15% of A431 cells vs. 2% of the MDA-468 cells were stained positive by AnnexinV, which is a marker for apoptosis. The induction of apoptosis appeared to correlate with loss of TK1 expression, as assessed by western blotting and film densitometry. Thus, we reasoned that caspase-mediated degradation of TK1 could be at least partially responsible for the drug-induced suppression of FLT uptake. To test this, A431 cells were treated simultaneously with the caspase inhibitor ZVAD-fmk and erlotinib. ZVAD-fmk treatment reduced the drug-induced cell death, and this was associated with a corresponding attenuation of erlotinib-induced suppression of FLT uptake (Table 1). The effects of ZVAD-fmk on TK1 expression and apoptosis induction will be reported.

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Signaling mediators of bystander response are potential therapeutic targets for attenuating tumor relapse

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Background: Our objective is to understand whether radiation used to treat local tumor after surgery can, trigger a positive feed back signaling mechanism. This may cause the cells to have a memory of the initial irradiation insult for an extended period of time that may result in local/regional tumor recurrence at later time. In this study we determined whether exposure to radiation could initiate NF-kB and TNF-a signaling and maintain NF-kB >TNF-a > NF-kB feed back cycle in human epithelial cells.
Materials and Methods: To examine whether cells at normal tissue at the radiation-exposed treatment site could undergo these alterations, normal human epithelial cells were exposed to radiation at doses used in fractionated radiotherapy and examined for (a) the dose and time dependent activation of NF-kB by electrophoretic mobility shift assay (EMSA), (b) the kinetics of TNF-a expression by RT-PCR (mRNA expression) and ELISA (secreted protein expression), and (c) the involvement of reactive oxygen species (ROS) in TNF-a mediated NF-kB activation by FACS analysis. Blocking experiments were performed using specific inhibitors.
Results: EMSA of nuclear extracts from cells exposed to clinical doses of radiation revealed a bi-phasic time-dependent expression of NF-kB, reaching a first maximum at 3h and a second maximum at 48 h. The functional integrity of the radiation-induced NF-kB, determined by transient transfection with pNF-kB-Luc that expresses the luciferase reporter gene in an NF-kB-dependent manner showed a 3.8-folds compared to mock irradiated control indicating that NF-kB DNA-binding activity triggered by radiation exposure could initiate transcriptional activation of NF-kB–dependent genes. Cells either incubated with TNF-a soluble receptor or TNF-a neutralizing antibody blocked the second phase (24 & 48 h) induction of NF-kB activation. Similarly, TNF-a mRNA expression was observed at 8h and protein expression at 16 and 24 h post-exposure. The TNF-a expression both at mRNA and protein level were inhibited to constitutive level by pre-incubating the cells with NF-kB inhibitor NF-kB SN50 cell permeable inhibitory peptide (100 μg/ml) 1 h prior to radiation exposure. These results clearly indicated the occurrence of a positive feedback cycle initiated by the activation of NF-kB upon radiation exposure. This activated NF-kB signaling mechanism triggers the TNF-a production, which in turn induces the activation of NF-kB through generation of ROS in primary endothelial cells.
Conclusions: Reappearance of a local or regional tumor after treatment is a major limitation in achieving disease-free survival. Identifying and intervening the mediators involved in this mechanism may help to achieve a prolonged disease free survival. This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG03–02ER63449.

