Here, we describe the antitumor activity of PLX4032, the first drug designed to specifically inhibit B-Raf V600E without affecting wild-type Raf signaling. Immunoblotting analyses showed that sub-micromolar concentrations of PLX4032 initiated a prolonged inhibition of ERK phosphorylation in V600E cell lines, but did not affect ERK activity in wild-type B-Raf lines. Results from a series of FACS experiments demonstrated that inhibition of B-Raf V600E with PLX4032 caused sustained cell cycle arrest and subsequent apoptosis in melanoma cells possessing the mutation, while leaving cells with wild-type B-Raf unperturbed. MTT analyses on a panel of B-Raf mutant and wild-type melanoma cell lines further established the specificity of the compound, as measured by survival over 72 hours. Using a collagen-based 3D spheroid approach, PLX4032 also displayed antitumor activity against V600E melanoma cell lines, while B-Raf wild-type cells remained viable. Furthermore, the pharmaceutical properties of PLX4032 were optimized to inhibit V600E-initiated tumors *in vivo* when dosed orally. The collective results from these experiments argue that targeted, efficacious antitumor therapy may be achieved in a majority of melanoma patients with moderate dosages of PLX4032.

570 POSTER

Overcoming resistance to tyrosine kinase inhibitors (TKIs) through inhibition of Heat Shock Protein 90 (Hsp90) chaperone function in patients with metastatic GIST: results of a Phase I Trial of IPI-504, a water-soluble Hsp90 inhibitor

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Introduction: Prior work from our team has demonstrated that inhibition of the Hsp90 chaperone protein results in selective destruction of the mutated KIT kinase in molecularly-characterized human GIST cell lines. This novel strategy is associated with antitumor activity in cells harboring mutations which confer resistance to small molecule TKIs. To translate this into clinical testing, we are performing a phase I trial of IPI-504, a water-soluble inhibitor of Hsp90, in patients with metastatic GIST following failure of TKI therapy.
Methods: Patients (pts) with metastatic GIST were eligible for study entry following failure of prior TKI therapy such as imatinib and sunitinib. Patients received IPI-504, infused in 250 cc of normal saline over thirty minutes IV, on days 1, 4, 8 and 11 of a 21-day cycle. Serial monitoring with 18FDG-PET imaging at baseline, day 11, and day 21, as well as PK profiling of IPI-504 and its major active metabolites 17-AAG, and 17-AG, was performed on all pts.
Results: To date in this ongoing trial, 14 GIST pts have been enrolled at 4 dose levels (90 [n = 6], 150 [3], 225 [3], 300 [2] mg/m^2 IPI-504). One pt at 90 mg/m^2 had asymptomatic grade 3 lipase elevation possibly drug related, but no other grade 3 or 4 toxicities nor DLTs have been observed. Other adverse events possibly related to IPI-504 include Grade 1–2 elevation of alkaline phosphatase, fatigue and headache. PET imaging as a biomarker demonstrated decreases in tumor FDG avidity in 1/6 pts, 1/3, 2/3, and 2/2 at the respective dose levels. Although no RECIST-defined disease responses were noted, stable disease has allowed 7 pts to continue on study treatment for 3 or more cycles.
Conclusion: Targeting Hsp90 represents a novel therapeutic strategy in GIST resistant to TKIs, and the clinical evaluation of IPI-504 is ongoing to define the tolerability, MTD and clinical and biological activity of IPI-504 in this setting. The activity of IPI-504 in decreasing FDG avidity of GIST lesions is promising. Results to date demonstrate that IPI-504 is well-tolerated at doses up to 300 mg/m^2. Further results from this ongoing trial will be available for this meeting.

571 POSTER

AZD0530, a dual-specific Src/Abl tyrosine kinase inhibitor, inhibits migration and invasion without growth inhibition in head and neck squamous cell carcinomas with a mesenchymal phenotype

