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.

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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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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.

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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