Results: We found that oncogenic *ras* induces the PKCι-dependent activation of iPFK-2 in bronchial epithelial cells causing an increase in intracellular F2,6BP (+18.4±2.1 pmol/mg protein). Additionally we found that siRNA-silencing of iPFK-2 expression completely abrogated the formation of soft agar colonies by ras-transformed bronchial epithelial cells (control siRNA 123.4±23.1; anti-iPFK-2 siRNA 3.3±3.5) and attenuated the flux of glucose carbons into de novo nucleic acids and amino acids. Although iPFK-$2^{+/-}$ mice display a normal phenotype, isolated iPFK-$2^{+/-}$ lung fibroblasts were not able to be transformed with T antigen and oncogenic ras as evidenced by zero growth in soft agar or athymic mice. Conversely, 10^3 ras-transformed iPFK-$2^{+/+}$ lung fibroblasts grew as soft agar colonies (172.6±38.3) and as tumors in athymic mice.
Conclusions: iPFK-2 should prove useful as a novel molecular target for the development of anti-neoplastic agents that target the downstream metabolic effects of oncogenic ras.

70 POSTER

Therapeutic human monoclonal antibody targeting VEGFR-1 suppresses growth of human breast cancers

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Vascular endothelial growth factor receptor 1 (VEGFR-1) is activated by the ligands VEGF-A, VEGF-B and placental growth factor (PlGF) and has been shown to be a potential therapeutic target for treatment of tumors and angiogenesis-associated diseases. Studies have shown that VEGFR-1 plays not only an important role in regulating pathological angiogenesis for tumor growth but also a functional role in directly promoting growth of certain cancer cells. IMC-18F1 was generated from the KM strain of human Ig transgenic mice (Medarex). The variable regions of the antibody were engineered into a high expression vector for production of fully human IgG1κ ε antibody. IMC-18F1 binds human VEGFR-1 with a high affinity (K_D = 54 pM) and efficiently blocks the binding of PlGF, VEGF-A and VEGF-B to VEGFR-1 with an IC50 of 0.5, 0.6 and 0.8 nM, respectively. IMC-18F1 inhibited ligand-induced phosphorylation of VEGFR-1 and activation of MAP kinase and Akt downstream signaling pathways in VEGFR-1 expressing endothelial and human breast cancer cell lines. The antibody also inhibited VEGF and PlGF-stimulated growth of breast carcinoma cells *in vitro*. Pharmacokinetic analysis indicates thatIMC-18F1 has plasma T1/2 of 4.8 days. Pharmacodynamic studies showed that a threshold dose of IMC-18F1 for maximal inhibition of VEGFR-1-related tumor growth was 20 mg/kg twice a week and average steady state plasma 18F1 concentration was 454 μg/ml. Treatment of mice with IMC-18F1 significantly suppressed the growth of human breast tumors in several xenograft models. Histology analysis showed that IMC-18F1 treatment inhibited MAPK and /or Akt signaling in breast tumor xenograft. These results indicate that IMC-18F1 is a potent VEGFR-1 antagonist and warrant further investigation.

71 POSTER

In vivo efficacy of STI571 in xenografted human small cell cancer alone or combined with chemotherapy

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STI571 or imatinib selectively inhibits BCR/ABL, PDGFR and c-kit kinase activity. It has been reported that a large proportion of small cell lung cancer (SCLC) cell lines and tumors express the c-kit receptor and that STI571 inhibits tumor cell growth. We therefore investigated the therapeutic efficacy of STI571, alone or combined with chemotherapy, in human SCLC cells or tumors xenografted into *nude* mice. The level of c-kit mRNA expression was variable in SCLC tumors (positive for 2/4 xenografts), and C-kit protein was not detected by immunohistochemistry. STI571 induced inhibition of proliferation of the SCLC6 cell line without inducing apoptosis; in contrast, in combination with etoposide or topotecan, the growth inhibition of SCLC6 cells induced by STI571 was increased, with apoptotic DNA fragmentation. Four human SCLC xenografts (SCLC6, SCLC61, SCLC74, and SCLC108) were transplanted into mice. After intraperitoneal injection of STI571, we observed 80%, 40%, and 78% growth inhibition of SCLC6, SCLC61, and SCLC108 tumors, respectively, without any significant inhibition of SCLC74 tumor growth. In mice bearing responsive SCLC tumors, we observed an increase of growth inhibition induced by chemotherapy (etoposide + ifosfamide or topotecan) by concomitant and continuous administration of STI571, associated with an increase of toxic deaths. In SCLC6-bearing mice receiving sequential treatments, we observed a reduction of toxic deaths, but a decrease of synergistic anti-tumor efficacy. In conclusion, the efficacy of STI571 alone in SCLC xenografted tumors was variable and did not depend on c-kit expression. Moreover, a significant increase of chemotherapy-induced growth inhibition was obtained by concomitant administration of STI571 that should be carefully investigated in SCLC patients.

