insulin receptor. It had minimal activity against a panel of 30 other protein kinases. Compound-1 also abolished IGF-induced anchorage-independent growth and activation of downstream pathways pAkt, pErk1/2 and p-p70S6K in both IGF-IR transfectant cells and a GEO human colorectal cancer cell line. Analysis of GEO cells revealed an active IGF-II/IGF-IR autocrine loop. Robust anti-tumor efficacy was achieved in a GEO xenograft model with Compound-1 administrated orally once daily, which correlated with the degree and duration of inhibition of tumor IGF-IR phosphorylation by Compound-1. No substantial blood glucose changes were observed in mice at efficacious dose levels. Moreover, when Compound-1 was tested in EGFR inhibitor erlotinib (Tarceva®)-sensitive (H292), moderately sensitive (H441) and insensitive (H460) NSCLC lines alone or in combination with erlotinib *in vitro* and *in vivo,* synergistic effects were achieved in both H292 and H441 cell lines, whereas minimal potentiation was observed in H460 cells. Analysis of downstream pathways to EGFR and IGF-IR indicated that Compound-1 together with erlotinib effectively blocked pAkt and pErk1/2 in H292 and H441 cells, which in turn led to significant apoptosis, whereas in H460 cells there were minimal effects of the combination on the downstream pathways. Accordingly, tumor growth of H292 exhibited a durable cure in 3/8 mice when erlotinib was co-administrated with this IGF-IR inhibitor orally once daily. Significant tumor regression was also observed in H441 tumors in response to the combination treatment. Thus potent and selective IGF-IR kinase inhibitors like Compound-1 may have broad clinical utility for treatment of cancers as a single agent and in combination with EGFR inhibitors such as erlotinib.

547 POSTER

Novel small-molecule inhibitors of STAT3 that selectively induce antitumor cell activity

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The Signal Transducer and Activator of Transcription (STAT) family of proteins were originally discovered as mediators of cytokine and growth factor responses. Evidence has accumulated demonstrating that persistently-active STAT3, one of the family members, is a critical mediator of events that contribute to carcinogenesis and tumor progression. Aberrant STAT3 activity mediates dysregulated growth and survival, promotes angiogenesis and modulates immune responses. Of clinical significance, constitutive STAT3 activation is detected in breast, prostate, and colon cancers, non-small cell lung cancer, squamous cell carcinoma of the head and neck, as well as many other solid and hematological tumors. Inhibition of aberrant STAT3 induces antitumor cell activity and tumor regression in mouse model studies, thus providing therapeutic relevance to STAT3 inhibition and a validation of STAT3 as a cancer drug target. We have identified a diverse group of small-molecule STAT3 inhibitors by structure-based design with computational modeling that exploited the key structural requirements for binding of the native STAT3 phosphotyrosine (pTyr) peptide to the SH2 domain of STAT3. In addition, we performed random screening of compound libraries for binders of the STAT3 SH2 domain or inhibitors of STAT3 DNA-binding activity. Current inhibitors, such as non-peptide analogs of the STAT3 pTyr peptide (PpYLKTK) as disruptors of STAT3 dimerization, structurally-diverse compounds that bind the STAT3 SH2 domain, as well as novel platinum (IV) complexes that interact with the STAT3 DNA-binding domain, potently inhibit constitutive STAT3 activation and signaling in malignant cells. Moreover, these small-molecule STAT3 inhibitors selectively block tumor cell growth and induce apoptosis of malignant cells harboring aberrant STAT3 activity. Consistent with these findings, molecular analysis reveals down-regulation by STAT3 inhibitors of known STAT3-regulated genes, including Cyclin D1, Bcl-xL, Bcl-2 and Survivin, in malignant cells. Together, our studies demonstrate the feasibility of current efforts to develop small-molecule STAT3 inhibitors as anticancer drugs. Our findings also provide proof-of-concept for the antitumor cell effects of novel small-molecules that inhibit aberrant STAT3.

