vascular defects. To determine the role of EphB4 in tumor angiogenesis as well as the therapeutic index for inhibiting EphB4 in the most pharmaceutically relevant setting, we have developed a unique chemical genetics-based mouse model in which wild-type EphB4 is replaced by a functionally intact analog sensitive kinase allele (ASKA) of EphB4 through gene targeting. We have demonstrated that the embryonic lethal EphB4 knockout phenotype is fully rescued in EphB4 ASKA mice, and that ASKA EphB4 is potently and selectively inhibited *in vivo* by the small molecule ASKA inhibitor, 1-NaPP1. These EphB4 ASKA mice are currently being studied in a number of oncology models to determine the effect of specific inhibition of EphB4 on tumor angiogenesis. We have also initiated a drug discovery program to identify small molecule inhibitors against wild-type EphB4. Using medicinal and high-speed analog chemistry, we have created proprietary compound libraries around scaffolds predicted to have kinase inhibitory activity as well as good "drug-like" properties. Screening of these libraries and subsequent medicinal chemistry optimization has generated multiple chemical series of lead inhibitors that demonstrate potent activity in both biochemical and cell-based assays on EphB4. While the compounds show a very favorable selectivity profile (minimal activity on a 25 kinase cross-screen panel), further characterization has demonstrated that they also potently inhibit VEGFR2 and Tie2, two EC RTKs critically involved in tumor angiogenesis. Preliminary *in vivo* testing indicates that these lead compounds have good PK/tox profiles and the ability to inhibit tumor growth. Since tumor angiogenesis is a multi-pathway process, strategies targeting multiple angiogenic kinases are likely to produce maximum clinical efficacy for treating cancer. Multiplex inhibitors of EphB4, VEGFR2, and Tie2, therefore, represent potentially improved new therapies for the treatment of many types of human cancer.

40 POSTER

RAD001 sensitizes tumor cells to cisplatin-induced apoptosis in an mTOR dependent manner by inhibition of p53-induced p21 protein expression

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The use of DNA damaging agents, such as cisplatin, as antitumor agents has revolutionized chemotherapy against a wide variety of solid tumors. However, a narrow therapeutic window combined with the potential for severe side effects has greatly limited their broader application. This has lead to the search for drugs, which sensitize tumors to lower doses of DNA damaging agents, potentially increasing their clinical efficacy. Here we show that RAD001 (everolimus), an orally bio-available derivative of rapamycin currently in phase **II** clinical trials, dramatically enhances cell death when A549 – (lung carcinoma) or MCF7 (breast carcinoma) cells are treated with sub-optimal concentrations of DNA-damaging agents such as cisplatin or gemcitabine. The enhanced loss of cell viability was defined as apoptosis as judged by poly (ADP-ribose) polymerase (PARP) cleavage, a direct measure of caspase 3 activation. Interestingly, wild type status of the tumor suppressor protein p53 (A549/MCF7) correlated with the enhancement of apoptosis since RAD001 was unable to significantly enhance cisplatin-induced cell death in cells lacking (PC3M) or expressing mutant forms of p53 (DU145/HCT15). Through the use of isogenic tumor cell lines generated to stably express either a wild type allele of mTOR or an allele of mTOR that does not bind RAD001, we demonstrate that the effects of RAD001 on both proliferation and the enhancement of apoptosis are directly through the inhibition of mTOR function. Extensive biochemical analysis revealed that RAD001 impeded the induction of the cell cycle regulator p21, a target gene transactivated by p53 as a response to DNA-damage provoked by cisplatin. With the matched tumor cell lines and the use of RNA interference, we further show that the reduced expression of p53-induced p21 is directly responsible for the enhanced sensitivity of the cells to the RAD001/cisplatin combination. Unexpectedly, the effects of RAD001 are not through inhibition of transcription or translation of p21 mRNA, nor through decreased p21 half-life, but instead through inhibition of global translation combined with the high turnover rate of cellular p21 protein. These findings provide the molecular rationale for combining DNA damaging agents with a sensitizing agent such as RAD001, and suggest that such combination strategies will enhance the efficacy of DNA damaging agents in the treatment of cancer patients with solid tumors.

