toxicities. We report the results of one of these studies with analysis EGFR mutation status of all responders.
Methods: Patients (pts) with advanced solid malignancies were enrolled. BIBW 2992 was given orally as a continuous once daily dose from 10 mg, doubled in successive cohorts until drug-related toxicity > grade 2, when escalation of no more than 50 % was allowed. All pts had pharmacokinetic sampling. DNA sequencing of tumour cell EGFR and HER2 was performed on patients achieving objective response.
Results: Thirty-three pts have been treated (15 M/18 F). Median age: 53 (range: 30−68). ECOG PS 0/1: 9/23. Nineteen pts continued beyond cycle 1. Three dose-limiting toxicities (DLT) were seen in cycle 1. One pt with HER2+ breast cancer treated previously with trastuzumab and lapatinib, developed dyspnoea with radiological interstitial changes at 30 mg of BIBW 2992 and fully recovered on drug discontinuation. The 2 other DLTs were CTC grade 3 acneiform skin rash, at doses of 40 mg and 50 mg daily. Adverse events (AE) resolved on drug discontinuation and pts were dose reduced to 30 mg and 40 mg, respectively. One patient treated at 50 mg developed grade 3 diarrhoea in cycle 2 and was dose reduced to 40 mg with resolution of the AE. Other AEs were mild (grade 1 or 2); nausea, diarrhoea, mucositis and fatigue. Further dose escalation beyond 50 mg daily was not pursued.
Two female patients with lung adenocarcinoma treated with 10 mg and 40 mg daily had confirmed partial responses (PR) and remain on treatment beyond 20 and 10 months, respectively. Both patients have similar EGFR mutations. The patient on 10 mg has deletion and missense mutation of 4 amino acids in the kinase domain (WT: KELREATSPKANKEILD; Patient: KEP - - - - SPRANKEILD).
The patient on 40 mg has an in-frame deletion of 5 amino acids in the same region of the kinase domain (WT: KELREATSPKANKEILD; Patient: K - - - - - TSPRANKEILD).
Conclusion: BIBW2992 is well tolerated and can be dosed continuously at 50 mg/day. Durable partial responses have been seen in patients with mutated EGFR.

574 POSTER

Wnt1 transactivates EGFR in human breast cancer cells

T. Schlange, Y. Matsuda, N. Hynes. *Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland*

Background: Wnt growth factors induce a number of signaling pathways that play context dependent roles in the development of several types of cancer. While mutations in the "destruction" complex for beta-catenin and in the N-terminus of beta-catenin itself are causative for the majority of colorectal cancers, de-regulation of the Wnt pathway seems to occur in an autocrine fashion in breast cancer. Previous work from our lab linked Wnt signaling to the activation of EGFR receptor tyrosine kinase in non-transformed mouse mammary epithelial cells. Activation of EGFR in human breast cancers is discussed as a possible mechanism for development of resistance to targeted treatment against estrogen receptor (ER)-postive and ErbB2 over-expressing cancer cells in the clinic.
Materials and Methods: Therefore, we analyzed the effect of Wnt mediated EGFR transactivation in human breast cancer cell lines treated with 4-hydroxytamoxifen (4-HT) or trastuzumab/4D5 in vitro. sFRP1 was used as a naturally occurring inhibitor of Wnt signaling in conditioned medium and stably transfected breast cancer cells to analyze the effect of inhibition of Wnt signaling in vitro and in vivo (tumor xenografts and tail vein injection).
Results: We provide evidence for a cross-talk of a non-canonical Wnt signal with EGFR that is mediated in a similar fashion as transactivation of this receptor tyrosine kinase (RTK) by different GPCR ligands. The mechanism depends on the activity of heterotrimeric G proteins, PLC, Src kinase and metalloprotease activity, but is apparently independent of beta-catenin. Expression data reveal that human breast cancer cell lines express several Wnt ligands, which may account for the autocrine activation of different signaling pathways downstream of the Wnt ligands. Furthermore, we show that inhibition of autocrine Wnt signaling by the secreted inhibitor sFRP1 reduces the growth rate of human breast cancer cells in vitro and in vivo.
Conclusions: Data presented imply that targeting the Wnt pathway in breast cancer may slow the growth of tumor cells and may provide a new therapeutic tool to prevent development of resistance against established treatments.
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575 POSTER

