Drugs of the Future 2006, 31(10): 854-858

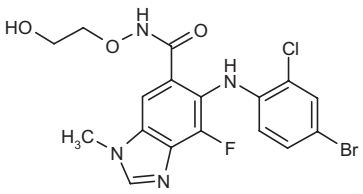
CCC: 0377-8282/2006
DOI: 10.1358/dof.2006.031.10.1040269

AZD-6244

MEK1/2 Inhibitor
Oncolytic

ARRY-142886

5-(4-Bromo-2-chlorophenylamino)-4-fluoro-1-methyl-1*H*-benzimidazole-6-carbohydroxamic acid 2-hydroxyethyl ester



$C_{17}H_{15}BrClFN_4O_3$
Mol wt: 457.6813

CAS: 606143-52-6

EN: 355417

Abstract

The MEK/ERK-dependent mitogen-activated protein (MAP) kinase pathway mediates cellular responses to growth signals, and aberrant regulation of the pathway has been implicated in many human cancers. Because of its key position as the only known activator of ERK, and its location downstream of the oncogenes *ras* and *raf*, MEK offers an attractive target for chemotherapeutic intervention. AZD-6244 (ARRY-142886) is an orally active, highly specific inhibitor of MEK that has shown tumor-suppressive activity in a wide range of preclinical models of human cancer. AZD-6244 is currently in phase II development for the treatment of melanoma, non-small cell lung and pancreatic cancer.

Synthesis

AZD-6244 is synthesized as follows:

Nitration of 2,3,4-trifluorobenzoic acid (I), followed by selective displacement with aqueous ammonia of the 4-fluoride in the resulting nitrobenzoic acid (II) gives 4-amino-2,3-difluoro-5-nitrobenzoic acid (III), which is further esterified to (IV) upon treatment with trimethylsilyldiazomethane. Subsequent condensation of (IV) with aniline (V) in hot xylene yields the diaminobenzoate (VI). The reduction of the nitroamine (VI) in the presence of formic acid and Pearlman's catalyst under transfer hydrogenation conditions proceeds with concomitant cyclization to produce the benzimidazole (VII). The anilino moiety in (VII) is stepwise brominated to (VIII) with *N*-bromosuccinimide (NBS) and then chlorinated with NCS to afford the 4-bromo-2-chlorophenylamino derivative (IX). Alternatively, the intermediate (IX) is obtained by condensation of difluorobenzoate (IV) with 2-chloroaniline (X), followed by reduction of the resulting diaminonitrobenzoate (XI) with Zn dust in AcOH, cyclization of the *ortho*-diamine (XII) to the benzimidazole (XIII) by means of formamidine acetate, and bromination with NBS. After methylation of the benzimidazole (IX) with iodomethane and K_2CO_3, the methyl ester (XIV) is hydrolyzed to the benzimidazolecarboxylic acid (XV) under alkaline conditions. Coupling of acid (XV) with *O*-(2-vinyloxyethyl)hydroxylamine (XVI) in the presence of EDC/HOBt gives the vinyl-protected hydroxamate (XVII), which is finally deprotected by hydrolysis with HCl in aqueous ethanol (1). Scheme 1.

