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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.

Results: A dose and exposure dependent inhibition of p-EGFR, p-MAPK and Ki67 in skin was observed across dose levels (25–100 mg). Up to 50 mg (8 pts), mean decrease in p-EGFR was ⩽33%, in p-MAPK ⩽13%, and in Ki67 ⩽37%. At 100 mg (2 pt), p-EGFR, p-MAPK and Ki67 were inhibited by average of 83, 41 and 58%, respectively. Total EGFR and TGF-α were unchanged. Tumor inhibition of p-EGFR, p-MAPK and Ki67 was 90%, 70% and 10% respectively at 100 mg (1 pt). The remaining two paired tumor samples at 25 mg and 50 mg did not show any biomarker inhibition. An Emax model adequately characterized the effect-exposure relationships on p-EGFR, p-MAPK and Ki67. The model-fitted average concentration during a dose interval at half maximum inhibition (ICav50) of p-EGFR was 0.018 μM (AEE788), 0.041 μM (AQM674) and 0.024 μM (composite of AEE788+AQM674).
Conclusion: Dose and exposure dependent responses were observed in signaling pathways to the primary targets of AEE788 and AQM674 in skin. cell proliferation (Ki67) was also inhibited. PK-PD modeling of the effect-exposure relationships revealed serum $ICav_{50}$ of inhibition of p-EGFR in skin is similar to the *in vitro* IC_{50} (0.011 μM) for inhibition of p-EGFR in A431 tumor cell line. Enrollment is ongoing.

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Demonstration of broad in vivo anti-tumor activity of ARRY-142886 (AZD-6244), a potent and selective MEK inhibitor

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Activation of the Ras/Raf/MEK/MAP kinase pathway has been implicated in uncontrolled cell proliferation and tumor growth and MEK1,2, dual specific kinases that activate ERK1/2, are key players in this pathway. We have discovered ARRY-142886 (AZD-6244), a novel, potent and selective inhibitor of MEK 1,2, which is non-competitive with respect to ATP. It inhibits both basal and induced levels of ERK1/2 phosphorylation in numerous cancer cell lines with IC_{50}s as low as 8 nM. We have previously reported that ARRY-142886 (AZD-6244) has demonstrated efficacy in several murine xenograft tumor models, including HT29, BxPC3, MIA PaCa2, A549, and PANC-1. We have now evaluated additional tumor models [Colon26, LoVo, Calu6, HCT116, MDA-MB-231, and LOX] for inhibition of tumor growth and/or effects on tumor pERK levels. In the Colon26 model, tumor cells were implanted subcutaneously in the flank of Balb/c mice. For the human tumor cell lines, female nude mice were inoculated subcutaneously in the flank. Tumor size was measured at regular intervals for up to 30 days. Animals received oral doses of ARRY-142886 (AZD-6244) ranging from 2 to 200 mg/kg/d. In all of these models, ARRY-142886(AZD-6244) showed significant tumor growth inhibition and, in some models [Colon26, HCT116, MDA-MB-231 and LOX], tumor regression. In HCT116, pERK levels were significantly reduced in tumors 4 hours after the last dose. In an HT29 human colon carcinoma model, dose-dependent inhibition of tumor growth was observed. Doses of 10 mg/kg, BID, *p.o.* resulted in greater than 50% tumor growth inhibition. Examination of tumor pERK, by Western blot analysis, following a single dose of 30 mg/kg showed >99%, 90% and >80% inhibition 4, 12 and 24 hours after dosing, respectively. Consistent with the mechanism of action of ARRY-142886(AZD-6244), tumor growth inhibition correlates with decreased phospho-ERK levels in tumors. ARRY-142886 (AZD-6244) has entered clinical development.

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A phase II, pharmacokinetic (PK) and biological correlative study of OSI-774 (Tarceva) in patients with advanced renal cell carcinoma, with FDG-PET imaging: evidence of durable stable disease and antitumor activity