548 POSTER

Active mutant epidermal growth factor receptor undergoes less protein degradation due to diminished binding to c-Cbl ubiquitin ligase

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Background: Recently, new research has revealed that gefitinib (Iressa) sensitivity in non-small-cell lung cancer (NSCLC) is associated with activating mutations in the epidermal growth factor receptor (EGFR). Previously, we identified a 15-bp deletion mutation (2411 to 2425) in a NSCLC cell line, PC-9 (AACR 2004, abs 1221). This cell line is 1000 times more sensitive to gefitinib as compared with another NSCLC cell line, PC-14, which expresses wild-type EGFR. It has been reported that autophosphorylation of mutant EGFR is significantly prolonged as compared with the wild type EGFR (Lynch et al NEJM 2004). The same observation is seen in PC-9. EGFR is known to be degraded by lysosomal proteases and 26S proteasome after autophosphorylation, and in the latter case, the degradation occurs through ubiquitinylation of EGFR by c-Cbl ubiquitin ligase. To explore the mechanism of sustained autophosphorylation of the mutant EGFR, we examined the difference in EGFR degradation activities between the wild type EGFR and the mutant EGFR.
Material and Methods: To clarify the mechanism, we used expression vectors of wild type EGFR and mutant EGFR with the 15-bp deletion found in PC-9, and established stable transfected cell lines, named 293_pEGFR and 293_pΔ15, respectively. EGFR internalization and degradation activities were measured by using ^{125}I-EGF. EGFR-bound c-Cbl was immunoprecipitated by EGFR-specific antibody and the binding activity was detected by immunobloting. Site specific c-Cbl phosphorylations were detected by immunobloting using respective anti phospho-c-Cbl antibodies.
Results: After TGF-α exposure, degradation rate of EGFR was about 10-fold higher in PC-14 as compared with that in PC-9. In 293_pΔ15, EGFR degradation activities and c-Cbl-binding to this receptor after TGF-α stimulation were significantly decreased as compared with 293_pEGFR. Although Tyr-700, Tyr-731, and Tyr-774 residues of c-Cbl were significantly phosphorylated by TGF-α exposure in 293_pEGFR, these residues, excluding Tyr-774, were not phosphorylated in 293_p15.
Conclusion: Based on these results, we concluded that mutant EGFR with a 15-bp deletion undergoes less protein degradation than wild type EGFR due to diminished binding to c-Cbl ubiquitin ligase. There is a possibility that the mutant EGFR alters substrate specificity and the alteration of phosphorylation status in c-Cbl possibly decreases its binding to EGFR.

549 POSTER

The RANKL inhibitor osteoprotegerin (OPG) inhibits tumor growth, prevents tumor-induced osteolysis, and significantly improves survival in a mouse model of breast cancer bone metastasis

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Background: Bone metastases from breast cancer cause severe skeletal morbidity including fractures and hypercalcemia. Tumor cells in bone can induce activation of osteoclasts, which mediate bone resorption and release of growth factors from the bone matrix, resulting in a cycle of bone breakdown and tumor proliferation. RANKL acts as an essential mediator of osteoclast function and survival, which is opposed by a soluble decoy receptor, OPG. RANKL inhibition by OPG-Fc can prevent tumor-induced osteolysis and decrease skeletal tumor burden. Using bioluminescence imaging (BLI) in a mouse model, we monitored the anti-tumor efficacy of RANKL inhibition on MDA-MB-231 human breast cancer cells longitudinally and tested the role of RANKL inhibition in overall survival.
Methods: After injection of luciferase-labeled MDA-231 cells into the left cardiac ventricle, tumor burden was tracked non-invasively by BLI. BLI measurements of the hind limbs were correlated to histological analysis of tumor growth, and osteolysis was analyzed by x-ray histomorphometry. Osteoclasts were measured by TRAP staining in sections of femurs and tibias. OPG-Fc (0.3 and 3.0 mg/kg, 2×/week) was administered in both prevention (begin day 0) and therapeutic (begin day 7) settings. In the therapeutic setting, mice with established bone metastases continued treatment until they became moribund and/or experienced hind limb paralysis, at which point they were euthanized (blinded analysis). Based on these criteria, a Kaplan Meier analysis of survival was performed.
Results: In the prevention setting, OPG-Fc (3.0 mg/kg) resulted in a 78% decrease ($p = 0.0002$) in BLI tumor burden vs. vehicle at day 25. Tumor burden as measured by histology also decreased by 78%