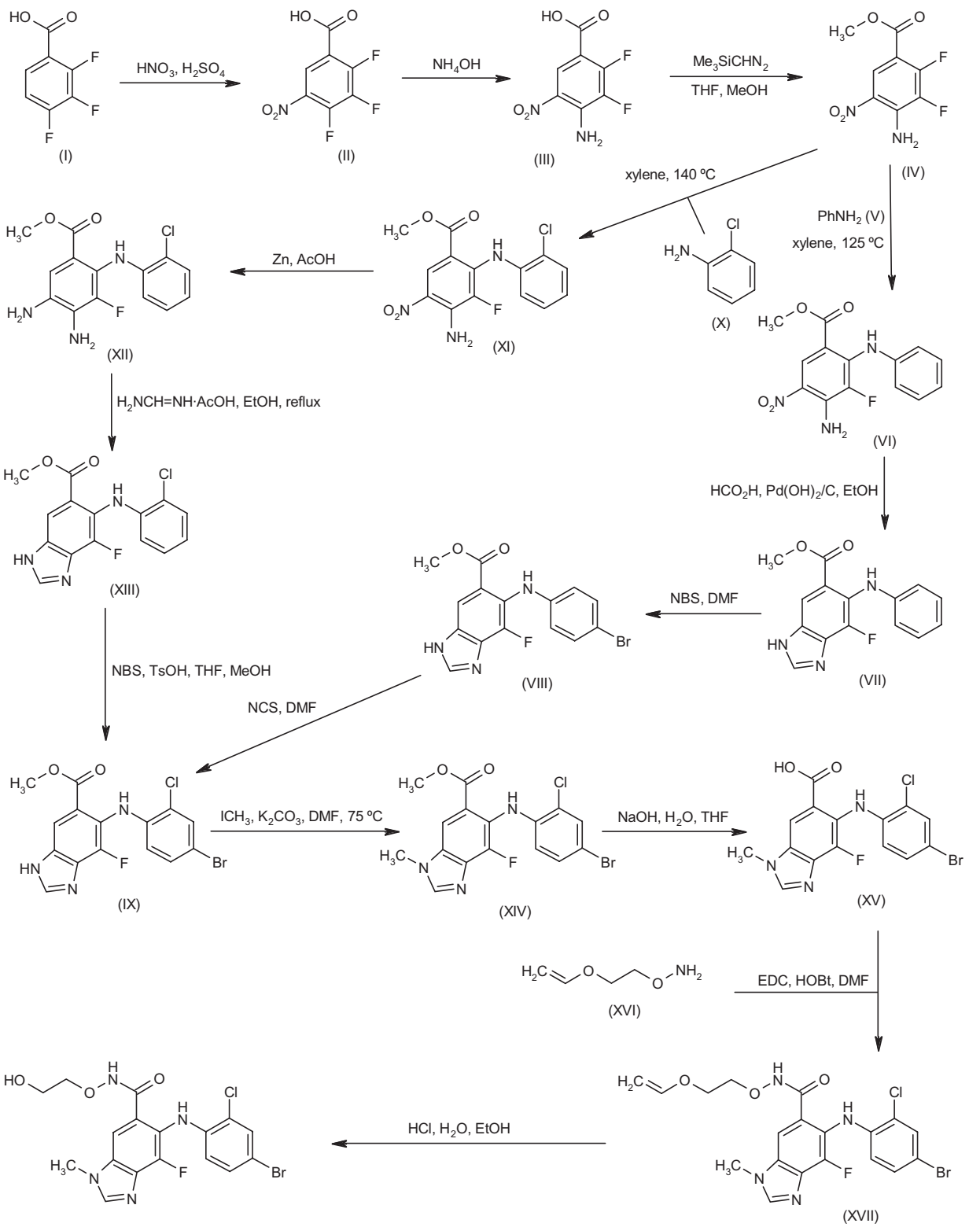
Background

Under normal circumstances, the regulation of cell growth and development is mediated by growth factors (*e.g.*, epidermal growth factor [EGF], erbB-2, vascular endothelial growth factor [VEGF], platelet-derived growth factor [PDGF]) that interact with their respective cell-surface receptor tyrosine kinases. The activated receptors coordinate the activation of mitogen-activated protein (MAP) kinase signal pathways (Fig. 1), leading to the appropriate changes in the cell (2).

One of the best understood MAP kinase pathways is the Ras/Raf/MEK/ERK cascade, and its constitutive activation has been implicated in aberrant cell growth and the development of many tumors (3-5). The cascade initiates with receptor tyrosine kinase-mediated activation of the G-protein Ras. GTP-Ras then phosphorylates Raf, which phosphorylates MEK1/2, which phosphorylates ERK1/2.