372 POSTER

A phase I/II trial of erlotinib and bexarotene in aerodigestive tract cancers

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Background: We have previously reported the overexpression of the epidermal growth factor receptor (EGFR) and cyclin D1 as early events in lung carcinogenesis. Classical retinoids prevent the carcinogenic transformation of human bronchial epithelial (HBE) cells at least partly through repression of these proteins. Clinical activity of classical retinoids is limited by the frequent repression of the critical retinoic acid receptor, RAR-beta. Non-classical retinoids such as the rexinoid, bexarotene, exist that repress cyclin D1 and EGFR expression but can signal independent of RAR-beta. We found that combining an EGFR tyrosine kinase inhibitor, erlotinib, with bexarotene induced at least additive suppression of growth and cyclin D1 expression in HBE cells which had silenced RAR beta. Based on these and other pre-clinical findings we performed a phase **I/II** clinical trial of this combination in patients with advanced aerodigestive tract cancers.
Materials and Methods: Patients with advanced aerodigestive tract cancers who had failed prior chemotherapy were enrolled onto this dose-escalation study. Three dose levels were utilized and at least three patients were enrolled at each level. Primary objectives were to determine the maximum tolerated dose. Secondary objectives were to determine activity, toxicity, and surrogate markers of response in buccal epithelial cells.
Results: Twenty patients were enrolled and sixteen are evaluable. Toxicities have generally been mild with asymptomatic elevations of cholesterol and triglycerides occurring frequently. No cases of pancreatitis were observed. One case of dose-limiting rash (grade 3) and one case of dose-limiting diarrhea (grade 3) were observed. To date, three patients have had radiographic responses (2 PR, 1MR), one of which has lasted more than 1 year. Four additional patients remain on study with stable disease. Two have had stable disease for more than three months. Changes in surrogate markers of response in buccal epithelial cells are being determined.
Conclusions: The combination of erlotinib and bexarotene is well tolerated and appears active for the treatment of advanced aerodigestive tract cancer resistant to chemotherapy. A confirmatory trial comparing this regimen to erlotinib alone is warranted to determine the efficacy of this regimen.

373 POSTER

p38/JTV-1 is a novel modulator of TGF-beta required for the downregulation of c-myc and lung cell differentiation: its functional association with lung cancer formation

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p38/JTV-1 is known to be an essential scaffold protein for the formation of macromolecular tRNA synthetase complex (ref1). Interestingly. The mice lacking the gene encoding p38 were developed with severe hyperplasia of the alveolar epithelial cells in lung that caused immediate death after birth due to respiratory dysplasia (ref2). Molecular screening revealed that p38 interacts with FBP (FUSE-binding protein) that is a transcriptional activator of protooncogene, c-myc. The binding of p38 enhanced the ubiquitin-mediated degradation of FBP, resulting in downregulation of c-myc, which is required for the functional differentiation of alveolar type **II** cells. The ectopic expression of p38 suppressed proliferation and restored the differentiation markers in lung carcinoma cells. The cellular level of p38 was increased by TGF-beta to mediate cell growth arrest. In reverse, the loss of p38 led to resistance to TGF-induced cell cycle inhibition. The mice with reduced expression of p38 showed higher susceptibility to tumorigenesis, and the severe reduction of p38 level was frequently found in lung cancer cell lines and clinical cancer tissues. The working mechanism and association with tumor formation in animal model and human cancer patients strongly suggest p38/JTV-1 as a novel tumor suppressor.

374 POSTER

Inhibition of PI3K/AKT pathway by rhabdastrellic acid-A induced caspase-3-dependent apoptosis in leukemia cells

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Background: It has been demonstrated that PI3K/AKT signaling is aberrantly activated in AML cells. It is a promising strategy to target PI3K/AKT pathway for cancer treatment. Rhabdastrellic acid-A, an isomalabaricane triterpenoid, isolated from the sponge Rhabdastrella globostellata. The aim of this study is to explore effect of Rhabdastrellic acid-A on PI3K/AKT pathway and apoptosis.
Methods: Cytotoxicity was determined by MTT assay. Immunoblot analysis was employed to detect protein expression. DNA fragmentation was analyzed using agrose gel electrophoresis.
Results: Our investigation indicated that Rhabdastrellic acid-A inhibited proliferation of HL-60 cells with IC_{50} value of 0.64 μg/ml and induced apoptosis of HL-60 cells. Also, Rhabdastrellic acid-A induced cleavage of the death substrate poly (ADP-ribose) polymerase (PARP) and caspase-3. Pretreatment of HL-60 cells with caspase-3 specific inhibitor DEVD-CHO prevented Rhabdastrellic acid-A-induced DNA fragmentation, PARP cleavage. The expression levels of protein bcl-2, bax have no change in response to Rhabdastrellic acid-A treatment in HL-60 cells, whereas activated PI3K had significantly a decrease after treatment with

Rhabdastrellic acid-A in HL-60 cells. Activation of downstream molecule of PI3K pathway such as AKT also was inhibited following Rhabdastrellic acid-A treatment.
Conclusion: It concludes from these results that Rhabdastrellic acid-A inhibits PI3K/Akt survival pathway and induces caspase-3-dependent apoptosis in leukemia cells.