($p < 0.05$). When given therapeutically, both doses of OPG-Fc caused ~80% reduction of tumor burden by day 25 ($p < 0.03$). OPG-Fc dose-dependently reduced osteolysis in both settings, with the 3.0 mg/kg groups showing a complete absence of tumor-induced lesions. TRAP staining confirmed the reduction (with 0.3 mg/kg OPG-Fc) or absence (with 3.0 mg/kg OPG-Fc) of osteoclasts. Finally, therapeutic treatment of OPG-Fc (3.0 mg/kg) significantly ($p = 0.004$) increased the median survival time by 17% vs. vehicle.
Conclusions: RANKL inhibition reduces MDA-231 breast cancer-induced bone lesions and skeletal tumor burden. These data for the first time show that RANKL blockade in a bone metastasis model leads to an overall improvement in survival.

550 POSTER

Dual inhibition of the MAPK pathway by combination targeted therapy: a phase I trial of sorafenib (SOR) and erlotinib (ERL) in advanced solid tumors

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Background: SOR and ERL are potent, orally available receptor tyrosine kinase (RTK) inhibitors; SOR targets multiple RTKs (VEGFR-2 and PDGFR-β) and serine-threonine kinase including Raf isoforms, while ERL reversibly blocks EGFR. Dual targeting of the MAPK pathway enhanced inhibition of signal transduction and downstream effector processes in preclinical models (Huang, et al. Cancer Res, 2004; Matar et al. Clin Cancer Res, 2004). Given their inhibitory targets profile and efficacy as single agents, the combination of SOR and ERL is of considerable interest in solid malignancies. This study aimed to determine the recommended phase II dose (RPTD) of this targeted combination, their toxicity profile, pharmacokinetic interaction, pharmacodynamic and preliminary clinical activities.
Methods: SOR was administered for a one week run-in period, and then SOR and ERL were given together continuously, with every 4 weeks considered as a cycle. Three dose levels were assessed.
Results: Seventeen patients (pts) were enrolled with median age = 56 (range 30−77), M:F = 9:8 and ECOG 0:1:2 = 6:10:1. To date, 30 cycles (median = 2) have been administered to 16 pts; 1 pt was inevaluable for dose-limiting toxicity (DLT) due to removal from study for an adverse event (AE) during the run-in period. The most common AEs of all grades (as % of cycles) were: fatigue (93%), diarrhea (77%), lymphopenia (73%), hypophosphatemia (70%) and acneiform rash (60%). The most common grade 3 AEs of all causalities (as % of cycles) were: hypophosphatemia (30%), lymphopenia (17%), dyspnea (13%), GGT (13%), fatigue (10%) and hypokalemia (10%). There were no grade 4 or 5 AEs. DLTs at each dose level are listed in table below. The RPTD of this combination was SOR 400 mg bid and ERL 150 mg qd. Among 13 pts evaluable for response thus far, there were 3 PR (1 cholangiocarcinoma, 1 neuroendocrine tumor and 1 small bowel adenocarcinoma), 8 SD and 2 PD.
Conclusions: Vertical signaling inhibition by this combination of SOR and ERL is feasible at the full recommended doses of both agents with acceptable toxicity. Electrolyte abnormalities such as hypophosphatemia may occur and require replacement. Promising clinical activity was observed in several tumor types. Pharmacokinetic evaluations are ongoing and will be presented.

Dose level	SOR dose (mg bid)	ERL dose (mg qd)	Pts with DLT/ Evaluable pts at dose level (n/n)	DLT
1	200	100	0/3	–
2	200	150	1/7	Gr 3 hypophosphatemia
2	400	150	1/6	Gr 2 intolerable diarrhea and anorexia