P. Revill, N. Serradell, J. Bolós, J. Bozzo. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of AZD-6244



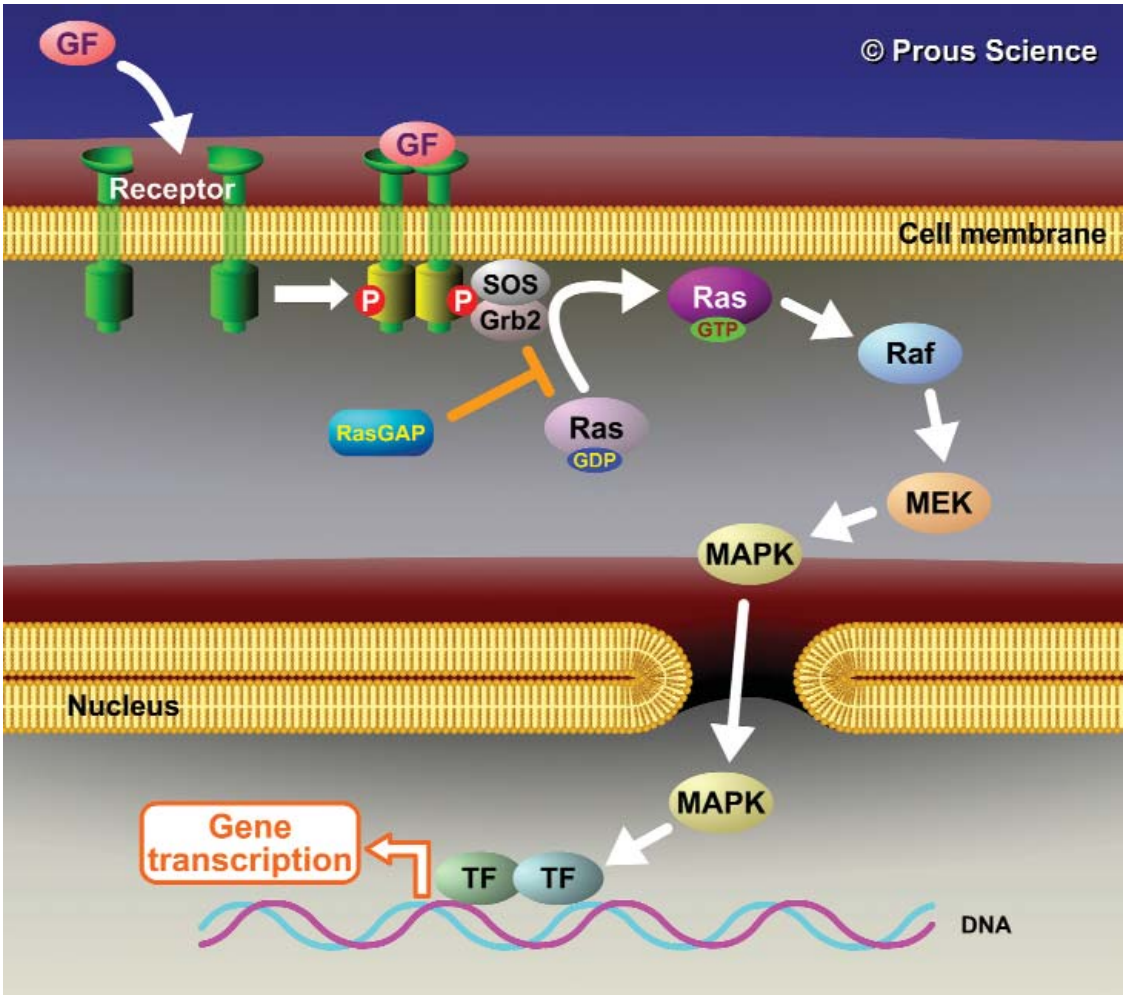


Fig. 1. Mitogen-activated protein kinase (MAPK) signaling pathway. Cell signaling is triggered when an inducer molecule such as a growth factor (GF) binds to its enzyme-linked receptor. This leads to receptor dimerization and phosphorylation of the cytoplasmic domain. The phosphorylated receptor activates and recruits the small adaptor protein Grb2, which induces the binding of the guanylnucleotide exchange factor SOS. The small G-protein Ras is then recruited to the plasma membrane by the Grb2-SOS complex. Ras becomes activated through the release of bound GDP, allowing a GTP molecule to bind in its place. The GTP-bound form of Ras can be negatively regulated by interacting directly with RasGAP. Activated Ras phosphorylates and activates Raf protein. Activated Raf phosphorylates and activates MEK, which in turn phosphorylates and activates MAPK. Activated MAPK migrates to the nucleus, where it phosphorylates gene transcription factors (TF).

Phosphorylated ERK1 and ERK2 dimerize and translocate to the nucleus, where they in turn phosphorylate a number of proteins that regulate cytoskeletal proteins, metabolism, chromatin remodeling and numerous transcription factors (2, 6, 7).

