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.

370 POSTER

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,

15% of A431 cells vs. 2% of the MDA-468 cells were stained positive by AnnexinV, which is a marker for apoptosis. The induction of apoptosis appeared to correlate with loss of TK1 expression, as assessed by western blotting and film densitometry. Thus, we reasoned that caspase-mediated degradation of TK1 could be at least partially responsible for the drug-induced suppression of FLT uptake. To test this, A431 cells were treated simultaneously with the caspase inhibitor ZVAD-fmk and erlotinib. ZVAD-fmk treatment reduced the drug-induced cell death, and this was associated with a corresponding attenuation of erlotinib-induced suppression of FLT uptake (Table 1). The effects of ZVAD-fmk on TK1 expression and apoptosis induction will be reported.

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Signaling mediators of bystander response are potential therapeutic targets for attenuating tumor relapse

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Background: Our objective is to understand whether radiation used to treat local tumor after surgery can, trigger a positive feed back signaling mechanism. This may cause the cells to have a memory of the initial irradiation insult for an extended period of time that may result in local/regional tumor recurrence at later time. In this study we determined whether exposure to radiation could initiate NF-kB and TNF-a signaling and maintain NF-kB >TNF-a > NF-kB feed back cycle in human epithelial cells.
Materials and Methods: To examine whether cells at normal tissue at the radiation-exposed treatment site could undergo these alterations, normal human epithelial cells were exposed to radiation at doses used in fractionated radiotherapy and examined for (a) the dose and time dependent activation of NF-kB by electrophoretic mobility shift assay (EMSA), (b) the kinetics of TNF-a expression by RT-PCR (mRNA expression) and ELISA (secreted protein expression), and (c) the involvement of reactive oxygen species (ROS) in TNF-a mediated NF-kB activation by FACS analysis. Blocking experiments were performed using specific inhibitors.
Results: EMSA of nuclear extracts from cells exposed to clinical doses of radiation revealed a bi-phasic time-dependent expression of NF-kB, reaching a first maximum at 3h and a second maximum at 48 h. The functional integrity of the radiation-induced NF-kB, determined by transient transfection with pNF-kB-Luc that expresses the luciferase reporter gene in an NF-kB-dependent manner showed a 3.8-folds compared to mock irradiated control indicating that NF-kB DNA-binding activity triggered by radiation exposure could initiate transcriptional activation of NF-kB–dependent genes. Cells either incubated with TNF-a soluble receptor or TNF-a neutralizing antibody blocked the second phase (24 & 48 h) induction of NF-kB activation. Similarly, TNF-a mRNA expression was observed at 8h and protein expression at 16 and 24 h post-exposure. The TNF-a expression both at mRNA and protein level were inhibited to constitutive level by pre-incubating the cells with NF-kB inhibitor NF-kB SN50 cell permeable inhibitory peptide (100 μg/ml) 1 h prior to radiation exposure. These results clearly indicated the occurrence of a positive feedback cycle initiated by the activation of NF-kB upon radiation exposure. This activated NF-kB signaling mechanism triggers the TNF-a production, which in turn induces the activation of NF-kB through generation of ROS in primary endothelial cells.
Conclusions: Reappearance of a local or regional tumor after treatment is a major limitation in achieving disease-free survival. Identifying and intervening the mediators involved in this mechanism may help to achieve a prolonged disease free survival. This research was supported by the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG03–02ER63449.

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A phase I/II trial of erlotinib and bexarotene in aerodigestive tract cancers

