In vivo study was performed in BALB/c nude mice. Six weeks old nude mice were divided 4 groups, 10 mice of each group, control, SG135, 5 mg/kg treated, SG135, 20 mg/kg treated and SG135 60 mg/kg treated groups. For the mice of control group were fed saline through out the experimental period. For the mice of three kinds of SG135 treated groups were fed SG at the dose of 5, 20 and 60 mg/kg/day, p.o. three times a week. After medication of SG135 for 12 days, tumor cells, 1×10^7 cells/mouse, were inoculated by s.c. on the flank of mouse. The tumor sizes were measured twice a week. The tumor growth was inhibited in all SG135, 5, 20 and 60 mg/kg/day treated groups, 21.0%, 26.0% and 25.0%, respectively, on day 49 after tumor inoculation when compared with control group. The survival rate were prominently increased in the mice of SG 20 and 60 mg/kg/day treated groups, 150.0% and 200.0%, respectively, on day 120 after tumor inoculation when compared with control group. These data presented that SG135 treatment was most effective in tumor growth inhibition and prominently effective in increase of average survival rate.

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Down-regulation of Sphingosine 1-Phosphate Receptor-1 in intestinal tumorigenesis

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Introduction: The bioactive sphingolipid, Sphingosine 1-Phosphate (S1P) is implicated in the regulation of cellular proliferation, migration, and survival via its G protein-coupled receptors S1P1–5. While animal models of intestinal neoplasia have demonstrated a beneficial effect of dietary sphingolipids in chemoprevention, the role of S1P in colon cancer is still unclear. The purpose of this study was to help define the role of the S1P receptor-ligand system in colon cancer.
Methods: Small intestine specimens of bigenic S1P1+/−Apcmin and S1P1+/+Apcmin mice were compared to determine effect of S1P1 heterozygosity on polyp number. Growth inhibition of RIE-1 cells was assessed using enforced expression of S1P1 receptor by adenoviral vector followed by treatment with S1P. Matched human normal and cancer colon tissue were obtained from surgical specimens.
Differential expression of S1P1 between the tissues was evaluated utilizing western blot analysis and immunohistochemistry.
Results: Bigenic S1P1+/−Apcmin mice revealed a 27% increase in polyp number when compared to control mice. Induced expression of S1P1 in RIE-1 cells caused growth inhibition with treatment of S1P.
Western blot analysis and immunohistochemistry revealed an increased expression of S1P1 in the human normal tissue as compared with tumor tissue.
Conclusions: Our results suggest that S1P1 receptor functions in the intestinal epithelium to inhibit tumorigenesis. Down-regulation of S1P1 in colorectal cancer may have functional consequences in the proliferation and or metastatic spread of cancer. Further evaluation of Sphingosine 1-phosphate receptor-1 is necessary to determine its potential for therapeutic intervention in colon cancer.

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Vitamin E succinate inhibits the in vitro growth of pancreatic cancer cells

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Background: Vitamin E Succinate (VES, α-tocopheryl succinate) is the most potent anti-tumor analog of Vitamin E that selectively induces apoptosis in cancer cells by modulating the expression of Bcl-2 family proteins. Despite its role being studied as a chemopreventive, chemotherapeutic and chemosensitizing agent in various cancers, there are scarce studies of VES in pancreatic cancer. Pancreatic cancer is the number four killer in the US and about 32,000 new cases are reported every year. The five year survival rate is only 5%. In this study, we investigated the effects of VES in three pancreatic cancer cell lines, ASPC-1, COLO-357 and PANC-1. We also assessed the synergistic growth inhibitory effect of VES along with two known cytotoxic drugs, Etoposide and Gemcitabine.
Methods: Cells were treated with varying concentrations (5 μM to 100 μM) of VES alone or in combination with Etoposide or Gemcitabine for different time periods. WST-1 cell proliferation reagent (Roche) was used to determine the cytotoxicity after the treatment. We studied the expression pattern of Bcl-2 family proteins in response to VES in ASPC-1 cells.
Results: VES inhibits the cell proliferation of all the three pancreatic cancer cell lines in a time and dose dependent manner. Our data also demonstrates that VES synergistically inhibits the cell growth in combination with 80 μM etoposide and 0.5 μg/ml Gemcitabine. In ASPC-1 cells, we observed a dose dependent decrease in the expression of Bcl-XL in response to VES.
Conclusion: This study demonstrates that (a) VES inhibits the *in vitro* growth of pancreatic cancer cell lines (b) Vitamin E succinate synergistically inhibits the growth of pancreatic cancer cells in combination with cytotoxic drugs Etoposide or Gemcitabine and (c) VES down-regulates the expression of antiapoptotic protein Bcl-XL in ASPC-1 cells.

