response, six (4%) had minor response and 75 (55%) had stable disease (SD) as best response. Median overall survival (OS) was 9.5 months and median TTP was 5.6 months. Progression-free survival (PFS) was 37% and 24% at 6 and 12 months, respectively. Median OS was higher in patients with SD (10.4 months) than progressive disease (PD; 4.6 months), and was 11 months in CP A pts. Of 103 (75%) pts with elevated AFP baseline values, 25 (25%) had >50% reductions in AFP levels. Multivariate analysis showed three of 14 disease characteristics tested had significant prognostic value for PFS in response to treatment with BAY 43–9006: AFP (>400 ng/mL), sodium (>140 mmol/L) and CP status. The most common grade 3/4 drug-related toxicities were fatigue (9%), diarrhea (8%), hand–foot skin reaction (5%) and abnormal AST (5%). No significant difference in safety was observed in pts with CP A vs. B. None of the 28 deaths (20 due to PD or liver failure and eight due to other AEs) during treatment or within 30 days of last administration were considered drug-related. PK data do not suggest an association between drug exposure and toxicity. Preliminary data suggest a correlation between pre-treatment tumor phospho-ERK levels, Affymetrix gene expression profiling and patient response.
Conclusions: These data indicate that BAY 43–9006 has modest activity in HCC and a favorable toxicity profile that was predictable and manageable. The data available warrant further evaluation of BAY 43–9006 in combination with other active agents in HCC.

43 POSTER

Proteasome inhibition activates a p38 MAPK-dependent anti-apoptotic program involving MKP-1 and Akt

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The proteasome, a multi-catalytic proteinase complex which is part of the ubiquitin-proteasome pathway, is involved in regulated intracellular protein degradation in eukaryotes. Proteasome inhibitors have anti-tumor efficacy in part through activation of programmed cell death, but less is known about the induction of possible anti-apoptotic pathways. Since inhibition of p38 mitogen activated protein kinase (MAPK) had been reported to enhance apoptosis due to proteasome inhibitors, we pursued studies to determine the mechanism of this effect. Exposure of A1N4-*myc* human mammary epithelial cells overexpressing c-Myc, and BT-474 breast carcinoma cells to the proteasome inhibitor PS-341 (bortezomib) in conjunction with the p38 inhibitor SB 203580 resulted in enhanced apoptosis compared with controls. Overexpression of dominant negative p38 isoforms confirmed that p38 inhibition alone was sufficient to enhance apoptosis, and that the beta isoform was important in this process. Inhibition of p38 resulted in enhanced levels of the activated, phosphorylated forms of c-Jun-N-terminal kinase (JNK), which plays a role in proteasome inhibitor-mediated apoptosis. Studies of the upstream JNK kinase MKK4 did not reveal consistent elevations of the activated form, however, suggesting the involvement of a JNK-interacting phosphatase. Since MAPK phosphatase (MKP)-1 has this ability, and can be induced in a p38-dependent fashion, we evaluated the possibility that MKP-1 induction is anti-apoptotic. Consistent with this hypothesis, inhibition of p38 with SB 203580 down-regulated both MKP-1 promoter activity and MKP-1 protein expression. Moreover, infection of cells treated with the PS-341/SB 203580 combination with Adenovirus (Ad) inducing MKP-1 and green fluorescent protein (GFP) suppressed apoptosis and phospho-JNK levels compared with the Ad-GFP controls. Treatment of MKP-1 knockout cells with PS-341/SB 203580 still resulted in enhanced apoptosis, however, suggesting a contribution from other pathways. Further downstream targets of p38 MAPK were therefore studied, and PS-341 was noted to activate phosphorylation of both heat shock protein (HSP)-27 and the AKT8 virus oncogene cellular homolog (Akt). Inhibition of p38 MAPK with SB 203580 resulted in decreased phospho-HSP-27 and phospho-Akt levels, while down-regulation of HSP-27 with a small interfering RNA enhanced apoptosis and decreased phosphorylation of Akt. Finally, inhibition of Akt with the phosphatidylinositol 3 kinase inhibitor LY294002 down-regulated Akt phosphorylation and increased apoptosis. These studies support the possibility that proteasome inhibitors activate an anti-apoptotic survival program through p38 MAPK that involves MKP-1 and Akt. Further, they suggest that strategies targeting MKP-1 and Akt could enhance the *in vitro* and *in vivo* anti-tumor efficacy of proteasome inhibitors.

