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**

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**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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**References**

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**575** POSTER

**Molecular pathways regulating AZD0530 reduction of human colon tumor metastasis**

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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**

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**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