Clinical methodology

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Preliminary results of a accelerated dose escalation phase I trial with a novel anthracycline derivative (RTA-744) in patients with primary brain tumors

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Background: RTA 744 is an anthracycline derivative that was shown in preclinical studies to cross the blood-brain barrier, not be a substrate for p-GP or MRP mediated efflux and improve survival in an orthotopic murine model of glioblastoma. A trial of RTA 744 was initiated at M. D. Anderson Cancer Center in patients with primary, high-grade gliomas.
Methods: RTA 744 is being administered as a 2-hour intravenous infusion on each of the first three days of a 21-day cycle. Dose escalation is proceeding according to an accelerated titration design, with single patient cohorts and 100% dose escalations until evidence of drug-related Grade 2 or greater toxicities are observed. Standard determinants of MTD are being employed. The MTD is being determined first in patients who do not take enzyme-inducing anti-convulsants. Pharmacokinetic samples are being taken at multiple time points on days 1–5 of Cycle 1. Tumor activity is being assessed according to the MacDonald criteria.
Results: As of May, 2006, RTA 744 has been administered to a total of 7 patients (pts) at dose levels of 1.2 (1 pt), 2.4 (3 pts), 4.8 (2 pts), and 9.6 mg/m^2/day (2 pts) (corresponding to 3.6, 7.2, 14.2, and 28.4 mg/m^2/cycle). No Grade $\geqslant$2 drug-related toxicities have been observed at doses of 4.8 mg/m^2 and below; results at 9.6 mg/m^2 have shown the first Grade 2 toxicities (platelets, lymphopenia and elevated SGPT). As a result the 9.6 mg/m^2/day cohort will be expanded and the percent of dose escalation for subsequent cohorts will be reduced. The pharmacokinetic profile indicates dose proportionality, with some accumulation by Day 3. Mean plasma half-life of RTA 744 thus far is approximately 34 hours. Three of the first four patients received at least four cycles, and one of these patients remains on study. Evidence of clinical activity was also seen in the first four patients, including 2 Minor Responses (2.4 mg/m^2) and 1 Stable Disease (1.2 mg/m^2). The most recent patient received a dose 4 times the level at which tumor regression was first documented. MRI results from the two most recently enrolled patients are pending.
Conclusions: RTA 744 is well tolerated up to doses of 9.6 mg/m^2/day, has predictable pharmacokinetics, and shows early evidence of activity. Full results of this trial should be available by the fall of 2006. Based on the activity seen to date, Phase 2 studies of this novel agent in primary and metastatic brain tumors appear warranted.

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Phase II trial of Sequenced Bevacizumab and Erlotinib with Bevacizumab and Chemotherapy for 1st Line Stage IIIB or IV Non-Small Cell Lung Cancer (NSCLC)

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Background: Recent evidence suggests that bevacizumab added to erlotinib increases activity in 2nd line metastatic NSCLC and the addition of bevacizumab to chemotherapy improves survival in 1st line metastatic NSCLC. Bevacizumab plus erlotinib (B+E) has never been tested in 1st line NSCLC. Furthermore, administration of 4 cycles of B+E prior to bevacizumab plus chemotherapy would allow selection of patients who could benefit from consolidation B+E.