375 POSTER

HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer cells in conjunction with blockade of androgen receptor, STAT3, and AKT signaling

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This study found that HIV-1 protease inhibitors (PIs), including nelfinavir, ritonavir, and saquinavir induced growth arrest and apoptosis of human prostate cancer cells (LNCaP, DU145 and PC-3 cells), as measured by MTT and TUNEL assay, respectively on the third day of culture. In addition, PIs blocked androgen receptor (AR) signaling in association with down-regulation of nuclear levels of AR in LNCaP cells as measured by reporter assay and Western blot analysis. As expected, PIs down-regulated the level of the AR target molecule prostate specific antigen in these cells. Moreover, PIs disrupted STAT3 signaling; PIs blocked IL-6-induced phosphorylation of STAT3 and inhibited STAT3 DNA binding activity in LNCaP and DU145 cells, as measured by Western blot analysis and ELISA-based assay, respectively. Furthermore, PIs blocked AKT signaling in prostate cancer cells as measured by kinase assay with GSK-3α /β as a substrate. Taken together, PIs inhibited proliferation of prostate cancer cells in conjunction with blockade of signaling by AR, STAT3, and AKT suggesting that this family of compounds might be useful for the treatment of individuals with prostate cancer.

376 POSTER

Tolerability results with the novel oral prenyl transferase inhibitor AZD3409 following single and multiple doses in volunteer studies

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Background: AZD3409 is a novel, oral, antitumour agent that acts as a prenyl transferase inhibitor. Here we report preliminary results of the tolerability assessments conducted during two studies of AZD3409 in healthy human volunteers.
Methods: In the single ascending dose study, a maximum of eight healthy male volunteers were dosed at each dose level (6 active, 2 placebo) in a randomised, double-blind, alternating panel design with doses escalated from 20 mg to 2500 mg. In the multiple dose study, a maximum of 16 volunteers (12 active, 4 placebo) were administered the same once-daily dose for 7 consecutive days at the following ascending doses for three consecutive cohorts: 500 mg, 1000 mg, and 1750 mg. The following were monitored: vital signs, ECG, clinical chemistry, haematology and urinalysis, and adverse events for 21 days after each dose. Data remain blinded and the analysis is ongoing.
Results: The maximum tolerated single dose of AZD3409 was 1750 mg, which was also tolerated on multiple dosing. In the multiple dose study, there have been no significant safety or tolerability issues identified with 500 mg, 1000 mg, or 1750 mg multiple doses. Possible drug-related adverse events include loose stools, abdominal discomfort, light-headedness, nausea, and transient rash. These adverse events were generally mild (CTC grade 1) and resolved without treatment. One subject in each of the 1000 mg and 1750 mg cohorts had loose stools graded as moderate (CTC grade 2) in the first half of the dosing week. The 'moderate severity' grading lasted no more than 1 day in both cases and both volunteers completed the full 7-day treatment schedule. The incidence, but not the severity, of gastrointestinal adverse events appears to correlate with increasing dose. No clinically important changes in clinical assessment, ECG, or routine laboratory safety data have been detected.
Conclusions: Based upon these results, 1750 mg of AZD3409 once daily for 7 days is well tolerated in healthy volunteers.

