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The SRC-kinase inhibitor AZD0530 efficiently counteracts the transformation potential of BCR/ABL by targeting its kinase activity

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Background: More than 95% of chronic myeloid leukaemias (CMLs) as well as 20–25% of adult acute lymphoid leukaemias (ALLs) are Philadelphia chromosome-positive (Ph+), the cytogenetic correlate of the t(9;22). In BCR/ABL, the t(9;22) translocation product, the BCR sequences fused to ABL constitutively induce ABL-kinase activity that autophosphorylates BCR/ABL. Autophosphorylated BCR/ABL is able to transform cells. The abolition of ABL-kinase activity by the ABL-kinase inhibitor imatinib induces apoptosis in cells transformed by BCR/ABL. The treatment of patients suffering from Ph+ leukaemia with imatinib induces a very high rate of complete remissions. However, the onset of resistance is rapid, especially in Ph+ ALL, due to mechanisms such as mutation in the BCR/ABL fusion protein and amplification of BCR/ABL. Therefore there is an urgent need for other compounds that inhibit the transformation potential of BCR/ABL. Activation of Src-kinases is believed to be involved in the BCR/ABL-induced transformation as well as in resistance mechanisms against imatinib.
Methods: we investigated the effect of AZD0530, a potent Src-kinase inhibitor (inhibits Src-kinases at a concentration of 50 nM), on the biology of leukaemia cells with a focus on ALL cells, especially Ph+ ALL.
Results: i) AZD0530 (0.2 μM) significantly inhibited the migration of factor-dependent TF-1 cells in a three-dimensional spheroid model of stroma cells, without interfering with their proliferation or viability; ii) AZD0530 did not inhibit growth of Ph-lymphoblastic cell lines such as Nalm-6 or Ba/F3 even at a concentration of 10 μM; iii) AZD0530 induced cell growth arrest and a high apoptosis rate (30–80%) in Ph+ lymphoblasts (SupB15, TMD-5 and BV173) at 0.5 μM and higher doses in a dose-dependent manner; iv) combination with imatinib did not have an additional effect on the apoptosis rate induced by 0.5 μM of AZD0530; v) AZD0530 reduced the transformation potential of BCR/ABL as shown by the decrease of factor-independence of Ba/F3 cells retrovirally transduced with BCR/ABL; vi) AZD0530 directly targeted the kinase activity of BCR/ABL in the Ph+ ALL cell lines as well as in BCR/ABL-transduced Ba/F3 cells, inhibiting autophosphorylation to a similar extent to imatinib.
Conclusions: Taken together, these data establish AZD0530 as a very promising agent for the therapy of Ph+ leukaemia due to its capacity specifically to target the kinase activity of BCR/ABL. The inhibitory effect of AZD0530 on migration may be clinically important due to the high rate of CNS involvement in patients suffering from Ph+ ALL.

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Identification of a novel TGF-beta-induced signaling cascade involved in EMT and invasion of breast cancer cells

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Transforming growth factor-beta 1 (TGF-β1) is well documented to induce epithelial to mesenchymal transition (EMT) and promote cell invasion, both of which have been observed to play a role in breast cancer metastasis. The intracellular signaling cascades responsible for these roles of TGF-β have been partially identified in specific cell systems, and appear to involve the classic TGF-β-induced nuclear translocation of the SMAD signaling molecules. However, nothing is known regarding the connection of such signaling cascades and the actual molecular changes leading to EMT/invasion; e.g. how does TGF-β signaling lead to the disruption of intercellular junctions at the molecular level and how is the TGF-β signal linked to the migration complex formed by the small GTPases Rho, Rac and Cdc42. Using high throughput screens, our laboratory has recently characterized a novel signaling cascade responsible for TGF-β induced EMT/invasion, which appears to interlink these missing points, and more interestingly, in a manner independent of the classic SMAD pathway. Furthermore, we have identified a member of the tight junction/polarity complex, Par6 (partitioning defective 6) as a key modulator of TGF-β-induced EMT. Using a series of molecular analyses, including tryptic phosphopeptide mapping, molecular interaction studies and immunofluorescence, we demonstrate that the phosphorylation of Par6 by the TGF-β receptor at the carboxy terminal tail is essential for its role in TGF-β signaling. Mutations directed at this phosphorylation site block TGF-β-induced EMT in non-transformed mouse mammary epithelial cells (NMuMG), and also block the EMT phenotype and invasiveness of transformed mouse mammary epithelial cells (Emt-6, which produce autocrine TGF-β) in three-dimensional culture conditions. Additionally, we also observed, that the role of Par6 in EMT is not specific to TGF-β signaling, suggesting that it may function as a convergent point to other growth factors responsible for cell invasion. Taken together, the results suggest that Par6 represents a novel molecular target for cancer invasion.