Molecular pathways regulating AZD0530 reduction of human colon tumor metastasis

R. Irby, C. Kline. *Penn State Cancer Institute, Penn State College of Medicine, Hershey, PA, USA*

Colorectal cancer kills more than 65,000 people in the US each year, usually as a result of metastasis. Reducing the metastatic potential of colon cancer cells would be a key step in reducing the incidence of death from metastatic colorectal cancer. Src kinase has long been associated with the progression and metastasis of colorectal cancer and provides an attractive target for chemotherapeutic intervention. AZD0530 is a novel, orally potent, once-daily, highly selective and dual-specific Src/Abl kinase inhibitor with potential for activity in a wide range of tumors. We conducted *in vitro* and *in vivo* experiments using AZD0530 with two human colon cancer cell lines: SW480, both wild type and c-Src transfected, and HT29, which has a high native level of Src expression. We found that *in vitro*, AZD0530 reduces migration and invasion of each cell line tested by 90%. *In vivo*, we used HT29 cells for intrasplenic injection in nude mice. Mice were treated orally with AZD0530 daily from the day prior to injection (Day −1) or from 7 days post injection (Day 7). Four weeks post-injection, mice treated with AZD0530 had developed fewer metastatic lesions than mice treated with vehicle; 32% and 42% of mice treated with AZD0530 from Day −1 and Day 7, respectively, developed tumors compared with 60% of mice treated with vehicle. We performed proteomic and genomic analyses (using Ciphergen SELDI-TOF and Affymetrix U133 2.0 Plus gene chips, respectively) of tumors isolated from all mice to determine the pattern of Src-regulated gene expression in the metastatic tumors. Analysis of microarray data showed 40 genes that were upregulated and 49 that were downregulated in tumors from mice treated with AZD0530. Gene Ontology significant genes include members of surface signaling pathways; specifically, 5 of 53 genes fall into the integrin/adhesion group. Previous studies using a tiered microarray technique to investigate Src regulated invasion identified upregulated genes that enhanced invasion. In the tumors isolated from mice, several of the same genes were downregulated by AZD0530, including ADAM21 (disintegrin and metalloproteinase protein 21) and DEAD/H transcription factor Dp-1. Correlation between the proteomic and genomic data allows identification of biomarkers at two levels. The data is being validated with PCR arrays (SuperArray Bioscience). We conclude that inhibition of Src with AZD0530 selectively affects genes regulating the invasive phenotype of colon cancer cells.

576 POSTER

Inhibiting orphan nuclear receptor mediated transcription: implications for controlling drug metabolism

H. Li, H. Wang, G. Kalpana, S. Mani. *Albert Einstein College of Medicine, Cancer Center, Bronx, USA*

Background: We and others have shown that variations in biotransformation and elimination of microtubule binding drugs is a major cause of unpredictable side-effects during cancer therapy. Since microtubule binding drugs activate SXR (steroid and xenobiotic receptor, an orphan receptor that coordinately regulates the expression of drug metabolizing and transport enzymes), inhibiting this process could improve therapeutic outcome.
Materials and Methods: Multiple primary and neoplastic liver and intestinal cells lines were used. Real-time RT-PCR, northern and immunoblots of SXR target genes were performed in the presence or absence of SXR ligands with or without ketoconazole. Mechanism of action of ketoconazole was assessed using scintillation proximity assays for SXR ligand binding, in vivo nuclear receptor transcription assays, yeast and mammalian two-hybrid studies, electromobility shift assays (EMSA), and CoActivator Receptor Ligand binding Assays (CARLA). Loss of righting reflex (LORR) assays as well as paclitaxel pharmacology was assessed in *PXR* wild-type, *PXR* null and *humanized PXR* mice to determine the nature of interaction of ketoconazole with orphan nuclear receptors in vivo.
Results: Using in vitro RNA and transcription based assays in multiple cell culture models, we show that that transcriptional activation of genes regulating biotransformation and transport by the *liganded* orphan nuclear receptors, PXR, CAR, FXR and LXR was inhibited by a commonly used antifungal, ketoconazole and its selected analogs, enilconazole and fluconazole. Ketoconazole had no consistent effect on unliganded receptors or on ligand mediated activation of ERα or PPARγ. Using yeast and mammalian two-hybrid studies, EMSA, as well as in vitro ligand binding and protein interaction studies (CARLA), we show direct evidence that ketoconazole binds to a distinct site on PXR that is independent of the ligand binding pocket but overlaps with coregulator binding. In mice, high dose ketoconazole inhibited PXR mediated loss of righting reflex to tribromoethanol anesthesia and paclitaxel metabolism. High

dose ketoconazole normalized PCN (mPXR ligand) induced changes in paclitaxel metabolism.
Conclusions: These studies demonstrate that ketoconazole and commonly used anti-fungal analogs repress the co-ordinated activation of several genes involved in drug metabolism by blocking PXR activation. We show a completely novel mechanism of inhibitor action on PXR by ketoconazole. Our observation may lead to the development of new strategies to improve the clinical efficacy of drugs, reduce therapeutic side effects and prevent unwanted drug-drug interactions during cancer treatment by specifically targeting liganded orphan nuclear receptors.

