antibody have been used to label tumour cells, with a CD45 (pan-leucocyte marker) antibody used to exclude white blood cells from the analysis. The individual antibodies were optimised using 3 cell lines with increasing levels of α-FR expression (JEG-3, IGROV-1 and KB cells). BGC 945 causes increasingly high levels of α-FR mediated growth inhibition in these cell lines. The three antibody protocol successfully measured α-FR expression levels in cell line samples spiked with blood. CellQuant calibrator beads were used to semi-quantify antigen sites/cell. KB cells expressed around 1×10^6 antigen sites/cell and IGROV-1 and JEG-3 cells around 5.5 and 0.7×10^5 sites/cell respectively (~50% and 7% of KB cells). Tumour cells were obtained from ascites in 19 patients with relapsed ovarian cancer. In each case sufficient cells were harvested to isolate a tumour cell population by this method in order to estimate the number of binding sites/cell. The majority of samples (13/19) had expression levels between 0.6×10^5 and 4.9×10^5 binding sites/cell, which lie between the JEG-3 and the IGROV-1 cell lines. A smaller number (4/19) formed a population of lower expressors with $<1\times10^4$ binding sites/cell. The final 2 samples lie in between these groups. These data may be useful in identifying a cohort of patients more likely to respond to α-FR targeted therapy.

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421 POSTER

New low-toxic analogs of vitamin D in the treatment mice bearing lung carcinoma

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A proper level of the steroid hormone 1,25-(OH)2D3 (1,25-dihydroxy-vitamin D3 (calcitriol) – the most potent metabolite of vitamin D3) is important not only in regulating calcium homeostasis and bone metabolism, but also in protecting against the development of cancer. Calcitriol and several synthetic vitamin D derivatives showing reduced calcemic activity inhibit the growth of a number of different cancer cells (epithelial, melanoma, soft tissue sarcoma, and leukemic) by inducing cell cycle arrest or apoptosis. Calcipotriol is a synthetic vitamin D3 analog that binds to vitamin D receptors. In vitro studies have shown that calcipotriol exerts similar effects on cell proliferation and differentiation to those of calcitriol, but has less effect on calcium metabolism.

The aim of our study was to examine the toxicity and antitumor activity of new vitamin D analogues selected during in vitro experiments, i.e. PRI-2202 (24R calcipotriol) and PRI-2205 (5,6-trans calcipotriol).

Subacute toxicity after 5 subcutaneous (s.c.) administrations was determined. We also compared antitumor activity (LLC tumor model) of calcitriol (in the dose 2 μg/kg/day) and PRI-2201 with PRI-2202 and PRI-2205 (20 μg/kg/day) injected s.c. or applied s.c. in various doses (1, 10, 50 i 100 μg/kg/day).

The toxicity studies showed, that PRI-2202 and PRI-2205 were very low-toxic analogs. Even in doses of 2.5–5.0 mg/kg (in 5 daily doses), no changes in body weight were observed. Calcitriol and tacalcitol showed toxicity in the same model system at 100-times lower doses. LD50 for calcitriol was 7.4 and for tacalcitol 21.0 μg/kg/day (total: 37 and 105 μg/kg, respectively). Also, cacipotriol caused death of all mice (mean life-span ± SD: 7.4±1.1 days) when the total dose of 5.0 mg/kg was administered.

Next we tested the antitumor activity of these analogs in the LLC mice tumor model. We show that the analog PRI-2205 is more active than both calcitriol and calcipotriol as well as PRI-2202. It revealed no calcemic activity in the doses which inhibit tumor growth nor at higher doses.

These data demonstrate that the analogs PRI-2202 and PRI-2205 are non-toxic and potent inhibitors of cancer growth. In particular, their role in combined treatment with cytostatics is considered for further study.