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Evaluation of Gefitinib biological effects in patients with solid tumors amenable to sequential biopsies

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Background: Biological effects of gefitinib are not well understood. The goal of this study is to determine the effects of gefitinib on the EGFR signaling pathways in tumor and normal tissues in patients treated with gefitinib as well as to explore potential mechanisms of resistance. The relationship between gefitinib dose and parameters of exposure and pharmacodynamic activities will be explored.
Methods: Patients with a diagnosis of a solid tumor potentially susceptible to gefitinib and lesions amenable to serial tumor biopsies are treated with gefitinib in two sequential cohorts of 12 patients each at doses of 250 and 500 mg given orally once daily. Biopsies of tumor and skin as well as plasma and oral buccal swabs are collected at baseline and at sequential time points after treatment for biological and pharmacological studies. Plasma samples for pharmacokinetic (PK) studies are obtained on d1(from 0–8h), and pre-treatment trough samples are obtained on d2, d3, d8, d15, d22, and d28 of cycle 1. Treatment is continued unless the development of severe drug related toxicity or disease progression.
Results: 10 patients (7 men, 3 women) with a median age of 61 years (range 29–77) and performance status 0–2 have been treated at the 250 mg dose. Tumor types included colo-rectal (4), pancreas (2), lung (1), carcinoid (1), breast (1), and head and neck (1). Grade 1–2 toxicities included rash, diarrhea, nausea, anorexia, and fatigue. No grade 3 or 4 toxicities due to gefitinib were observed. Average unbound pre-treatment concentrations (average of days 8, 15, 22, and 28) were highly variable (6-fold) and ranged from 122–739 ng/mL (mean±SD, 436±240 ng/mL). From compartmental modeling of day 1 PK data and simulation analysis to estimate pre-treatment concentrations, gefitinib concentrations were steadily accumulating 1.5 to 5-fold higher than predicted by the PK model during 28 days of treatment, suggesting gefitinib may be inhibiting its own clearance. Quantitative IHC revealed a marked reduction in MAPK and Akt activity in tumor biopsies and an increase in p27 expression.
Conclusions: The preliminary pharmacodynamic findings are provocative. Patient enrollment continues and further testing of tumor and normal tissues is ongoing. Studies to determine factors influencing variable exposure to gefitinib and the relation to biological effects and toxicity are in progress.

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Emerging pharmacokinetic (PK)–pharmacodynamic (PD) profile of AEE788, a novel multi-targeted inhibitor of ErbB and VEGF receptor family tyrosine kinases

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Background: AEE788 is an orally active, reversible, small molecule multi-targeted kinase inhibitor with potent inhibitory activity against ErbB and VEGF receptor family of tyrosine kinases. It has an IC_{50} of less than 100 nM against EGFR, ErbB2, VEGFR2. This phase I multicenter study was to assess the safety, pharmacokinetics (PK), MTD/DLT dose levels, and optimal biological dose of AEE788 in patients (pts) with advanced solid tumors who received daily doses of AEE788 at 25, 50, and 100 mg.
Methods: A total of 16 pts have been enrolled to date in the specified dose cohorts. Of these, 12 pts have evaluable skin biopsy samples; 10 pts have completed skin biopsy analysis, 2 pts have samples pending analysis. There were 3 pts with evaluable tumor biopsies pre- and post-treatment with AEE788. Biopsy samples were evaluated by immunohistochemistry (IHC). Pharmacodynamic (PD) effects of AEE788 for EGFR/p-EGFR, ErbB-2, KDR/p-KDR, p-AKT, p-MAPK, p-STAT3, Ki67 and p27 were evaluated. A TUNEL assay was performed on tumor samples to assess apoptosis. A 24 hr PK profile was obtained on days 1, 15 and 28, with trough sampling on days 8 and 22 to determine drug serum concentrations using a validated LC/MS/MS assay. PK parameters of AEE788, AQM674 (active metabolite) and the active composite sum of AEE788 and AQM674 were computed by model independent methods. An Emax model for PK/PD modeling was used to characterize effect-exposure relationships.