissolved in saline and intraperitoneally injected at doses of 250 (CA4P), 50 (Oxi4503), and 20 (DMXAA) mg/kg. Tumour oxygenation was determined using the Eppendorf polarographic electrode; the endpoint being the percentage of oxygen (pO2) values below 5 mmHg. Tumours were also locally irradiated (230 kV x-rays) in either single or fractionated (10 fractions in 12 days) schedules. The percentage of mice in each treatment group with local control at 90 days was recorded and the TDC50 values (radiation dose to control 50% of tumours) estimated from full radiation dose response curves. A Student's t-test (Eppendorf) or Chi-squared test (TCD50) were used for statistical analysis (significance level of $p < 0.05$).
Results: The average (with 1 S.E.) percent pO2 values below 5 mmHg was 45% (40−50) for control tumours. After injecting the VDAs, this significantly increased to around 90%. The TCD50 value (with 95% confidence intervals) for single radiation treatments was 53 Gy (51−55). Injecting VDAs immediately or within a few hours after irradiating significantly reduced this value to 46 Gy (42−49) and 45 Gy (41−49) for CA4P and DMXAA, respectively. This enhancement was lost if CA4P or DMXAA were injected immediately prior to irradiation. With Oxi4503, the TCD50 values were around 41 Gy (38−45) regardless of the time interval or sequence of the treatments. The TCD50 value for fractionated radiation was 76 Gy (73−9). Irradiating tumours and then injecting CA4DP or Oxi4503 after 5 and 10 radiation fractions significantly reduced the respective TCD50s to 66 Gy (62−69) and 67 Gy (63−71).
Conclusions: VDAs increase tumour hypoxia that can reduce the efficacy of radiation given shortly after drug treatment. However, hypoxia is not a problem if the VDA is given after irradiating or one uses a VDA like Oxi4503 that is also cytotoxic and thus can kill any induced hypoxic cells.

1161 POSTER

Evaluation of Drug Response of Trastuzumab Treated Cultivated Breast Cancer Tissue Slices

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Background: The aim of this study was to advance the previously developed preclinical model of cultivated cancer tissue slices to the application of therapeutic antibodies such as Trastuzumab (Herceptin®) thus allowing detailed drug testing in a natural tumour microenvironment.

Material and Methods: Tumour tissue slices from colorectal cancer and breast cancer patients were prepared and cultured according to Indivumed's standard operating protocols. For antibody diffusion assays tissue slices were incubated with different commercially available antibodies followed by immunofluorescence staining and microscopy. Functional effects of Trastuzumab treatment were examined in breast cancer cell lines (BT474 and MCF-7 cells) and breast cancer tissue slices using the expression level of pAkt as readout, shown by western blotting. Her-2 expression levels of cells and tissue slices were analyzed by immunohistochemistry.
Results: The antibody diffusion assays showed a time-dependent penetration of antibodies into and through 400 μm thick cultured tissue slices within 24 hours. Treatment of breast cancer cell lines and breast cancer tissue slices with different concentrations of Trastuzumab revealed a dose-dependent reduction of pAkt only in Her-2 positive cells and tissues. Thus, drug effects of therapeutic antibody Trastuzumab could be demonstrated in secondary cell lines and verified in organoid cultures.
Conclusions: Overall the data revealed that antibodies diffuse into 400 μm thick cultured tissue slices reaching their target within 24 hours. Furthermore, we demonstrated that functional drug effects of therapeutic antibodies could be validated in organoid cultures. Therefore, the preclinical model based on cultured cancer tissue slices developed by Indivumed is suitable to examine not only the effects of classical chemotherapeutics, as we have shown recently, but also of larger molecules, such as antibodies. Thus, this model representing the natural tumour environment is a promising and important tool to prioritise drugs, support dose finding and to individualise therapy.

1162 POSTER

Frequency of Mitochondrial Point Mutations and Deletions in Late Stage Colorectal Cancer Patients

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Objective & Background: Defects in mitochondrial function have been proposed to contribute to progression of cancer. This dysfunction may result from impairment in cellular respiration, forcing the cell to revert to glycolysis for energy. This switch causes cells to become undifferentiated and cancerous. This study examined the frequency of random mitochondrial mutations and deletions in late stage colorectal cancer tumours and their corresponding normal tissue. This analysis was performed using a novel random mutation capture assay which has the sensitivity to detect one mutation in one hundred million base pairs.
Methods: Mitochondrial DNA was extracted from 20 patients with late stage colon cancer and adjacent normal tissue. This DNA was and digested with TAQ 1α for 10 hours replacing the enzyme every hour. QPCR was performed across the TAQ1α restriction sites. The primer sequences used for identifying random mitochondrial mutations are 5′-ACAGTTTATGTAGCTTACCTCC-3′ and 5′-TTGCTGCGTGCTTGATGCTTGT-3′. The primer sequences used to determine mitochondrial DNA copy number are 5′-ACAGTTTATGTAGCTTACC TCC-3′ and 5′-TTGCTGCGTGCTTGATGCTTGT-3′. PCR was performed to assess single nucleotide polymorphisms. We will assess mutation frequency of TAQ1 restriction site (TCGA) located in the gene encoding the 12S rRNA subunit (bp 634–637). By flanking multiple TAQ1 restriction sites with a primer pair, the detection prevalence of the RMC assay can be skewed towards DNA deletions. As a result, the expected frequency of a PCR product due to mtDNA point mutations drops exponentially to 1×10^{-15}. In contrast, mtDNA deletions occur at a higher rate, and hence, every mutation detected with these primer pairs will be a deletion.
Results: A statistically significant increase in the frequency of deletions was detected in normal versus tumour tissue ($p = 0.021$), however levels of point mutations did not differ between tumour and normal.
Conclusions: The lower rate of deletions seen in tumour tissue versus adjacent normal may be explained by the Warburg theory where anaerobic metabolism is predominant in cancer. In contrast to normal cells, which generate energy by the oxidative phosporylation, tumours and cancer cells generate energy through glycolysis which could result in lower levels of mitochondrial deletions in tumours.

