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.

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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