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Background: EGFR is over-expressed in >80% of renal neoplasms and is implicated in tumor initiation and progression. Antitumor activity against renal cell carcinoma in the phase I study of OSI-774, a selective oral quinazoline inhibitor of EGFR tyrosine kinase (EGFR-TK) activity, has lead to a 2-stage Phase II evaluation in patients (pts) with advanced RCC.
Methods: OSI-774 (150mg) was administered daily using 28-day courses, until disease progression. A single dose reduction to 100 mg daily was allowed for ⩾ grade-3 toxicity. Primary end point was objective response rate (CR+PR+SD). Secondary endpoints were progression free survival (PFS), overall survival, toxicity and response correlation with post-receptor effects of EGFR-TK inhibition. The utility of FDG-PET imaging, as an early predictor of response, was also assessed with serial scans pre-treatment and after completion of course 1.
Results: One patient in the initial 19 patients had a partial response necessitating expanded accrual to stage 2. A total of forty pts; 31♂/9♀; median age – 57 (range 38–73); ECOG PS-0 (9)/1 (27)/2 (4); received 198 courses (median-3; range 1–15). Tumor histology was: clear cell (77%) and granular (13%). Median number of prior therapies was 2 (range 0–4): nephrectomy 90%, immunotherapy 83%, radiation 37% and chemotherapy 20%. Prolonged stable disease (SD) lasting more than 6 months was noted in 7 pts (23%) including 4 patients who remained on treatment for 9, 14, 14, and 15 months. Four pts underwent dose reduction for reversible grade 3 toxicities: skin rash (2), hand-foot syndrome (1) and PT prolongation (1). No other grade 3–4 toxicities have occurred. Minimum plasma steady state concentration of OSI-774 and biological correlatives such as pERK, pAkt and p27 are being assessed in all pts. Co-registration analysis of paired FDG-PET images performed pretreatment and on day-28 on patients treated in stage 2, reveals preliminary evidence of significant metabolic differences between patients that obtain clinical benefit (responders and stable disease) and non-responders. Furthermore, these results appear to be congruent with the results of conventional CT scans performed pretreatment and after 2 courses of treatment on the same set of patients.
Conclusion: OSI-774 induces prolonged stable disease (>6 months) and antitumor response in a significant subset of patients with metastatic renal cell carcinoma. Preliminary data suggests that FDG-PET imaging may be a useful early predictor of treatment outcome in this patient population.

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Cellular uptake of fluoro-2-deoxythymidine (FLT), a novel PET tracer, correlates with induction of apoptosis by erlotinib in A431 cells

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Introduction: Cellular retention of ^{18}F-FLT, a novel PET radiotracer, is dependent on thymidine kinase 1 (TK1) activity, and TK1 is maximally-expressed during S-phase of the cell cycle. On this basis, we hypothesized that changes in FLT uptake could be used to assess the cell cycle effects of EGFR inhibitor therapy, and to distinguish between inhibitor-sensitive vs. resistant tumors.
Materials and Methods: As a test of this hypothesis, we monitored changes in ^{3}H-FLT cellular uptake following treatment of two EGFR-amplified tumor cell lines that differ in sensitivity to erlotinib.
Conclusions: These data suggest that therapeutic agents that suppress TK1 expression can be monitored by FLT uptake, and that FLT PET has potential for use as a non-invasive method for monitoring drug-induced apoptosis.

Table 1: Effects of drug treatment on adherent cells

Treatment	Relative cell #	FLT uptake/cell	TK1 level	% AnnexinV
A431				
0 μM erlotinib	1	1	1	1
1 μM erlotinib	0.55±0.06	0.77±0.19	0.43	5
10 μM erlotinib	*0.20±0.05	0.23±0.12**	0.01	15
MDA-468				
0 μM erlotinib	1	1	pending	1.5
1 μM erlotinib	0.81±0.03	1.19±0.25	pending	1.5
10 μM erlotinib	*0.66±0.07	0.64±0.08**	pending	2
A431 + ZVAD-fmk experiment				
0 μM erlotinib	1	1		
10 μM erlotinib	0.16	0.09		
0 μM+ZVAD	1.02	0.94		
10 μM+ZVAD	0.38	0.48		

*, **: Difference between A431 and MDA-468 cells significantly different, p=0.05 by Rank-sum test.

Results: Despite significant inhibition of EGFR auto-phosphorylation on Tyr-1068 in both cell lines, 10 μM erlotinib was substantially more effective at suppressing cell number, as measured by a methylene blue absorption assay, in the sensitive A431 cell line as compared to the resistant MDA-468 cell line (Table 1). Consistent with our hypothesis, the effect of EGFR inhibitor therapy was more substantial on FLT uptake in A431 cells. A significant proportion of A431 cells, but not MDA-468 cells, detached from the dish following inhibitor therapy. Of the remaining adherent cells,