577 POSTER

TKI258 is an effective multitargeted receptor tyrosine kinase (RTK) inhibitor against prostate cancer models via potent inhibition of FGFR kinase

D. Lopes de Menezes, P. Hollenbach, Y. Tang, H. Ye, N. Aziz, M. Faure, H. Niu, P. Garcia, E. Garrett, C. Heise. *Novartis, Emeryville, USA*

Background: TKI258 (formerly CHIR-258) is an oral, multitargeted RTK inhibitor of FGFR, VEGFR, PDGFRβ, FLT3 and cKIT currently in clinical trials. FGFs and their cognate receptors FGFR1–4 are dysregulated in multiple cancer types and have been implicated in tumorigenesis and angiogenesis. Previous studies have confirmed the antiangiogenic activity and inhibition of other target RTKs. Preclinical evaluation of TKI258 against FGFR was undertaken in prostate cancer models to investigate the therapeutic rationale for clinical studies in this patient population.
Materials and Methods: Prostate (PXRF 1369, DU145, PC3M, MRIH 1579, 22RV1, PNT1A) cell lines and tumor xenografts were characterized for expression levels of FGFR1–4 by Western blot analyses. Mutational analyses and FGFR4 gene polymorphism (Arg388) were evaluated by PCR sequencing. To dissect the mechanism of TKI258 against FGFR, anti-proliferative activity (using clonogenic assays) and signaling (pFRS2 and pERK) was examined. Anti-tumor activity of TKI258, Taxotere and the combination was evaluated in DU145 and 22RV1 xenograft models *in vivo*.
Results: Prostate cells differentially expressed FGFR1–4. All prostate cells were generally responsive to TKI258, modulated downstream FGFR signaling of pFRS2 and pERK, and sensitivity independent of expression pattern of FGFRs and/or FGFR4 (Arg388) polymorphism. The addition of TKI258 increased the sensitivity (decreased EC50) of DU145, 22RV1 and MRIH 1579 cells to Taxotere. Single agent activity (tumor inhibition and regressions) with TKI258 (10–100 mg/kg, daily) was observed in all prostate xenograft models tested (PC3, DU145 and 22RV1), including inhibition of metastatic growth of PC3M-luc cells *in vivo*. TKI258 was more potent than selective kinase inhibitors ZD1839 (EGFR inhibitor), and SU5416 (VEGFR and PDGFRβ inhibitor) in the DU145 tumor model. The addition of Taxotere (20 or 30 mg/kg once/week) to TKI258 (20 – 40 mg/kg, daily) augmented anti-tumor responses in both DU145 and 22RV1 xenograft models, resulting in additive drug effects. In the PSA-secreting 22RV1 tumor model, drug efficacy (tumor volumes) correlated with reduction of serum PSA levels.
Conclusion: The multitargeted activity of TKI258, arising from the direct inhibition of FGFR in addition to its anti-angiogenic activity, contributes significantly to its effectiveness as both a monotherapy or combined with Taxotere in $FGFR^+$ cancers.

578 POSTER

A potential for combining the mTOR inhibitor RAD001 (everolimus) with the ErbB2 receptor tyrosine kinase inhibitory antibody trastuzumab in breast cancer

H. Lane[1], C. Stephan[1], S. Zumstein-Mecker[1], A. Welsch[1], W. Tinetto[1], M. Klopfenstein[1], C. Chateny-Rivauday[1], T. O'Reilly[1], N. Hynes[2], J. Baselga[3]. *[1]Novartis Institutes for BioMedical Research, Oncology, Novartis Pharma AG, Basel, Switzerland; [2]Friedrich Mescher Instititute for BioMedical Research, Basel, Switzerland; [3]Oncology Research Laboraory, Medical Oncology Service Vall d'Hebron Univrsity Hospital, Barcelona, Spain*