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Restoration of wild-type p53 in malignant melanoma

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The binding of S100B to p53 down-regulates wild-type p53 tumor suppressor activity in cancer cells such as malignant melanoma, so a search for small molecules that bind S100B and prevent S100B-p53 complex formation was undertaken. Chemical databases were computationally searched for potential inhibitors of S100B, and 60 compounds were selected for testing based upon energy scoring, commercial availability, and chemical similarity clustering. Seven of these compounds bound to S100B as determined by steady state fluorescence spectroscopy (1.0 uM = KD = 120 uM) and five inhibited the growth of primary malignant melanoma cells (C8146A) at comparable concentrations (1.0 uM = IC50 = 50 uM). Additionally, Saturation Transfer Difference (STD) NMR experiments confirmed binding and qualitatively identified protons from the small molecule at the small molecule-S100B interface. Heteronuclear Single Quantum Coherence (HSQC) NMR titrations indicate that these compounds interact with the p53 binding site on S100B. A model of one such inhibitor, pentamidine, bound to calium-loaded S100B was calculated using intermolecular NOE data between S100B and the drug, and indicates that pentamidine binds into the p53 binding site on S100B defined by helices 3, 4, and loop 2 (termed the hinge region).

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Inhibition of choline kinase is a highly specific and selective cytotoxic antitumoral strategy

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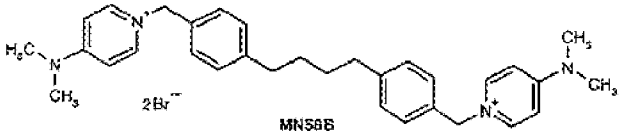
Choline kinase (ChoK), is responsible for the generation of phosphorylcholine, a proposed second messenger required for DNA synthesis induced by growth factors. ChoK levels are increased in different tumor-derived cell lines and in several human tumors when compared to their corresponding normal tissues (1). Moreover, ChoK inhibition has drastic inhibitory effects on cell proliferation and prevents tumor growth in mice (2). The aim of this work was to assess the specificity of the ChoK inhibitor MN58b and to provide a rational understanding for its antitumoral activity.
We have analysed the effects of a previously described ChoK inhibitor, MN58b (3) on different human tumor-derived cell lines compared to their appropriate primary, non transformed, counterparts. The effects on cell growth, cell cycle and the differential response in terms of cell signalling and lipid metabolism have been evaluated.



MN58B

A dramatic difference in the response of primary, non transformed human cells when compared to tumor-derived cell lines was observed. In normal cells, blockage of de novo phosphorylcholine synthesis by inhibition of ChoK promotes the dephosphorylation of pRb, resulting in a reversible cell cycle arrest at G0/G1 phase. In contrast, ChoK inhibition in tumoral cells renders cells unable to arrest at G0/G1 as manifested by a lack of pRb dephosphorylation. Furthermore, tumors cells specifically suffered a drastic wobble in the metabolism of main membrane lipids phosphatidylcholine (PC) and sphingomelin (SM). This lipid disruption results in the enlargement of the intracellular levels of ceramides. As a consequence, human tumor-derived cells are promoted to apoptosis while their normal counterparts remain unaffected. These results provide the evidence that MN58b is a specific and selective antitumoral strategy that works by specifically inducing apoptosis and killing tumoral cells without affecting normal cells.

Thus, ChoK inhibitors can be potencially useful in the clinic as a new tool for cancer therapy.