MEK offers two advantages as a potential target for therapeutic intervention in the treatment of cancer: 1) it is the only known activator of ERK1/2; and 2) it is downstream of both Raf and Ras. AZD-6244 (ARRY-142886) is an orally active inhibitor of MEK with high specificity for this enzyme. AZD-6244 has shown tumor-suppressive activity in a wide range of preclinical models of human cancer with aberrant expression of the ERK pathway, including melanoma, pancreatic, colon, lung and breast cancers, and is undergoing clinical development for the treatment of cancer (8-11).

Preclinical Pharmacology

AZD-6244 inhibits purified MEK1 with an IC_{50} of 12 nM, and is noncompetitive with respect to ATP. The agent is highly selective for MEK, and at 10 μM it did not inhibit any of over 40 purified tyrosine and serine/threonine kinases, including the cyclin-dependent kinase CDK2, c-Raf, IκB kinase β (IKKβ), c-Src, c-Jun *N*-terminal kinase (JNK), protein kinase C (PKC), EGFR, insulin-like growth factor-1 receptor (IGF-1R) and Lyn. In cell-based assays, it also potently and selectively inhibited the MEK pathway (IC_{50} = 10 nM), but not the phosphatidylinositol 3-kinase (PI3K), p38 or JNK pathways at up to 10 μM, and its selectivity for the MEK1/2/ERK1/2 pathway *versus* the MEK5/ERK5 pathway was also demonstrated in tumor cell lines (8-10).

AZD-6244 was shown to inhibit basal and stimulated ERK phosphorylation in cancer ell lines, with IC_{50} values as low as 8 nM (9, 10, 12). It was also found to be active against numerous cancer cell lines with Ras and B-Raf mutations, including the human colon carcinoma cell line HT-29, the melanoma cell lines Malme-3M, SK-MEL-28 and SK-MEL-2, and the pancreatic cancer cell line MIA PaCa-2 (IC_{50} = 50-270 nM). The cells were arrested at the G1/S phase of the cell cycle, and apoptosis was induced in the Malme-3M and SK-MEL-2 cell lines. The proliferation of Malme-3 cells (the normal counterpart of Malme-3M) was not inhibited, indicating that AZD-6244 specifically targets cells with activated MEK (9, 12).

Experiments using the human breast cancer MCF7 cell line (estrogen receptor-positive/HER2-negative, tamoxifen-sensitive) and the estrogen receptor/HER2-positive, tamoxifen-resistant MCF7/HER2 line indicated that AZD-6244 may be able to enhance the endocrine responsiveness of estrogen receptor-positive breast cancer, especially in cancers with activated MEK1/2 and increased ERK phosphorylation (13).

AZD-6244 was active in several murine human tumor xenograft models, including the HT-29 (tumor growth inhibition at 10 mg/kg p.o. b.i.d. and above), COLO 205 (tumor regression at < 25 mg/kg/day), HCT 116 (tumor growth inhibition at < 20 mg/kg/day) and SW620 (tumor growth inhibition at < 25 mg/kg/day) colorectal carcinoma models, the NSCLC A549 (tumor growth inhibition at 15 mg/kg/day) and Calu-6 (tumor growth inhibition at < 20 mg/kg/day) carcinoma models, the pancreatic BxPC-3 (tumor regression at 10-50 mg/kg p.o. b.i.d.), PANC-1 (tumor growth inhibition at 20-40 mg/kg/day) and MIA PaCa-2 (tumor regression at 40 mg/kg/day) carcinoma models, the breast MDA-MB-231 (tumor growth inhibition at < 20 mg/kg/day) and ZR-75-1 (tumor growth inhibition at 22 mg/kg/day) carcinoma models, and the LOX melanoma model (tumor regression at < 20 mg/kg/day) (9, 10, 14, 15).

Consistent with its proposed mechanism of action, tumor growth inhibition was correlated with a reduction in phosphorylated ERK in tumors (10, 14, 15).

Upon withdrawal of the drug in the BxPC-3 mouse model, tumor regrowth was seen, but when treatment was resumed, the tumors again regressed, indicating that they remained sensitive to the drug (9, 10, 15).