422 POSTER

Endosialin/TEM 1 a tumor stromal target in stem cells, progenitor cells and pericytes

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Background: Endosialin was originally identified as a cell surface protein expressed by reactive tumor stroma. Later, TEM 1 (Tumor Endothelial Marker 1) was described as a cell surface protein expressed by tumor endothelial cells (EC). Endosialin and TEM 1 are the same protein. The investigation of TEM 1 and other TEM expression has expanded to several distinct tumor stromal cells types including endothelial precursor cells (EPC), mesenchymal stem cells (MSC) and pericytes as well as tumor cells of mesenchymal origin.

Materials and Methods: Cells from various tissues were analyzed for TEM 1 expression prior to use in experiments. TEM 1 was abundantly expressed by EPC and MSC derived from human bone marrow. By RT-PCR, the message for TEM 1 was present at negligible levels in CD133+/CD34+ precursor cells, was abundantly expressed when these cell differentiated to EPC and is expressed at very low levels by fully differentiated EC such as HUVEC/HMVEC. Immunohistochemistry (IHC) was employed to evaluate TEM 1 expression in clinical samples.

Results: Exposure to rabbit polyclonal anti-TEM 1 inhibited EPC migration and tube formation in culture. In an in vivo Matrigel™ plug assay, EPC continued to express TEM 1 abundantly. TEM 1 protein expression was determined by IHC in human normal tissues and in frozen and paraffin-embedded human tumors. TEM 1 was expressed primarily in the vasculature of many tumor types especially bladder, sarcomas, colon, breast and non-small cell lung cancer. In most specimens it appeared that pericytes had the most intense expression of TEM 1 with additional expression in EC and reactive stroma which may be carcinoma-associated fibroblasts. Pericytes isolated from fresh human non-small cell lung cancer specimens also express TEM 1 as determined by flow cytometry. Some malignant cells of mesenchymal origin express TEM 1. For specific tumor-types TEM 1 was expressed in 90–100% of the specimens examined. In some normal tissues TEM 1 expression was observed in occasional cells that had a spindloid appearance.

Conclusions: TEM 1/endosialin is a potentially interesting therapeutic target that is selectively expressed in tumor vasculature. The development of new therapies directed toward the non-malignant cellular components of the disease process such as EC, pericytes and cancer-associated fibroblasts may yield may yield therapeutics with a high degree of tumor selectivity and limited normal tissue effects.

423 POSTER

Metformin is an AMP-kinase dependent growth inhibitor for breast cancer cells

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Background: Recent population studies provide clues that the use of metformin may be associated with reduced incidence and improved prognosis of certain cancers. This drug is widely used in the treatment of type 2 diabetes, where it is often referred to as an 'insulin sensitizer' because it not only lowers blood glucose but also reduces the hyperinsulinemia associated with insulin resistance. As insulin and insulin-like growth factors stimulate proliferation of many normal and transformed cell types, agents that facilitate signalling through these receptors would be expected to enhance proliferation.

Methods: Breast cell lines were treated with metformin for 3 days and/or AMP kinase siRNA. Proliferation assays were performed using Alamar reducing dye. AMP kinase downstream signalling pathway protein levels and phosphorylation were evaluated by Western blots.

Results: We demonstrate here that metformin acts as a growth inhibitor rather than an insulin sensitizer for epithelial cells. Breast cancer cells can be protected against metformin-induced growth inhibition by siRNA against AMP kinase. This demonstrates that AMP kinase pathway activation by metformin, recently shown to be necessary for metformin inhibition of gluconeogenesis in hepatocytes, is also involved in metformin-induced growth inhibition of epithelial cells. The growth inhibition was associated with decreased mTOR and S6 Kinase activation, and a general decrease in mRNA translation.

Conclusion: These results provide evidence for a mechanism that may contribute to the antineoplastic effects of metformin suggested by recent population studies, and justify further work to explore potential roles for activators of AMP kinase in cancer prevention and treatment.

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Effects of statins on IGF-IR signaling in normal and transformed breast epithelial cells

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Background: The 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely used cholesterol lowering drugs. Some epidemiologic studies imply that individuals taking statin decrease their cancer risk. Statins disrupt cellular processes such as (iso)-prenylation (required for the activity of proteins such as Ras and Rho) or dolichol synthesis (required for correct N-glycosylation of proteins such as insulin