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,