

of IL-8, an inflammatory chemokine that has been shown to play an important role in tumor growth and angiogenesis. Moreover, the mutant *BRAF* V600E gene was introduced into WT *BRAF* melanoma cell lines to directly determine the effects of inheritance of a mutant *BRAF* gene on sensitivity to PD0325901, VEGF/IL-8 secretion, and angiogenesis. In summary, the novel MEK inhibitor PD0325901 is endowed with potent growth-inhibitory, pro-apoptotic, and anti-angiogenic activity in preclinical models of human melanoma. Molecular mechanisms of action are currently under investigation, but preliminary results warrant further preclinical/clinical development of this compound.

566 POSTER Anti-leukemic activity of the novel MEK inhibitor PD0325901

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The Raf/MEK/ERK signaling module is frequently dysregulated in hematological malignancies. We investigated the effects of PD0325901, a novel MEK inhibitor, on phospho-protein expression, gene expression profiles, cell proliferation, and apoptosis in cell line models of AML, ALL, multiple myeloma (MM), ex vivo-cultured primary AML blasts, and oncogene-transformed hematopoietic cells. AML cell lines (OCI-AML2, OCI-AML3, HL-60) were strikingly sensitive to PD0325901 (IC₅₀: 5–19 nM), NB4 (APL) and U266 (MM) showed intermediate sensitivity (IC₅₀: 822 and 724 nM), while all the lymphoid cell lines tested and the myeloid cell lines U937 and KG1 were resistant (IC₅₀ > 1000 nM). Cell growth inhibition was due to inhibition of cell cycle progression and induction of apoptosis. A statistically significant reduction in the proportion of S-phase cells ($p=0.01$) and increase in the percentage of apoptotic cells ($p=0.019$) was also observed in 18 primary AML samples in response to 100 nM PD0325901. PD0325901 effects were also examined in a panel of IL-3-dependent murine myeloid FDC-P1 cell lines transformed to grow in response to 11 different oncogenes in the absence of IL-3. Fms-, Ras-, Raf-1-, B-Raf-, MEK1-, IGF-1R-, and STAT5a-transformed FDC-P1 cells were very sensitive to PD0325901 (IC₅₀: ~1 nM), while A-Raf-, BCR-ABL-, EGFR- or Src-transformed cells were 10 to 100 fold less sensitive (IC₅₀: 10 to 100 nM); the parental, IL-3 dependent FDC-P1 cell line had an IC₅₀ > 1000 nM. Analysis of the phosphorylation levels of 18 different target proteins after treatment with 10 nM PD0325901 showed a 5- to 8-fold reduction in ERK-1/2 and a 2-fold reduction in JNK and STAT3 phosphorylation. Conversely, increased phosphorylation in response to PD0325901 was observed for Raf-1 (2.5-fold), MEK1/2 (2.4-fold), AKT (2-fold), and p70^{S6K} (2-fold). PD0325901 (10 nM) treatment also profoundly altered the gene expression profile of the sensitive cell line OCI-AML3: 96 genes were modulated after 24 h (37 up- and 59 down-regulated), most of which involved in cell cycle regulation. Changes in cyclin D1 and D3, cyclin E, and cdc 25A were also validated at the protein level. Overall, PD0325901 shows potent growth-inhibitory and pro-apoptotic activity, indicating that MEK may be an appropriate therapeutic target in an array of different hematological malignancies. Further preclinical/clinical development of this compound is warranted, particularly in myeloid leukemias.

567 POSTER Efficacy of BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor, in combination with cytotoxic agents

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BIBW 2992 is an orally active irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor which is currently in clinical development. In preclinical models, BIBW 2992 has demonstrated single-agent activity in a variety of xenograft models of human cancer (e.g., A431 squamous cell carcinoma, MDA-MB-453 breast, NCI-N87 gastric and SKOV-3 ovarian carcinomas). Combinations of EGFR and/or HER2 targeted agents with standard chemotherapeutic agents have demonstrated efficacy in clinical trials. The present study aimed to assess the effect of BIBW 2992 in combination with established drugs *in vitro* and *in vivo*. Colony forming assays in soft agar revealed that concomitant treatment with BIBW 2992 and either docetaxel, doxorubicin, or 5-fluorouracil induces supra-additive inhibitory effects in SKOV-3 ovarian carcinoma cells. For cisplatin and