377 POSTER

Tolerability and limited activity of perifosine in patients with advanced soft tissue sarcoma (STS): a multi-center phase 2 consortium (P2C) study

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Background: Current treatments in STS are largely palliative and novel agents need exploration to improve patient (pt) outcome. A prolonged (>12 mos) objective response in a refractory STS pt during a phase I trial prompted our phase II evaluation of the 6-month progression-free rate of perifosine in pts with advanced STS. The study design required 4 of 15 evaluable pts to be progression-free at 6 mos. to enroll 27 additional pts.
Methods: Pts received a perifosine loading dose of 150 mg p.o. every 6 hours $\times$ 4 for day 1, followed by 100 mg once daily for d2–28. Subsequent 28 day cycles were the same, excluding the loading dose. Eligible pts had measurable disease and adequate organ function (total bilirubin and creatinine $\leqslant$ UNL, PLT > 100,000 μL, ANC > 1,500 μL). Serum was collected for PK analyses.
Results: 23 pts were enrolled. A majority had prior treatment: 1–2 chemotherapy regimens (87%), surgery (96%), and radiotherapy (52%). 22% presented with liver metastasis. Pts are aged 24–77 yrs (median 53); 65% are female; and a majority (56%) ECOG PS 1 (vs 0). 19 of 23 pts received at least 2 cycles of therapy (range 2–8). All pts are evaluable for toxicity. One pt had Gr. 4 ileus. 6 pts (26%) had Gr. 3 toxicity, including fatigue (2 pts) and 1 patient each of anemia, infection, muscle weakness, pain, rash, anorexia, dehydration, and diarrhea. 6 pts (26%) have died, all of which are non-treatment related. A pt having myxosarcoma had a partial response lasting 5+ mos. Two (1-myxosarcoma, 1-desmoid) pts are progression-free at 6 months [15%, 95% CI (2–41%)].
Conclusions: Although this study failed to satisfy the criteria to proceed with accrual, the regimen was tolerable. The preliminary observation of another potential prolonged responder raises the question of whether specific histologies or tumor characteristics might predict a more sensitive sub-population of STS pts. PK analyses are underway. Supported by N01-CM-17104.

378 POSTER

In vivo and in vitro enhanced antitumor activity of Oxaliplatin in combination with cetuximab (C225), a chimeric monoclonal antibody anti-epidermal growth factor receptor on a panel of human colorectal tumor xenografts

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Advanced colorectal carcinoma is a major cause of morbidity and mortality in the developed countries. Colorectal cancers frequently express the epidermal growth factor receptor (EGFR), which has been correlated with more aggressive disease and poor prognosis. Several EGFR inhibitors such as C225 (cetuximab), a chimeric anti-EGFR monoclonal antibody, are being developed in various indications. Oxaliplatin (L-OHP), is a major compound in the treatment of colorectal carcinoma but has not been yet evaluated in preclinical studies in association with C225.These two drugs have demonstrated efficacy as single agent in the inhibition of tumor growth and apoptosis induction in colon cancer *in vivo* and *in vitro*.
The aim of our study was to evaluate the effect of the combination of C225 and L-OHP on a panel of L-OHP-insensitive colon cancer cell lines.
These studies were performed both *in vivo* and *in vitro* on 4 colon cancer cell lines HCT-8; HT-29, SW620, HCT-116 showing different levels of EGF-R expression in WB analysis. We first assessed the cell growth and IC_{50} of L-OHP, C225 monotherapy or combination of both. The combination of L-OHP and C225 led us to observe an inhibition of tumor growth and a decrease in IC_{50} of L-OHP in HCT-8, and HT-29. On the other hand, the combination of C225 and L-OHP did not show any major modification in IC50 of L-OHP in SW 620 (EGF-R negative) or in HCT-116 (EGF-R positive).
Xenografts in nude mice were established by subcutaneous injection of 10 $X10^6$ human colon cancer cells in both flanks. Mice were then randomized into four treatment groups: control, anti-EGFR (C225), L-OHP or C225 plus L-OHP. C225 was administered i.p. at the dose of 0.5 mg. three times a week. L-OHP was infused at the dose of 10 mg/kg by i.v. route 7 days after implantation.The combination of C225 (0.5 mg) and L-OHP (10 mg/kg) strongly inhibited the growth of HCT-8 and had a slight effect on HT-29 established tumors. In a refractory tumor model SW620 and HCT-116, the