551 POSTER

Hemangioma is induced by sustained Akt signaling and inhibited by rapamycin

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Background: Infantile hemangiomas are the most common soft-tissue tumor of infancy. However, little is known about their pathogenesis. Our goal is to understand the signaling mechanisms that regulate infantile hemangioma development. We hypothesize that sustained Akt activation in endothelial cells is necessary for the development of hemangiomas, and inhibition of mammalian target of rapamycin (mTOR) activity, which is a major downstream effector of Akt, blocks the growth of hemangiomas.
Material and Methods: We generated double transgenic mice with tetracycline-inducible and endothelial cell-specific expression of constitutively active myristylated Akt (myrAkt) and grafted the skin from these mice onto immunocompromised nu/nu mice. Nu/nu mice were then taken off tetracycline to induce myrAkt expression. Some were treated +/− rapamycin (4 mg/kg/day) for 4 weeks. To set up explant cultures, infantile hemangioma tissue was cut into 2 mm^3 pieces, then placed between two layers of fibrin matrix, covered with media, and incubated for 7–10 days.
Results: There was increased Akt and mTOR activation in infantile hemangioma tissue and purified endothelial cells. Induction of endothelial myrAkt expression led to the development of hemangiomas in the skin grafts in nu/nu mice, whereas repression of myrAkt expression resulted in gradual regression/involution of these tumors. Treatment of skin graft recipients with rapamycin, an inhibitor of mTOR, resulted in a significant reduction in hemangioma growth. Furthermore, rapamycin also inhibited the outgrowth of cells in explant cultures of infantile hemangiomas. Investigation into the mechanism of rapamycin action in hemangiomas revealed that in addition to inhibiting S6 kinase, rapamycin also blocked Akt phosphorylation in both cultured human and mouse hemangioma endothelial cells, suggesting that rapamycin inhibition of Akt may in part account for its anti-angiogenic properties.
Conclusions: These findings indicate that Akt is necessary for hemangioma formation. Furthermore, they show the possible clinical utility of rapamycin as an angiogenesis inhibitor in the treatment of hemangiomas and other vascular tumors with hyperactivated Akt/mTOR, and support a novel pathway for rapamycin action via Akt inhibition.

552 POSTER

Integrated population pharmacokinetic analysis of temsirolimus in cancer patients following weekly IV treatments

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Background: Temsirolimus (TEMSR), a novel anticancer agent, blocks activity of mammalian target of rapamycin (mTOR), a key mediator of cell signaling in the PI3K pathway. This inhibition blocks G1 to S phase transition of the cell cycle. Since pharmacokinetic (PK) variability may influence target signaling and patient (pt) response to treatment, an integrated population pharmacokinetic (PPK) analysis was performed to characterize the variability and to assess covariate effects of weekly IV TEMSR treatment for pts with advanced renal cell carcinoma (RCC).
Materials and Methods: PPK models for TEMSR and its major active metabolite sirolimus (SIR) were individually developed using NONMEM. Mechanistic description for TEMSR in blood and plasma used a 4-compartment model with saturable distribution to blood cells and peripheral tissue. For SIR, a separate, linear 2-compartment model with first-order input was used with factors for TEMSR dose (in mcg) based on structural parameters. PK data in healthy volunteers (following IV TEMSR 1–25 mg/wk), in pts with RCC receiving TEMSR alone or with interferon-alfa, and in pts with breast cancer (BrCA) (IV $\leqslant$ 250 mg/wk) yielded final data for TEMSR of 1153 observations from 90 subjects and for SIR of 1312 from 211 SIR subjects. Covariate factors included age, race, sex, weight, hematocrit, albumin, AST, ALT, bilirubin, creatinine clearance, concomitant interferon-alfa, and study protocol. Typical pt was a 49-year-old white man weighing 81.1 kg.
Results: TEMSR typical value expressions were TVCL (L/hr) = $116 \cdot (1 - 0.377\text{RAFL}) \cdot (1 - 0.619\text{BrCA})$ and volume of distribution plasma TVPL (L) = $9.92 \cdot (1 - 0.377\text{RAFL}) \cdot (1 - 0.619\text{BrCA})$ in which RAFL = 1 for nonwhites, 0 for whites; BrCA = 1 for BrCA and 0 for other studies. SIR apparent TVCL = $6.23 \cdot (\text{dose}/25000)^{0.527}(1 + 0.248\text{RCC})$ and TVV2(L) = $228 \cdot (\text{dose}/25000)^{-0.0265}(1 + 0.191\text{BrCA})$ in which RCC = 1 for RCC pts receiving TEMSR alone, 0 for other. Monte Carlo simulation was used