44 POSTER

Selective small molecule inhibitors of ADAM metalloproteases as a novel approach for modulating ErbB pathways in cancer

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The ErbB family of receptor tyrosine kinases and the ligands that bind to them are important regulators of cell proliferation, differentiation and survival. Dysregulation of this pathway through overexpression and/or genetic alterations results in strongly enhanced signal transduction and has been observed in numerous cancers, including breast, lung, colon and prostate. As such, the ErbB pathways represent targets for therapeutic intervention and have resulted in the development of a number of agents that are currently used in the clinic. These include antibodies directed against ErbB1/EGFR (Erbitux®) and ErbB-2/Her-2 (Herceptin®), as well as small molecule inhibitors of the ErbB1 tyrosine kinase (Iressa®, Tarceva®). An alternative approach to reduce the mitogenic and survival signals from the ErbB pathways is to identify inhibitors of the proteases responsible for the cleavage and activation of the ligands that bind to and activate the ErbB receptors. This proteolytic processing, termed ectodomain shedding, has emerged as a critical step for the functional activation of EGFR ligands and is mediated by members of the ADAM family of zinc-dependent metalloproteases. To this end, we have identified selective, orally bioavailable small molecule inhibitors of ADAM proteases that block shedding of a number of EGFR ligands (e.g. TGF α, HB-EGF, amphiregulin), thereby blocking the activation of multiple ErbB receptors. The selective ADAM inhibitor, INCB3619, blocks EGFR ligand shedding with potencies in the low nanomolar range in vitro and significantly inhibits tumor growth in vivo, equivalent to that achieved with the EGFR kinase inhibitor, gefitinib (Iressa®). Additionally, tumor specimens from compound treated animals had reduced Ki67 staining, a marker of cell proliferation, and decreased AKT activity, similar to what was observed following treatment with agents that directly target growth factor receptors. Importantly, the compounds show no toxicities in a two-week rodent safety study and show no evidence of fibroplasia or tendonitis, the dose-limiting toxicities associated with matrix metalloprotease inhibitors. These results demonstrate that inhibitors of proteases responsible for activating ErbB pathways, through ligand cleavage, may offer a potentially novel therapeutic for the treatment of human cancers.

45 POSTER

Eukaryotic translation initiation factor eIF-4E is consistently upregulated with human prostate cancer progression: inhibition by siRNA or ASO therapy suppresses CaP xenograft growth

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Eukaryotic translation initiation factor 4E, eIF-4E, binds the 5′ cap structure of cellular mRNAs and recruits these mRNAs to the eIF-4F translation initiation complex. The eIF-4F complex then scans 5′–3′ through the 5′ untranslated region (5′ UTR) unwinding secondary structure to reveal the translation initiation codon and to enable ribosome loading. Messenger RNAs with short unstructured 5′ UTRs are more easily translated than mRNAs harboring lengthy, highly structured 5′ UTRs, as the latter prohibit efficient scanning and start codon recognition. As such, the translation of these mRNAs, which typically encode proteins involved in angiogenesis (e.g. VEGF), tumor growth (cyclin D1) and survival (Bcl-2), is suppressed except when eIF-4E is engaged with the eIF-4F complex – ; a common event in many human and experimental cancers resulting from overexpression of eIF-4E and/ or enhanced signaling through the AKT/ mTOR pathway. We now show data implicating enhanced eIF-4E function as a common event in prostate cancer (CaP) progression in human prostate cancer tissues (n=138), the TRAMP transgenic mouse CaP model and two congenic androgen-dependent/ independent human CaP cell lines. Compared to normal mouse prostate, TRAMP tumors show marked upregulation of eIF-4E expression in concert with increased cyclin D1 protein expression. Similarly, in human prostate tissues, eIF-4E expression is significantly upregulated with advancing disease (Trend analysis, $p<0.001$). Phosphorylation of the inhibitory eIF-4E binding protein 4E-BP1 is also significantly upregulated in prostate cancers relative to normal human prostate tissue. In androgen-independent derivatives of the androgen-sensitive/dependent LNCaP cells, the activity of the AKT/ mTOR pathway is enhanced, which leads to liberation of eIF-4E from the inhibitory binding protein 4EBP1. In the PTEN+ CWR-22/22R CaP model, expression of eIF-4E is directly upregulated more than 3 fold with androgen-independent progression, in concert with increased protein expression of