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Background: We have previously reported the overexpression of the epidermal growth factor receptor (EGFR) and cyclin D1 as early events in lung carcinogenesis. Classical retinoids prevent the carcinogenic transformation of human bronchial epithelial (HBE) cells at least partly through repression of these proteins. Clinical activity of classical retinoids is limited by the frequent repression of the critical retinoic acid receptor, RAR-beta. Non-classical retinoids such as the rexinoid, bexarotene, exist that repress cyclin D1 and EGFR expression but can signal independent of RAR-beta. We found that combining an EGFR tyrosine kinase inhibitor, erlotinib, with bexarotene induced at least additive suppression of growth and cyclin D1 expression in HBE cells which had silenced RAR beta. Based on these and other pre-clinical findings we performed a phase **I/II** clinical trial of this combination in patients with advanced aerodigestive tract cancers.
Materials and Methods: Patients with advanced aerodigestive tract cancers who had failed prior chemotherapy were enrolled onto this dose-escalation study. Three dose levels were utilized and at least three patients were enrolled at each level. Primary objectives were to determine the maximum tolerated dose. Secondary objectives were to determine activity, toxicity, and surrogate markers of response in buccal epithelial cells.
Results: Twenty patients were enrolled and sixteen are evaluable. Toxicities have generally been mild with asymptomatic elevations of cholesterol and triglycerides occurring frequently. No cases of pancreatitis were observed. One case of dose-limiting rash (grade 3) and one case of dose-limiting diarrhea (grade 3) were observed. To date, three patients have had radiographic responses (2 PR, 1MR), one of which has lasted more than 1 year. Four additional patients remain on study with stable disease. Two have had stable disease for more than three months. Changes in surrogate markers of response in buccal epithelial cells are being determined.
Conclusions: The combination of erlotinib and bexarotene is well tolerated and appears active for the treatment of advanced aerodigestive tract cancer resistant to chemotherapy. A confirmatory trial comparing this regimen to erlotinib alone is warranted to determine the efficacy of this regimen.

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p38/JTV-1 is a novel modulator of TGF-beta required for the downregulation of c-myc and lung cell differentiation: its functional association with lung cancer formation

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p38/JTV-1 is known to be an essential scaffold protein for the formation of macromolecular tRNA synthetase complex (ref1). Interestingly. The mice lacking the gene encoding p38 were developed with severe hyperplasia of the alveolar epithelial cells in lung that caused immediate death after birth due to respiratory dysplasia (ref2). Molecular screening revealed that p38 interacts with FBP (FUSE-binding protein) that is a transcriptional activator of protooncogene, c-myc. The binding of p38 enhanced the ubiquitin-mediated degradation of FBP, resulting in downregulation of c-myc, which is required for the functional differentiation of alveolar type **II** cells. The ectopic expression of p38 suppressed proliferation and restored the differentiation markers in lung carcinoma cells. The cellular level of p38 was increased by TGF-beta to mediate cell growth arrest. In reverse, the loss of p38 led to resistance to TGF-induced cell cycle inhibition. The mice with reduced expression of p38 showed higher susceptibility to tumorigenesis, and the severe reduction of p38 level was frequently found in lung cancer cell lines and clinical cancer tissues. The working mechanism and association with tumor formation in animal model and human cancer patients strongly suggest p38/JTV-1 as a novel tumor suppressor.

374 POSTER

Inhibition of PI3K/AKT pathway by rhabdastrellic acid-A induced caspase-3-dependent apoptosis in leukemia cells

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Background: It has been demonstrated that PI3K/AKT signaling is aberrantly activated in AML cells. It is a promising strategy to target PI3K/AKT pathway for cancer treatment. Rhabdastrellic acid-A, an isomalabaricane triterpenoid, isolated from the sponge Rhabdastrella globostellata. The aim of this study is to explore effect of Rhabdastrellic acid-A on PI3K/AKT pathway and apoptosis.
Methods: Cytotoxicity was determined by MTT assay. Immunoblot analysis was employed to detect protein expression. DNA fragmentation was analyzed using agrose gel electrophoresis.
Results: Our investigation indicated that Rhabdastrellic acid-A inhibited proliferation of HL-60 cells with IC_{50} value of 0.64 μg/ml and induced apoptosis of HL-60 cells. Also, Rhabdastrellic acid-A induced cleavage of the death substrate poly (ADP-ribose) polymerase (PARP) and caspase-3. Pretreatment of HL-60 cells with caspase-3 specific inhibitor DEVD-CHO prevented Rhabdastrellic acid-A-induced DNA fragmentation, PARP cleavage. The expression levels of protein bcl-2, bax have no change in response to Rhabdastrellic acid-A treatment in HL-60 cells, whereas activated PI3K had significantly a decrease after treatment with