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74 POSTER

Determinants of the synergistic interaction between TS inhibitors, IFN-gamma and Fas signaling in therapy of colon cancer

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We have demonstrated a Fas-dependent component in thymineless death of human colon carcinoma cell lines (cc). The cytotoxicity of 5-fluorouracil (FUra) + leucovorin (LV), or the pure thymidylate synthase (TS) inhibitor ZD9331, is synergistic when combined with interferon-gamma (IFN-g) in a panel of cc, dependent on the Fas death receptor, DNA damage, independent of p53. Synergism was also demonstrated in HCT116 cells treated with ZD9331 + IFN-g, demonstrating RNA-mediated FUra/LV cytotoxicity not potentiated by IFN-g. In HT29 cells, IFN-g (but not ZD9331) upregulated the expression of caspases −3, −4, −7 and −8, and ZD9331 + IFN-g enhanced caspase activation and PARP cleavage, not prevented by overexpression of Bcl-2. IFN-g increased proteasomal activity, leading to selective downregulation of the IAP protein survivin, as well as increasing Fas expression. The cyclin dependent kinase inhibitor p21Cip1 also regulated thymineless death. HCT116 wt and p53−/− cells underwent apoptosis and loss in clonogenic survival when exposed to ZD9331, while p21Cip1−/− cells were resistant. In contrast, IFN-g induced marked cytotoxicity in p21Cip1−/− cells only. Cell cycle analyses determined that HCT116 wt and p21Cip1−/− cells accumulated in S phase within 24 hr of ZD9331 exposure, however wt cells exited S-phase more rapidly, apoptosis occurring prior to mitosis, either in late S or G2. ZD9331 induced p21Cip1 upregulation in all cc examined, as did dThd deprivation in TS-deficient cells. Selective induction of p21Cip1 in RKO was also sufficient to induce apoptosis. Based on results from preclinical studies, a Phase I trial was conducted. FUra (370 mg/m2) and LV (200 mg/m2), i.v. bolus daily × 5 days, were combined with escalating doses of IFN-g (10–100 mg/m2) s.c. on days 1, 3 and 5, every 28 days. Twenty-five patients with carcinomas were enrolled. MR or SD were observed in 6/21 heavily pretreated patients. Three evaluable chemo-naive patients demonstrated PR (2) or CR (1). Data demonstrate 1) several sites of interaction between the TS inhibitor, IFN-g and Fas signaling pathways including Fas, caspases, and an increased Fas/survivin ratio, independent of the mitochondria, 2) regulation of sensitivity to both TS inhibitors and IFN-g by p21Cip1, and 3) activity in a Phase I trial in colorectal carcinoma when FUra/LV was combined with IFN-g. This combination is to be evaluated in a Phase II trial. Supported by NCI awards CA 32613, CA 21765 and by ALSAC.

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Conjugates of lytic peptides target and destroy prostate cancer metastases

