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Preclinical activity of AZD0530, a novel, oral, potent and selective inhibitor of the Src family kinases

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Background: Overexpression and/or hyperactivity of Src kinases, in particular c-Src kinase, have been reported in many common human epithelial tumours. In normal cells, c-Src kinase functions as an intracellular signal transduction modulator, acting as a highly regulated 'gatekeeper' for many signal transduction pathways. In tumour cells c-Src kinase activity is often substantially increased compared with cells of the corresponding normal tissue. Although the precise nature of the mechanisms driving the hyperactivation of c-Src in tumours has not been fully elucidated, post-translational modification as a result of multiple growth factor activation events may be a significant component. A number of experimental studies suggest that the predominant consequence of elevated Src kinase activity is the promotion of a tumour invasive phenotype through effects on cytoskeleton reorganisation, cell: cell and cell: matrix adhesion. We report on the preclinical activity of AZD0530, a novel, oral, potent and selective inhibitor of Src kinases.
Methods: IC_{50}s were established in isolated enzyme assays and cell proliferation, adhesion, migration, invasion and wound healing assays.
Results: In isolated enzyme assays, AZD0530 demonstrated nM IC_{50} activity vs c-Src, c-Yes and Lck, and high selectivity against a panel of Tyr and Ser/Thr protein kinases (eg IC_{50}s: Kdr: 21 μM, MAPK: 14 μM, CDK2: 10 μM, c-kit: 5 μM, PDGFRTK: 1 μM) and also potently inhibited abl kinase (IC_{50} vs v-abl: 30 nM). In cell lines, AZD0530 potently inhibited proliferation in human c-Src Y530F transfected NIH3T3 fibroblasts (IC_{50}: 80 nM). In contrast, AZD0530 exhibited low and variable antiproliferative activity against a range of human tumour cell lines expressing endogenous active c-Src kinase. However, submicromolar IC_{50} activity was observed with AZD0530 in several of these cell lines, tested in adhesion, migration, invasion and wound healing assays, supporting a mechanistic role for endogenous active c-Src kinase in the invasion phenotype. Treatment with AZD0530 *in vivo* led to a potent, dose-dependent inhibition of proliferation of subcutaneously transplanted c-Src Y530F transfected NIH 3T3 mouse embryo cells in immunocompromised mice and rats. In contrast, in human tumour xenograft models only a small, dose-independent inhibition of tumour growth was observed with AZD0530, inconsistent with a direct anti-proliferative or anti-angiogenic response. In an orthotopic model of human pancreatic cancer (BxPC3 cells), AZD0530-dosed animals demonstrated a significant increase in survival compared with control animals (38% prolonged survival; p=0.04).
Conclusions: AZD0530 is a potent, selective and orally active inhibitor of Src kinase, with potential clinical utility to inhibit the invasive phenotype associated with the hyperactivated Src kinase demonstrated in many human epithelial tumours.

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Binding of gefitinib (ZD1839) to human plasma proteins: in vitro and clinical pharmacokinetic (PK) studies