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Background: Overexpression and activation of Src tyrosine kinase has been associated with disease progression in head and neck squamous cell carcinomas (HNSCC), and consequently presents a potential target for therapeutic intervention. Src regulates signals from cell surface molecules, including growth factor receptors and G-protein coupled receptors, and mediates proliferation, survival and motility. AZD0530 is a highly selective, orally available, dual-specific Src/Abl kinase inhibitor.
Methods: The effects of AZD0530 were assessed in 19 HNSCC cell lines by 5-day MTT viability assays, cell cycle progression by flow cytometry, apoptosis by FACS analysis, cell migration/invasion, and immunoblotting to monitor specific signaling molecules.
Results: Cell lines were first characterized for anti-proliferative and viability responses to gefitinib. Gene expression profiles suggested that gefitinib-sensitive lines (IC50 < 1 μM) had an epithelial phenotype typified by the expression of E-cadherin and the tight junction proteins claudins 4 and 7. Resistant lines did not express these proteins and expressed the mesenchymal protein marker vimentin. *In vitro* growth sensitivity to AZD0530 followed a similar trend. Lines with a mesenchymal phenotype had IC50s > 7 μM, while epithelial lines were sensitive to AZD0530 (IC50 < 1 μM). AZD0530 (1 μM for 24 hours) inhibited phosphorylation of Src (Tyr 416) in all cell lines tested. In cell lines with IC50s of <1 μM, EGFR (Tyr845), and p44/p42 MAPK (Thr202/Tyr204) were inhibited, and G1 cell cycle arrest was induced.
In contrast to the differential effect observed using proliferation and viability as endpoints, AZD0530 inhibited migration and invasion in both epithelial and mesenchymal cell lines treated with 1 μM AZD0530 for 24 hours. Expression of proteins associated with adhesion (E-cadherin) and tight junctions (claudins 4 and 7) increased in treated epithelial lines.
Conclusions: AZD0530 decreases HNSCC viability in cell lines with an epithelial phenotype by inhibiting Src downstream signaling and inducing cell cycle arrest. Growth of mesenchymal lines is not inhibited by AZD0530, but Src-signaling inhibition results in decreased migration and invasion. These results suggest that this inhibition may be associated with induced expression of E-cadherin and claudins 4 and 7. Studies are in progress to determine the mechanism of increased E-cadherin and tight junction protein expression in HNSCC cell lines when treated with AZD0530.

572 POSTER

Sorafenib (BAY 43–9006) inhibits imatinib-resistant mutant KIT signaling

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Activating point mutations in KIT are found in a number of neoplasias including cases of seminoma, acute myelogenous leukemia, mast cell disorders and gastrointestinal stromal tumors (GIST). Particularly high levels of KIT activation are found in GIST with over 80% harboring activating mutations in KIT, the most common occurring in the juxtamembrane region encompassing amino acid residues 550–580. Imatinib mesylate (Gleevec®) is a potent small-molecule kinase inhibitor with activity against wild-type and certain mutant forms of KIT and is currently the frontline therapy for KIT-positive unresectable or metastatic GIST. However, acquired resistance to imatinib has been observed in GIST patients and has been associated with a number of secondary missense mutations in KIT. Previously, we have reported that the multikinase inhibitor sorafenib is a potent inhibitor of KIT kinase activity. To characterize the effects of sorafenib on imatinib-resistant KIT isoforms, KIT proteins containing an activating juxtamembrane mutation or the juxtamembrane mutation in combination with a secondary mutation (V654A, T670I, D816G, N822K, or Y823D) were expressed in the IL-3-dependent pro-B cell line Ba/F3. Imatinib was found to potently inhibit the growth of cells expressing the juxtamembrane mutant KIT in a dose-dependent manner. This correlated with a dose-dependent decrease in KIT autophosphorylation. Cell lines harboring imatinib-resistant mutations were found to be approximately 10−40-fold less sensitive to imatinib in assays measuring cellular proliferation and KIT autophosphorylation. In contrast, sorafenib was found to potently inhibit the growth of both the imatinib-sensitive and -resistant cell lines in a dose-dependent manner. The effect of sorafenib on cellular proliferation correlated well with inhibition of KIT autophosphorylation. These data indicate that sorafenib can inhibit both activating KIT mutants and imatinib-resistant isoforms and may provide an additional therapeutic option for patients with imatinib-resistant or -intolerant KIT-dependent tumors.

573 POSTER

Phase I study of BIBW2992, an oral irreversible dual EGFR/HER2 inhibitor, showing activity in tumours with mutated EGFR

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Background: BIBW 2992 is a novel, potent, orally bioavailable irreversible inhibitor of EGFR and HER2 receptor tyrosine kinases with IC_{50} values of 0.5 and 14 nM, respectively. Phase I studies in the US and UK have identified daily dosing with 50 mg as the optimal schedule to be further explored in phase II trials with rash and diarrhoea as the dose limiting

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.
Funded by the Swiss Cancer League (Onco Suisse 01445−12–2003) and the Novartis Research Foundation.

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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