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In previous studies hormone resistant breast, prostate and ovarian xenografts were destroyed by targeting their receptors for chorionic gonadotropin (CG) with membrane disrupting peptide conjugates (Leuschner et al. Prostate 46, 116–125, 2001, Gawronska et al. Gynecol Oncol 85, 45–52, 2002, Leuschner et al Breast Cancer Research and Treatment, 78, 17–27, 2003).
Objective: To test the efficacy of a lytic peptide conjugate, Phor14-betaCG-ala, to destroy human prostate cancer cells and their metastases in vivo.
Methods: Phor14 [$(KFAKFAK)_2$] was conjugated to a modification of the 15-amino acid segment (81–95) of betaCG in which the cysteines were replaced by alanines. Luciferase transfected PC-3. luc prostate cancer cells were injected s.c. in a Matrigel suspension into nude mice. In the first experiment, the efficacy of Phor14-betaCG-ala at concentrations of 0.02, 0.2, 2, 5 or 10 mg/kg was tested in PC-3. luc tumor bearing mice. The mice received single (1× per week) or multiple (3× per week) injections through the lateral tail vein for 3 weeks starting on day 35 after tumor inoculation. In a second experiment the primary tumors were removed and the mice treated with Phor14-betaCG-ala at concentrations of 0.02, 0.2 and 2 mg/kg in single or multiple weekly injections. The tumor weights (in tumor bearing mice) were determined at necropsy. Metastases in lymph nodes (LN) were determined as luciferase positive cells through luciferase assays.
Results: In the single injection group 8 out of 50 tumor bearing mice were tumor free, whereas 16 out of 50 mice were tumor free in the multiple injection group. Tumor weights were reduced from 1.6±0.2 g in control mice to 0.5±0.03 g (0.2 mg/kg), and 0.2±0.08 g (10 mg/kg) in single injection groups, and to 0.3±0.04 g to 0.15±0.02 g in multiple injection groups ($p<0.01$ vs saline control; $p<0.05$ single vs multiple injections). LN metastases were significantly lower at all concentrations in the multiple injection groups than in the single injection groups ($p<0.002$, N=10). Phor14-betaCG-ala (0.02 mg/kg; multiple injections) was highly effective in destroying LN metastases in interscapular, axillary, hepatic and mesenteric LN ($p<0.002$).
Resection of the primary tumor stimulated metastatic progression in axillary interscapular LN (luciferase positive cells/LN) from 210±64 to 929±396 in saline controls and from 164±44 to 1154±405 in interscalular LN ($p<0.05$, N=10). Multiple injections of Phor14-betaCG-ala reduced the metastatic load in axillary and interscapular LN by 99% at concentrations as low as 0.02 mg/kg ($p<0.03$).
Conclusion: Phor14-betaCG-ala destroys primary tumors and lymph node metastases at concentrations as low as 0.02 mg/kg. Multiple injections are more effective than single injections. Lymph node metastases are directly targeted and destroyed by the lytic peptide conjugate.

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ZD4054: assessment of endothelin A receptor specificity following single dose administration in healthy volunteers

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In pre-clinical studies ZD4054 has been shown to be an orally active, potent, specific endothelin A receptor (ET_A) antagonist with potential utility in prostate cancer and metastatic bone disease (Curwen and Wilson. Eur J Cancer 2002;38[Suppl 7]: S102). Specific blockade of the ET_A receptor may be optimal in the oncology arena, since the anti-cancer effects of endothelin antagonists appear to be mediated via blockade of the ET_A receptor while concomitant inhibition of the endothelin B receptor (ET_B) may affect clearance of endothelin 1 (ET-1) and other beneficial processes such as apoptosis. In healthy volunteers, biological activity (objective pharmacodynamic activity and the adverse event profile) consistent with ET_A receptor blockade is seen with ZD4054 doses above 5mg.
Circulating ET-1 concentrations have been established as a biomarker of ET_B blockade *in vivo* in man e.g. Strachan et al (Hypertension 1999;33:581–5), and its measurement was utilised during the initial human studies with ZD4054 to determine its specificity for the ET_A receptor. This initial study comprised a randomised, ascending, double-blind, placebo-controlled design. At each dose level studied, six subjects were randomised to single doses of ZD4054 and two to placebo. Dose escalation was continued based on tolerability until the maximum tolerated dose had been defined. Doses of 2.5mg, 10mg, 20mg, 30mg, 60mg, 120mg, 150mg and 240mg ZD4054 were investigated within this study. Samples were collected for measurement of ET-1 and its precursor, Big-ET-1, at 4 hours and 24 hours post dose to assess the specificity of ZD4054 for ET_A versus ET_B. A rise in ET-1 without an accompanying rise in Big ET-1 would be taken as evidence for ET_B blockade *in vivo*.
Mean values for ET-1 at all doses, for both timepoints, were within the placebo range defined by the 2.5% and 97.5% percentiles of the pre-dose and placebo samples. No consistent profile was observed when comparing the 4 hour and 24 hour timepoints at each dose, and there was no evidence of a dose response based on a rise in mean values or percentage change from baseline.
In conclusion, this clinical study confirms preclinical findings that ZD4054 specifically antagonises ET_A, with no evidence for inhibition of ET_B. As a result of this specificity, ZD4054 has the potential to block the pathological processes in malignancy that are mediated by ET_A while allowing the beneficial processes mediated by ET_B to proceed.