both c-myc and cyclin D1. Together, these data show that prostate cancers consistently have elevated free eIF-4E – a consequence of elevated eIF-4E expression and/ or reduced 4EBP1 expression/ function. As such, eIF-4E represents a novel target for prostate cancer therapy. Indeed, treatment of prostate cancer cells with siRNAs or ASOs specific for eIF-4E dramatically reduces eIF-4E expression and suppresses proliferation in concert with markedly diminished expression of cyclin D1 protein. Moreover, systemic treatment of CaP xenografts with eIF4E-specific ASOs or siRNAs suppresses xenograft tumor growth. These data firmly implicate eIF-4E in the development and progression of both human and experimental prostatic adenocarcinomas and indicate the therapeutic utility of targeting eIF4E.

46 POSTER

Biological testing of the tyrphostin tyrosine kinase inhibitor Adaphostin (NSC 680410) as a vascular endothelial growth factor secretion inhibitor in human and murine tumor cells and tumor-activated bone marrow stromal cells

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Background: Vascular endothelial growth factor (VEGF) is a proinflammatory and proangiogenic factor whose production by host and tumor cells contributes to cancer progression and metastasis. Drugs decreasing endogenous VEGF secretion may contribute to cancer prevention and inhibition. However, production of VEGF by host and tumor cells has redundant and heterogeneous mechanisms that hamper discovery of the most efficient inhibitors and their targeted mechanisms. Preliminary studies on the tyrphostin tyrosine kinase inhibitor adaphostin (NSC 680410) suggest its potential ability to inhibit VEGF secretion in human T-lymphoblastic leukemia cell lines. Herein, the inhibitory activity of Adaphostin on VEGF secretion was tested in different solid tumor cell lines that secrete VEGF under basal and cytokine-induced conditions and in bone marrow stromal cells (BMSCs) activated by tumor-derived soluble factors.
Materials and Methods: Human (MDA-MB-435 breast carcinoma, PC3 prostate carcinoma, HT-29 colon carcinoma, SK-HEP-1 hepatoma) and a murine tumor cell line (B16 melanoma) characterized by the ability to secrete VEGF were used. Primary cultured BMSCs were obtained from human and murine organ samples. Adaphostin was added to cultured tumor and host cells at a 0.1-to-10 μM concentration range for 24 and 48 hours. Measurement of human and murine VEGF secretion in control and Adaphostin-treated cells was performed by ELISA. Cytotoxic and proapoptotic effects of Adaphostin were assessed in cultured cells by the MTT assay and apoptotic cell markers.
Results: Following 12 hour-culture under basal conditions with 1 μM Adaphostin, the secretion of VEGF from human cancer cells decreased by 40-to-50% in the absence of any evidence of toxicity or proapoptotic signs. Not statistically significant alterations of VEGF secretion were observed in normal human BMSCs cultured under basal conditions with 1 and 5 μM adaphostin for up to 48 hours. Addition of conditioned medium (CM) from MDA-MB-435 breast carcinoma cells, PC3 prostate carcinoma cells, and HT-29 colon carcinoma cells to human BMSCs for 1 hour increased VEGF secretion by 5-folds. Addition of Adaphostin to tumor-activated BMSCs decreased tumor-induced VEGF production by 50-to-100%. Two BMSC-derived cytokines (IL-18 and TNFalpha), given as recombinant murine proteins at 10 ng/ml for 4 hours, also significantly increased (by 50%) VEGF production from cultured B16 melanoma cells. Addition of 1 μM Adaphostin to B16 melanoma cells 30 min prior to cytokine stimulation completely abrogated cytokine-dependent VEGF secretion. Moreover, the VEGF concentration was also significantly increased by 3-fold in the 6 hour-supernatant of primary cultured murine BMSCs given B16 melanoma-CM for 1 hour. Tumor-induced VEGF was abolished by Adaphostin when added to BMSCs 30 min prior to tumor-CM.
Conclusions: Current results demonstrate the ability of Adaphostin to inhibit VEGF secretion by solid tumor cells and tumor-activated BMSCs. Combination of this tyrosine kinase inhibitor with other antiangiogenic drugs are under study to test possible synergistic drug regimens. This work was supported in part by NCI Contract NO1-CO-12400.