RAD001 (everolimus) is an mTOR pathway inhibitor exhibiting potent, antiproliferative/antitumor activity, which is currently in phase II clinical trials in oncology. Trastuzumab, is a humanized monoclonal antibody registered for the treatment of cancer patients with ErbB2-overexpressing, metastatic breast cancer. To investigate the potential for RAD001/trastuzumab combinations, trastuzumab-sensitive ErbB2-overexpressing breast carcinoma cells (BT474, SKBR3), with known sensitivity to RAD001 (IC50 for antiproliferative activity: 0.55±0.12 and 0.74±0.34 nM, respectively), were incubated with increasing concentrations of trastuzumab in the presence of an optimal RAD001 concentration (2 nM) and effects on proliferation were analyzed. In both lines, increased antiproliferative effects were observed with the combination as compared to the single agents. For example, as assessed by direct cell counting following 6 days incubation, treatment of BT474 cells with 10 nM (optimal) trastuzumab or 2 nM RAD001 resulted in a 64 % and 73 % decrease in cell number, respectively, as compared to control cultures. Interestingly, a more striking decrease (83 %) in cell number was observed with the combination. Similar results were obtained with SKBR3 cells, with a 33 % and 61 % decrease in cell number occurring with trastuzumab or RAD001 alone, and a 71 % decrease with the combination. In both cell lines, statistical analysis indicated significant positive interactions between RAD001 and trastuzumab ($p < 0.001$ BT474; $p = 0.035$ SKBR3, two-way ANOVA). Based on this promising in vitro data, the combination was also assessed in an athymic mouse BT474 orthotopic mammary tumor model. Ten estrogen pellet-bearing animals per group were treated three times per week (MWF) with RAD001 (5 mg/kg p.o.), trastuzumab (2 mg/kg i.p.), the combination or vehicle control. Antitumor activity (expressed as % T/C: mean increase of tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100) was observed with the single agents (T/C = 40 % for RAD001, 42 % for trastuzumab) and was increased with the combination (T/C = 14 %); a result indicating positive interaction between the two agents that reached near significance ($p = 0.060$, two-way ANOVA). A second experiment also showed increased activity of the combination. Based on body weights, treatments were well tolerated. Taken together, these data suggest that combinations of RAD001 and trastuzumab may have application in the treatment of ErbB2-overexpressing breast cancer patients, although further dose/regimen optimization may be required.

579 POSTER

NVP-TAE226, an inhibitor of FAK, induces regression of solid tumor

S. Hatakeyama[1], D. Tomioka[1], O. Ohmori[2], N. Matsuura[2], K. Masuya[2], T. Miyake[2], I. Umemura[2], T. Kanazawa[2], T. Honda[1], E. Kawahara[1]. *[1]Novartis Institutes for BioMedical Research, Discovery Biology, Tsukuba, Ibaraki, Japan; [2]Novartis Institutes for BioMedical Research, Global Discovery Biology, Tsukuba, Ibaraki, Japan*

Background: Focal Adhesion Kinase (FAK) is a non-receptor cytoplasmic tyrosine kinase that regulates multiple cell functions. Activation of integrins and the growth factor receptors result in FAK autophosphorylation at Y397 and the presentation of suitable binding sites for proteins containing either SH2 or phosphotyrosine binding domains. Elevated expression levels of FAK have been detected in various tumor samples and are closely correlated with invasive potential. Recent evidences indicate that FAK plays important roles in cancer cell proliferation and survival. Therefore, an inhibitor of FAK may selectively block the growth, migration, and survival of tumor cells.
Methods: In the present study, anti-cancer activity of a potent and selective FAK inhibitor, NVP-TAE226, was evaluated in cancer cell lines panel and various tumor models. All procedures in this study were in compliance with the regulations of Animal Welfare Committee in Novartis Institutes for BioMedical Research Tsukuba.
Results: NVP-TAE226 inhibited various cancer cell lines with mean GI_{50} value of 0.76 μmole/L. Expression of P-glycoprotein did not affect inhibition of cancer cell proliferation by NVP-TAE226, suggesting that the inhibitor is not served as a substrate of P-glycoprotein. Pancreas, colon tumors and melanoma growth were inhibited by oral administration of NVP-TAE226. Tumor stasis was observed at a dose of 30 mg/kg, furthermore regression of a pancreatic tumor was observed at a higher dose. The compound was also effective in an orthotopic model. All animals tolerated NVP-TAE226 treatment up to 100 mg/kg with no body weight loss. Inhibition of downstream signaling such as phosphorylation of Akt at Serine473 was accompanied by inhibition of FAK phosphorylation in human tumor cell lines.

Group	Regimen/Route	Dose (mg/kg)	ΔT/C (%)	Regression (%)	ΔBW[a] (%)	Dead/ Total
Vehicle	qd, po, 7×/wk	–	–	–	10±0.9	0/7
TAE226	qd, po, 7×/wk	10	50**	None	9.9±0.3	0/7
		30	13**	None	6.2±0.9	0/7
	qd, po, 5×/wk	100	<0	17##	7.0±1.2	0/7

MAI PaCa-2 model, efficacy in primary tumor, n = 7 @day 14
[a]BW, body weight. *$P < 0.05$, **$P < 0.01$ vs. vehicle control (Dunnett's test); #$P < 0.05$, ##$P < 0.01$ (paired t-test.)

Conclusion: NVP-TAE226 is a novel class of selective and small molecule kinase inhibitors with a potent in vivo activity and potential therapeutic application.