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A potential for combining the rapamycin derivative RAD001 (everolimus) with the EGF/ErbB2/VEGF receptor tyrosine kinase inhibitor AEE788 in human cancer

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RAD001 (everolimus) is an mTOR pathway inhibitor exhibiting potent antiproliferative/antitumor activity, which is currently in phase **II** clinical trials in oncology. AEE788 is a small molecule dual family inhibitor of EGF/ErbB2 and VEGF receptor tyrosine kinases (RTKs) in Phase **I** clinical studies. We addressed the role of the mTOR pathway *in vitro* as a function of ErbB receptor overexpression. The effect of mTOR inhibition on cell cycle progression (characterized by G1 accumulation) was dominant in tumor lines exhibiting low ErbB receptor expression (A549 lung, MCF7 breast). Specifically, exogenous ErbB ligands were unable to bypass the effects of mTOR pathway inhibition despite RTK activation. Strikingly, although two ErbB2-overexpressing lines exhibited a similar phenomenon (BT474 and MDA-MB-453 breast), bypass of the antiproliferative effects of RAD001 was observed in the EGFR/ErbB2- and ErbB2-overexpressing lines MKN7 gastric and SKBR3 breast, respectively. As autocrine receptor activation plays a major role in tumor cell proliferation, these data suggest that the antitumor efficacy of RAD001 could be compromised by the presence of ErbB ligands; arguing for the use of logical drug combination strategies in the context of EGFR/ErbB2-overexpressing tumors. To investigate the potential for RAD001/AEE788 combinations, ErbB2-overexpressing cells (BT474, SKBR3) were incubated with increasing concentrations of AEE788 in the presence of an optimal RAD001 concentration of 2 nM. In both lines, increased antiproliferative effects were observed with the combination as compared to the single agents; with dramatically increased cell death at optimal AEE788/RAD001 concentrations. For example, as assessed by YOPRO analysis following 72 hrs incubation, treatment of SKBR3 cells with 0.8 μM AEE788 (which caused almost total ErbB receptor inhibition) in combination with 2 nM RAD001 resulted in a 29% loss of cell viability (as compared to 0.5%, 4.5% and 0.8% with vehicle-, AEE788- or RAD001-treated cells, respectively). This increased cell death was defined as apoptosis by PARP/Lamin A cleavage analysis, and strongly suggests that RAD001 and AEE788 may elicit more potent antitumor effects in ErbB2-overexpressing tumors when used in combination. Furthermore, a more detailed analysis demonstrated that suboptimal AEE788 concentrations (0.2 μM: which did not totally inhibit ErbB receptor phosphorylation) in combination with 2 nM RAD001, although potentiating G1 accumulation (e.g. G1 population after 24 hrs: 92% combination; 78% RAD001; 66% AEE788; 63% vehicle), had little effect on cell viability as compared to the single agents. Taken together these data indicate that, in order to fully realize the potential of RAD001/AEE788 combinations in cancer patients, it may be necessary to totally inhibit ErbB RTK activity.

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Phase II study of BAY 43-9006 in patients with advanced hepatocellular carcinoma (HCC)

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Background: This multi-centre phase **II** study of BAY 43-9006, a novel Raf kinase and VEGFR inhibitor, was conducted to assess response rate, time to progression (TTP), toxicity, overall survival, pharmacokinetics (PK) and biomarker assessment in patients (pts) with advanced HCC.
Materials and Methods: Pts with inoperable HCC, no prior systemic treatment, Child-Pugh (CP) score A or B, and ECOG performance status =1, received oral BAY 43-9006 at 400 mg bid continuously in 4-week cycles. Tumor response was assessed every two cycles using revised WHO criteria. Biomarker assays (phospho-ERK levels via immunohistochemistry in pretreatment biopsies and Affymetrix gene expression profiling of blood cells from pretreatment draws) were each performed in approximately 25 pts.
Results: Of 137 pts enrolled (M: F=97:40; median age 69 years [range 28–86]), 98 (72%) had CP A and 39 (28%) CP B. Seven (5%) pts had partial

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