Oral administration of AZD-6244 in mouse models of primary human hepatocellular carcinoma resulted in dose-dependent inhibition of tumor growth, which was associated with inactivation of ERK1/2 and p90RSK, and upregulation of cleaved caspase-3, cleaved caspase-7, cleaved poly(ADP-ribose) polymerase (PARP) and phosphorylated MEK1/2 (16).

In a nude mouse model of BxPC-3 human pancreatic tumor xenografts, AZD-6244 caused tumor growth inhibition at 1 mg/kg p.o. b.i.d. and tumor regression at 3 and 10 mg/kg p.o. b.i.d. Inhibition of phosphorylated ERK in tumors was sustained at all time points after the two higher doses, but not at the low dose. Mean trough plasma levels of > 0.3 μg/ml were associated with activity (17).

In an analysis of four different murine human tumor xenograft models with varying sensitivity to AZD-6244, ERK1/2 phosphorylation was rapidly inhibited in all models after a single oral dose of 25 mg/kg. The two most sensitive models, COLO 205 and Calu-6, showed signs of apoptosis, whereas in the less responsive SW620 model, AZD-6244 appeared to inhibit proliferation and induce differentiation. PC-3 tumors were insensitive to AZD-6244 and showed no change in biomarkers of cell proliferation or apoptosis (10, 18).

The combination of AZD-6244 with gefitinib, an ATP-competitive inhibitor of EGFR, showed enhanced activity compared to either agent alone against human colorectal tumor LoVo xenografts (10, 19). Enhanced activity was also seen in combination with docetaxel in the SW620 xenograft model (10).

AZD-6244 exhibits other favorable characteristics, including minimal inhibition of cytochrome P-450 enzymes (> 10 μM), low hepatic clearance, no cytotoxic effects in normal cells and no changes in blood chemistry parameters. Moreover, it is negative in the Ames test and shows no activity at hERG channels (10).

Findings from cynomolgus monkeys, healthy volunteers and cancer patients treated with AZD-6244 indicated a strong correlation between drug plasma levels and inhibition of ERK phosphorylation in whole blood, suggesting the utility of pERK as a potential pharmacodynamic marker for MEK inhibition (10, 20).

Clinical Studies

In an open-label, multiple-dose phase I study, 23 patients with refractory solid tumors were administered oral doses of AZD-6244 of up to 300 mg b.i.d. The maximum tolerated dose (MTD) was found to be 200 mg b.i.d. The most common treatment-related adverse events were rash, diarrhea, nausea and fatigue, with dose-limiting toxicity (DLT) consisting of 1 case of hypoxia (200 mg b.i.d.), 1 case of diarrhea and 2 cases of rash (300 mg b.i.d.). After a single dose, t_{max} was reached in 1.3-2.6 h and the $t_{1/2}$ was 7-12 h. C_{max} and exposure were dose-dependent, and the mean C_{min} (12 h) at the 200 mg b.i.d. dose was 590 nM, well above the IC_{50} for inhibition of TPA-induced phosphorylation of ERK1/2. Stable disease was the best clinical response in this first part of the study, with Part B analyzing biomarkers (10, 21).

Based on findings from the phase I study demonstrating inhibition of MEK and downstream markers in tumors at well-tolerated doses, a phase II study of AZD-6244 *versus* temozolomide was initiated in stage III/IV melanoma patients (22, 23). An open, randomized phase II trial is also in progress comparing AZD-6244 to pemetrexed in patients with NSCLC who have failed 1 or 2 prior chemotherapy regimens (24), and another study is comparing AZD-6244 and capecitabine in patients with advanced metastatic pancreatic cancer who have failed first-line gemcitabine therapy (25).

Sources

Array BioPharma, Inc (US); licensed to AstraZeneca (GB).

References

1. Wallace, E.M., Lyssikatos, J.P., Hurley, B.T., Marlow, A.L. (Array BioPharma, Inc.). *N3 alkylated benzimidazole derivatives as MEK inhibitors*. EP 1482932, EP 1663210, JP 2005530709, US 2004116710, WO 03077914, WO 2005023251.

2. Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.E., Karandikar, M., Berman, K, Cobb, M.H. *Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions*. Endocr Rev 2001, 22(2): 153-83.