Methods: 3-stage phase II design with interim analyses planned after 10 and 20 subjects enrolled. Subjects with previously untreated stage IIIB with pleural effusion or IV NSCLC and normal organ function without hemoptysis or brain metastasis were treated with B (15 mg/kg every 21 days) plus E (150 mg OD) for 4 cycles followed by B (15 mg/kg), carboplatin (AUC 6) and paclitaxel (200 mg/m^2) every 21 days. Subjects who did not progress on initial B+E received further consolidation with B+E until progression.
Results: Twenty subjects have been enrolled thus far (11 male, 9 female; median age 61 years; 18 stage IV, 2 stage IIIB; 18 former smokers; 13 adenocarcinoma, 3 large cell carcinoma, 4 undifferentiated carcinoma; performance status 0 in 8 and 1 in 12). Sixteen subjects have completed at least 2 cycles of B+E with 3 partial responses (response rate 19%) and 8 stable disease (50%). Seven subjects have completed bevacizumab plus chemotherapy with best responses of 1 partial response and 5 stable disease. Toxicity observed during B+E has included grade 3 rash (4 subjects), grade 3 diarrhea (1 subject), grade 2 hypertension (1 subject), and grade 3 epistaxis (1 subject). Toxicity observed during bevacizumab/chemotherapy has included grade 3 neuropathy (2 subjects), grade 3 hypersensitivity reaction (1 subject), grade 4 neutropenia (1 subject), and grade 4 arterial thrombosis (1 subject).
Conclusions: The combination of B+E appears effective and well tolerated in 1st line stage IIIB/IV NSCLC. The administration of 4 cycles of B+E does not impair subsequent administration of cytotoxic chemotherapy. Accrual to a planned total of 48 subjects continues.

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Magnetic Resonance Perfusion Imaging predicts oligodendroglial cytogenetic subtypes and determines profiles of tumor angiogenesis

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Background: Chemosensitivity of oligodendroglial neoplasms and prolonged patient survival can be predicted based upon the loss of heterozygosity (LOH) of chromosomes 1p and 19q. Although the genes involved with oligodendroglial pathogenesis on chromosome 1p and 19q have not been identified, noninvasive characterization of the biological behavior of these tumors through advanced imaging techniques can direct the search for candidate genes. The relative tumor blood volume (rTBV) as determined by magnetic resonance (MR) perfusion-weighted imaging reflects the degree of neoplasm angiogenesis and metabolism. Therefore, the present study is aimed to correlate MR perfusion-weighted imaging data to molecular determinants of glial tumor malignancy and angiogenesis in distinct cytogenetic subsets of oligodendroglial tumors.
Materials and Methods: Thirty patients with oligodendroglial neoplasms were retrospectively selected for this study. Tumors were classified according to the current WHO histological criteria as either low-grade (II) or high-grade (III) and as either oligodendrogliomas or oligoastrocytomas. Tumors were divided into two groups: tumors with 1p or 1p/19q LOH (group 1) versus tumors with 19q LOH or intact alleles (group 2). MR studies were performed on a 1.5 T scanner using dynamic susceptibility-weighted methods (DSC) to determine rTBV. Cytogenetic tumor analyses were performed by either FISH or PCR methods. Paraffin-embedded tumor tissues were assessed for tumor expression of vascular endothelial growth factor (VEGF) using standard IHC techniques.
Results: In WHO grade II neoplasms, the rTBV was significantly greater ($p < 0.05$) in group 1 [n = 7; mean 2.63; (range 0.96–3.28)] compared to group 2 [n = 7; 1.71; (1.27–2.23)]. In grade III neoplasms, the differences between group 1 (n = 4; 2.83; (1.59–6.26)) and group 2 [n = 12; 2.88; (1.81–3.76)] were not significant. The rTBV was significantly greater ($p < 0.05$) in grade III neoplasms [n = 16; 2.88; (1.59–6.26)] compared to grade II neoplasms [n = 14; 1.99; (0.96–3.28)]. Interestingly, there was increased expression of VEGF and related angiogenic proteins in 1p/19q deleted tumors. It is possible that the genes on chromosomes 1p and 19q may include negative regulators of tumor angiogenesis and invasion.
Conclusions: Collectively, our data demonstrates the utility of advanced MR imaging in predicting molecular correlates of tumor malignancy and angiogenesis in distinct cytogenetic subsets of oligodendroglial tumors.