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Background: Gefitinib (ZD1839) is a small molecule inhibitor of epidermal growth factor receptor (EGFR) approved for oral administration to patients with refractory non-small cell lung cancer. It is generally believed that unbound drug concentration is more relevant to pharmacological and toxicological responses than total drug concentration. The purpose of this study was to investigate the binding of gefitinib to human plasma proteins, and to examine exposure to unbound drug in cancer patients.
Methods: Protein binding was determined using equilibrium dialysis on 96-well micro-dialysis system with 5 KDa cut-off membranes. Experiments were carried out with 200 μl of human plasma or isolated protein solutions containing a range of clinically relevant gefitinib concentrations (10–5000 ng/ml) and a tracer amount of ^{3}H-gefitinib against an equal volume of buffer (pH 7.4), equilibrated at 37ºC for 6 h. Drug concentrations in the buffer (Cb) and plasma (Cp) compartments at equilibrium were measured by liquid scintillation counting. The unbound drug fraction (fu) was calculated as: fu=Cb/Cp. For clinical PK studies, 10 cancer patients received monotherapy of gefitinib 250 mg once daily for 28 days and plasma samples were obtained on days 1 (serial sampling), 2, 3, 8, 15, 22, and 28. Unbound plasma concentration was calculated as fu * total drug concentration, determined by HPLC and tandem mass spectrometry.
Results: The present micro-equilibrium dialysis assay was reliable: within- and between-run precisions (quadruplicate on 5 days) were < 15%; Mean±SD recovery of total radioactivity was 93.6±4.2%. The binding of gifitinib to human plasma was extensive (fu, 3.4±0.2%) and independent of gefitinib concentration. Gefitinib was highly bound to human serum albumin (HSA) 40 mg/dL (90.2±7.6%) and alpha$_1$-acid glycoprotein (AAG) 1.4 mg/dL (77.8±7.0%), with binding constants of 1.66×10^4 M^{-1} and 1.03 x10^5 M^{-1}, respectively. At a gefitinib concentration of 500 ng/mL, fu decreased from 46.8±5.0% to 10.6±2.95% as AAG concentration was increased from 0.35 to 2.8 mg/mL. In 10 patients, the mean pre-treatment fu was 3.8% and varied 2-fold (range, 2.6 to 5.4%); fu did not vary (P>0.05) at each sampling time point over 28-days of treatment. Average unbound pre-treatment trough concentrations (average of days 8, 15, 22, and 28) were highly variable (6-fold) and ranged from 4–26 ng/mL (0.01–0.06 μM).
Conclusion: Gefitinib binding in human plasma is extensive (96.6%). In vitro studies indicate that varying concentrations of AAG among cancer patients would influence systemic exposure to gefitinib. The current assay will be used to determine the influence of gefitinib exposure (unbound and total) on EGFR kinetics and downstream pathway activation status, toxicity and antitumor activity.

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Targeting of PDGF receptors; combination effects in various solid tumors and identification of a PDGF dependent brain tumor subset

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PDGF receptors contribute to multiple tumor-associated processes, including stimulation of autocrine growth, recruitment of tumor stroma, and promotion of angiogenesis [1,2]. The availability of clinically useful PDGF antagonists, e.g. imatinib/STI571/Glivec and SU11248, has increased the interest in identification of PDGF dependent tumor-associated processes. We here report on novel markers for an imatinib-sensitive subset of glioblastoma-derived primary cultures and present novel findings that extend our earlier observations of a PDGF-antagonist mediated increase of tumor drug uptake. Finally, we demonstrate that PDGF-dependent tumor pericyte recruitment is associated with reduced sensitivity to VEGFR antagonists.
Primary cultures derived from 23 glioblastomas were characterized with regard to growth rate, PDGF receptor expression, gene expression profiles and imatinib-sensitivity. A strong correlation between PDGF receptor status and imatinib sensitivity was observed. Furthermore, for 10 selected primary cultures supervised analyses of gene expression profiles were used to identify expression markers predicting imatinib response. Gene lists, varying in length between 2 and 40 features, correctly classified all 10 cultures as determined with a weighted-voting algorithm and validation with a "leave-one-out" procedure.
Inhibition of PDGF receptors in tumor stroma is associated with reduced tumor interstitial fluid pressure (IFP) and concomitant increase in uptake of standard chemotherapy drugs like Taxol and 5-FU [3,4,5]. We now demonstrate that imatinib treatment also increase the uptake and improve the distribution of tumor targeting antibodies in subcutaneous experimental LS174T tumors. Also, a reduced tumor hypoxia was noted after imatinib treatment. As expected from these findings, the therapeutic effects of the radiolabelled tumor-targeting antibody B72.3 were dramatically enhanced by co-treatment with imatinib.
In experimental B16 mouse melanomas, paracrine stimulation of PDGF receptors on pericytes leads to increased pericyte coverage of tumor vessels and an associated increase in tumor growth rate[6]. We have used this model to investigate the effects of pericyte coverage on sensitivity to VEGF receptor targeting antibodies. Ongoing studies indicate that increased pericyte coverage is associated with a reduced sensitivity to therapy with DC101 antibodies. To what extent this resistance can be reverted with co-treatment with PDGF antagonists is presently investigated. In summary our studies identify a series of novel PDGF-dependent tumor associated processes that merits testing in clinical trials.
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