47 POSTER

CRx-026: discovery and clinical development of a syncretic anti-mitotic agent with significant anti-cancer activity

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Background: A 'syncretic' drug comprises multiple biologically active compounds that interact synergistically to provide an optimal therapeutic effect with minimal side effects. CRx-026, is a novel syncretic anti-cancer agent, comprising chlorpromazine and pentamidine, that achieves its synergistic effects through a dual action in mitosis, selectively inhibiting hsEg5/KSP, a mitotic kinesin essential for centrosome separation, and PRL phosphatases, which play an important role in regulating mitotic progression and proper chromosome separation. By modulating the activity of these two targets in a concerted fashion, CRx-026 achieves a synergistic effect greater than either component agent achieves on its own.
Methods &Results: *In vitro*, CRx-026 exerts anti-proliferative and cytotoxic effects across a spectrum of human cancer cell lines. Additionally, we have tested CRx-026 in pair-wise combination with 48 anti-cancer agents representing all major mechanistic classes. CRx-026 synergized with several chemotherapeutic classes – especially with taxanes and vinca alkaloids. *In vivo*, CRx-026 elicits substantial anti-tumor activity in the xenograft tumors, A549, HCT116 and HT29. Results demonstrate that CRx-026, but not its components, had significant anti-tumor activity which was comparable or superior to paclitaxel or 5-FU. Moreover, CRx-026 has a more favorable side effect profile than paclitaxel and 5-FU, with improved survival and overall lower toxicity effects. Finally, we have confirmed several *in vitro* synergies in xenografts where CRx-026 was synergistic both with paclitaxel and with vinorelbine, enhancing anti-tumor activity without increasing toxicity in xenografts.
Conclusions: CRx-026 is a novel anti-cancer agent with obvious anti-cancer activity in both *in vitro* and *in vivo*. The two components of CRx-026 are now being integrated into a novel pharmaceutical composition, and we are currently advancing this new anti-cancer drug through clinical development. Phase I studies are currently underway in a standard dose escalation schema for patients with solid tumors to determine safety and pharmacokinetics. Multiple treatment regimens are currently being explored to help optimize future clinical development. Data are expected in late 2004.

48 POSTER

In vivo selection of vascular extravasating phage: novel means to isolate prostate tumor-homing peptides

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Background: New tumor targeting agents are required to advance cancer diagnosis and therapy. Bacteriophage (phage) peptide display technology is an emerging approach to identify and improve peptides as tumor-targeting agents. However, only a few of the peptides identified by *in vitro* phage selections have been clinically useful. This failure may be attributed to poor peptide solubility, affinity, specificity, and stability *in vivo*. It is our hypothesis that the *in vivo* application of phage display would have the advantage over *in vitro* selection strategies in that one can "select" in the environment of the whole animal, peptides that can extravastate tumor vasculature and selectively and stably bind to a given tumor. To this end we developed *in vivo* schemes to select for phage that target prostate tumors in mice.
Material and Methods: First, the biodistribution and pharmacokinetics of a fUSE 5 phage library (15 amino acid peptides displayed on cpIII) in scid mice was determined. Next, the library was cleared through non-tumor bearing mice in an effort to enrich for extravasating phage. The pre-cleared library was injected into human PC-3 prostate carcinoma-bearing scid mice. After one hour, tumors were removed, phage were eluted with acidic or neutral buffers (with 0.5% CHAPs detergent) and amplified. After four rounds of selection phage clones were analyzed. Peptides displayed on phage with the highest titer for the tumors or PC-3 cultured cells were chemically synthesized and their binding properties analyzed.
Results: Biodistribution studies demonstrated that fUSE5 phage half-life in the blood was ~18 min with optimal tumor/tissue localization of ~1 h. Only those phage extracted from the prostate tumors with detergent exhibited specific binding to PC-3 carcinoma cells. The phage and synthesized peptides bound PC-3 cells and PC-3 xenografted tumor tissue but did not bind appreciably to human PC-3M (metastatic form of PC-3), OVCAR3 ovarian, MDA-435 breast, or LS174T colon carcinoma cell lines. The peptides exhibited a K_d of 100pM for binding to live PC-3 cells. While the peptide target(s) remain to be identified, they may represent new classes of prostate tumor biomarkers. Future work will employ the peptides as an affinity ligand for target identification and, once radiolabeled, as potential cancer imaging or therapeutic agents.