carboplatin, combination experiments with BIBW 2992 were not performed as the EC₅₀ values for the platinum-derived compounds were above 5000 nM in this assay system. Ensuing *in vivo* experiments in nude mice bearing subcutaneous SKOV-3 xenografts using docetaxel and doxorubicin in concomitant treatment combinations with daily doses of BIBW 2992 confirmed the *in vitro* observations. A refined assessment of docetaxel/BIBW 2992 combination schedules was performed using the SKOV-3 xenograft model. Pulsatile weekly treatment (qdx2) of tumor bearing mice for 5 weeks with BIBW 2992 at a dose of 35 mg/kg/d as single agent resulted in good anti-tumor activity (T/C=8 %). Weekly treatment with 10 mg/kg docetaxel also showed efficacy in this model (T/C=22%). The combination treatments irrespective of schedule resulted in better efficacy with T/C values between 1–3 %. The treatment schedule using docetaxel followed by BIBW 2992 resulted in tumor regressions (defined as V^{rel} < 50%) in all treated animals. For comparison the inverse treatment schedule resulted in regressions in 30% of the cases (p-value: 0.0072). Taken together, these observations suggest that clinical trials of BIBW 2992 in combination with established chemotherapeutic drugs are warranted.

568 POSTER In vitro and in vivo pharmacological properties of the potent phosphatidylinositol 3-kinase (PI3K) family inhibitor PI103

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PI103 (Plamed) is a potent and competitive pyridofuopyrimidine inhibitor of class I PI3K with IC₅₀ of 2–15 nM, as well as inhibiting mTOR. PI103 exhibited growth inhibitory (GI₅₀ = 0.1–1 μM) activity against a panel of human cancer cell lines, including prostate, lung, ovarian, colon and breast, that have different genetic abnormalities in the PI3K signalling pathway, eg PTEN deletion, PIK3CA mutation or over-expressed receptor tyrosine kinases. Consistent with inhibition of PI3K, treatment with 1× and 5× GI₅₀ concentrations of PI103 resulted in decreased phosphorylation of AKT on Ser473 in all cell lines. The effects of PI103 on the downstream components of the PI3 kinase pathway were rapid, with inhibition of phosphorylation of AKT on Ser473 and Thr308 and on Ser21 of GSK-3β as early as 10 min post treatment. In addition, PI103 induced redistribution of GFP-tagged FOXO4 from the cytoplasm to the nucleus (IC₅₀ = 30 nM) in U2OS cells. PI103 also inhibited the re-localisation of GFP-AKT1, 2 and 3 and GFP-PDK1 to the plasma membrane in CHO cells with IC₅₀s of 17, 13, 11 and 66 nM respectively. PI103 decreased cyclin D1 expression as early as 4h post-treatment, consistent with the G1 cell cycle arrest that was detected in all cancer cell lines tested, and this was both time- and concentration-dependent. Apoptosis was not seen, as measured by sub-G1 peak or by caspase 3/7 cleavage. PI103 inhibited the chemomigration and invasion properties *in vitro* of a variety of tumour cells (eg HCT116 colon carcinoma, MDA MB 468 breast carcinoma and PC3 prostate carcinoma (over 80% inhibition at 450 nM) and U87MG glioblastoma (44% inhibition at 50 nM). Despite extensive glucuronidation *in vitro* in microsomal incubations and *in vivo* in mice, PI103 distributed to liver, kidney, spleen and tumour (U87MG xenografts) resulting in tumour levels above GI₅₀ for 2–4h following 40 or 70 mg/kg PI103 ip. This resulted in target inhibition *in vivo* with decreased AKT phosphorylation on Ser473. Significant antitumour activity was observed in a number of human xenograft models including U87MG glioma, HCT116 colon, PC3 prostate, MDA MB-468 breast and MDA MB 435 breast cancers. In the latter case we also observed an inhibition of vascular and muscular invasion. In the late-stage orthotopic ovarian carcinoma model OVCAR-3, PI103 reduced tumour burden (T/C 60%) and tumour cells showed decreased levels of AKT phosphorylation. We also noted a substantial decrease in intraperitoneal invasion of all major sites, with complete control of liver, diaphragm, kidney and ovary invasion and lower incidences of mesenteric and lymph node spread. The results demonstrate the therapeutic potential of potent PI3 kinase inhibitors for the treatment of a range of cancers in which deregulation of the PI3 kinase pathway contributes to oncogenesis.

569 POSTER Antitumor activity of PLX4032, a novel B-Raf V600E Inhibitor

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The concept of targeted therapy in cancer treatment has become clouded with therapeutic compounds that inhibit entire pathways rather than mutated gene products exclusive to the oncogenic tumor itself. While nearly 70% of melanoma patients harbor an activating mutation in B-Raf (V600E) that renders constitutive activity to the MAPK signaling pathway, no compound to date has successfully inhibited this mutation without off-target effects.

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.

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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/m² IPI-504). One pt at 90 mg/m² 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/m². Further results from this ongoing trial will be available for this meeting.

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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.

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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 multitargeted 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.

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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 IC₅₀ 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