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Translation of in vitro markers of the anti-angiogenic and anti-tumor activity of the SOD1 inhibitor ATN-224 to clinical trials

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ATN-224 is a second generation analogue of the copper binding drug tetrathiomolybdate (TM) and is currently entering phase II trials in several oncology indications. ATN-224 inhibits CuZn superoxide dismutase (SOD1) having anti-angiogenic and anti-tumor effects (Juarez et al. (2006) Clinical Cancer Research, *in press*). SOD1 catalyzes the dismutation of superoxide anion into H_2O_2 and O_2. Hydrogen peroxide is a promiscuous second messenger that mediates mitogenic signaling and by inhibiting SOD1, ATN-224 suppresses the generation of H_2O_2 and down-regulates several mitogen-induced kinase cascades (VEGF, FGF-2, EGF, PDGF and IGF-1). The need for biomarkers to follow biological activity in patients or to select patients for clinical trial is obvious. Historically, the pharmacodynamics of TM have been followed by tracking ceruloplasmin (Cp), a biomarker for systemic copper. However, at least in mice, the inhibition of angiogenesis occurs before a measurable decrease in systemic copper is observed. In this study, we present the pre-clinical evaluation of three potential biomarkers and the translation of two of these in the clinic. We initially correlated the ATN-224 mediated inhibition of plasma, blood cells and tumor SOD as well as decrased in ERK 1/2 phosphorylation in animal tumor models with the inhibition of tumor growth and angiogenesis. Finally, we evaluated the effects of ATN-224 on circulating endothelial progenitors (EPCs) and circulating endothelial cells (CECs), which are established biomarkers for anti-angiogenic activity, in primates. Based on these pre-clinical studies, we evaluated the effects of ATN-224 on SOD activity in blood cells, Cp levels in plasma and CECs and EPCs in patients as part of a phase I clinical trial in advanced solid cancer (sponsored by Cancer Research UK). A dose-dependent decrease in SOD1 activity as well as CECs and EPCs was observed in ATN-224 treated patients. The inhibition of SOD activity occurred earlier than the depletion of Cp, suggesting that the inhibition of SOD activity is a more sensitive pharmacodynamic readout of ATN-224 activity than the depletion of systemic copper measured by Cp. Furthermore, changes in SOD activity were also more sensitive than Cp to ATN-224 dose adjustments. These results indicate that SOD activity and CEC/EPC measurements are useful biomarkers of ATN-224 activity and support the evaluation of these biomarkers in phase II trials for correlation to clinical benefit or tumor response.

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The reproducibility of perfusion parameters obtained from dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) in liver and lung tumors, and implications for sample size in clinical trials using DCE-MRI

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Background: DCE-MRI is being increasingly incorporated into Phase I/II clinical trials, particularly with antiangiogenic drugs. The aims of this study were to determine the reproducibility of measurements of perfusion parameters derived from DCE-MRI, and to evaluate their impact on trial design.
Material and Methods: DCE-MRI was undertaken on two occasions (2–7 days apart) in 25 patients with malignant tumors in the liver or lung. The main inclusion criteria were: no preceding therapy for >4 weeks, no inter-scan therapy, and lesions >3 cm. Dynamic gadolinium enhanced 3D fast spoiled gradient-echo images were acquired for approximately 4 minutes. Tumor blood normalized initial area under the curve ($IAUC_{BN}$) and transfer constant (K^{trans}) parameters were computed using a semi-automated algorithm. Coefficients of variation (CoV) were determined using the logtransformed K^{trans} and $IAUC_{BN}$ data, and the pooled between-visit variance. Sample size estimates used a general estimating equation model, single-sided 5% significance, 90% power and a 25% response rate.
Results: There were 24 evaluable patients (12 liver, 12 lung lesions). The medians (inter-quartile range) across patients for $IAUC_{BN}$ and K^{trans} for liver lesions were 0.23 (0.20–0.32) and 0.065 (0.056–0.096), and for lung lesions, 0.18 (0.14–0.26) and 0.053 (0.035–0.073), respectively. The CoV values for $IAUC_{BN}$ and K^{trans} were lower for liver than for lung lesions, estimate (95% range): 9.8% (6.8−17.3%) and 10.6% (7.4−18.9%) for liver, versus 18.9% (13.1−33.9%) and 19.3% (13.4−34.5%) for lung [p = 0.07 and 0.059], respectively. Sample size calculations with these data indicate that, for a sought after 25% therapeutic response, appropriate sample sizes are 4 and 12, for liver and lung tumors, respectively. A single patient can be evaluated, with 90% confidence, for a 25% and 40% change in DCE-MRI parameters for liver and lung lesions, respectively; and a 10% response in a single patient can be determined with 70% and 60% confidence, respectively (Table).
Conclusions: The reproducibility of perfusion parameters derived from DCE-MRI are in the range 10−20%, and appear better in the liver than