1163 POSTER

A Therapeutic Sphingosine 1-phosphate Antibody Inhibits Intratumoral Hypoxia and Sensitizes to Standard Chemotherapy in a Preclinical Model of Prostate Cancer

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Background: Hypoxia promotes neovascularization, metastasis, growth and resistance to treatments. The activation of HIF-1α has been identified as the master mechanism of adaptation to hypoxia. We recently identified the sphingosine kinase 1/sphingosine 1- phosphate (SphK1/S1P) pathway as a new modulator of HIF-1α activity under hypoxia in multiple cancer cell models (Ader et al, Cancer Res, 2008). S1P elicits proliferation, survival, or angiogenesis, and is believed to exert most of its actions as a ligand for a family of specific GPCRs to elicit paracrine or autocrine signaling. We have suggested that inhibiting SphK1/S1P signaling, which is up-regulated under hypoxia, may help normalizing the tumour microenvironment and increase sensitivity to chemotherapy, in the broader concept of "normalization of tumour vessels" as tumour oxygenation is known to enhance response to chemotherapy (Ader et al., Cancer Res, 2009).
Methods: Quantitation of hypoxia and angiogenesis, and treatment efficacy using an orthotopic (o.t) xenograft model of fluorescent HRPC cells.
Results: We provide *in vitro* evidence that inhibiting the S1P exogenous signaling, through pharmacological inhibition of its receptors or by taking advantage of a monoclonal antibody neutralizing S1P, blocks HIF-1α accumulation and its activity in prostate cancer cells under hypoxia. Second, using an o.t model of prostate cancer, we show that an anti-S1P antibody inhibits intratumoral hypoxia, modifies vessel architecture and improves tumour perfusion within 5 days of treatment. Third, we demonstrate that an anti-S1P strategy sensitizes to docetaxel, the 'gold standard' treatment for HRPC. A 5-day anti-S1P antibody pretreatment markedly sensitizes to docetaxel in an o.t. PC-3/GFP model established innude mice. The combination anti-S1P antibody together with docetaxel was not only accompanied by a smaller primary tumour volume compared to docetaxel alone, but also significantly reduced the occurrence and number of metastases.
Conclusions: These data establish the proof-of-concept that blocking the exogenous action of S1P reduces intratumoral hypoxia and sensitizes to chemotherapy in prostate cancer animal model.

1164 POSTER

The Impact of Ionizing Radiation on the Motility and Matrix Remodelling Properties of Carcinoma-associated Fibroblasts

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Purpose: Carcinoma-associated fibroblasts (CAFs) are key components of solid malignancies and play central roles in cancer sustainability. In this work we have investigated the invasive capacity and matrix remodelling properties of CAFs after ionizing radiation (IR).
Methods: CAFs were isolated from fresh lung tumour specimens from 14 different donors. Initially, radiation protocols were established by monitoring cellular viability at different doses of radiation. For further analyses, the migrative, invasive and adhesive capacities of CAFs were determined after a single dose of 18 Gy. Additionally, protein levels of secreted major matrix modulators represented by matrix metalloproteinases (MMPs)-1, -2, -3, -7, -8, -9, -13 and their endogenous inhibitors (TIMPs)-1, -2, -3, -4 were measured 4 to 6 days post-irradiation, as well as cell surface expression of various integrins.
Results: IR resulted in premature cellular senescence and caused moderate but significant inhibition of the proliferative, migrative and invasive capacity in CAFs. IR also promoted MMP-3 and inhibited MMP-1 appearance, whereas expression and activity of the gelatinases MMP-2 and MMP-9 were unchanged. Furthermore, the levels of TIMPs were not affected. Surface expression of integrins α2, β1, α5 was consistently enhanced.
Conclusions: Our data indicate that therapeutic doses of IR exert advantageous inhibitory effects on the proliferative, migratory and invasive capacity of lung CAFs, along with a prominent reduction of MMP-1 expression. However, the observed enhancement of MMP-3 could represent a negative outcome from radiation. Also, the altered surface expression of integrins in