3. Herrera, R., Sebolt-Leopold, J.S. *Unraveling the complexities of the Raf/MAP kinase pathway for pharmacological intervention*. Trends Mol Med 2002, 8(4, Suppl.): S27-31.

4. Kohno, M., Pouyssegur, J. *Targeting the ERK signaling pathway in cancer therapy*. Ann Med 2006, 38(3): 200-11.

5. Staehler, M., Rohrmann, K., Haseke, N., Stief, C.G., Siebels, M. *Targeted agents for the treatment of advanced renal cell carcinoma*. Curr Drug Targets 2005, 6(7): 835-46.

6. Imajo, M., Tsuchiya, Y., Nishida, E. *Regulatory mechanisms and functions of MAP kinase signaling pathways*. IUBMB Life 2006, 58(5-6): 312-7.

7. Lewis, T.S., Hunt, J.B., Aveline, L.D. et al. *Identification of novel MAP kinase pathway signaling targets by functional proteomics and mass spectrometry*. Mol Cell 2000, 6(6): 1343-54.

8. Lyssikatos, J., Yeh, T., Wallace, E. et al. *ARRY-142886, a potent and selective MEK inhibitor: I) ATP-independent inhibition results in high enzymatic and cellular selectivity*. Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 3888.

9. Wallace, E., Yeh, T., Lyssikatos, J. et al. *Preclinical development of ARRY-142886, a potent and selective MEK inhibitor*. Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 3891.

10. Lyssikatos, J. *ARRY-142886 (AZD6244): Preclinical and early clinical development of a potent, selective MEK inhibitor*. Proc Am Assoc Cancer Res (AACR) 2006, 47: Presentation April 3.

11. Koch, K. *ARRY-142886, a novel and highly selective MEK inhibitor in clinical trials*. Proc Am Assoc Cancer Res (AACR) 2004, 45: 1309.

12. Yeh, T., Wallace, E., Lyssikatos, J., Winkler, J. *ARRY-142886, a potent and selective MEK inhibitor: II) Potency against cellular MEK leads to inhibition of cellular proliferation and induction of apoptosis in cell lines with mutant Ras or B-Raf*. Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 3889.

13. Zhou, Y., Benz, C. *AZD6244 (ARRY-142886), a potent and selective MEK1/2 inhibitor, enhances tamoxifen sensitivity in resistant ER-positive/HER2-positive breast cancer cells with constitutively elevated MEK1/2*. Proc Am Assoc Cancer Res (AACR) 2006, 47: Abst 1344.

14. Lee, P., Wallace, E., Yeh, T. et al. *Demonstration of broad in vivo anti-tumor activity of ARRY142886 (AZD6244), a potent and selective MEK inhibitor*. Eur J Cancer - Suppl 2004, 2(8): Abst 368.

15. Lee, P., Wallace, E., Yeh, T. et al. *ARRY-142886, a potent and selective MEK inhibitor: III) Efficacy in murine xenograft models correlates with decreased ERK phosphorylation*. Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 3890.

16. Tran, E., Chee Soo, K., Chow, P., Huynh, H. *Targeted inhibition of the MEK-ERK signaling cascade by the selective MEK1/2 inhibitor AZD6244 (ARRAY-142886) for the treatment of hepatocellular carcinoma*. Proc Am Assoc Cancer Res (AACR) 2006, 47: Abst 5470.

17. Winkler, J., Lee, P., Wallace, E. et al. *Anti-tumor activity, pharmacokinetic and pharmacodynamic effects of the MEK inhibitor ARRY-142886 (AZD6244) in a BxPC3 pancreatic tumor xenograft model*. Eur J Cancer - Suppl 2004, 2(8): Abst 342.

18. Logie, A., Cockerill, M., Moore, S. et al. *In vivo evaluation of pharmacodynamic biomarkers and mode of action of AZD6244 (ARRY142886)*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther 2005, Abst A240.

19. Cockerill, M., Logie, A., Speake, G. et al. *Combination studies in vitro and in vivo with gefitinib (Iressa) and the MEK1/2 inhibitor AZD6244 (ARRY142886)*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther 2005, Abst A110.

20. Doyle, M., Yeh, T., Suzy, B. et al. *Validation and use of a biomarker for clinical development of the MEK1/2 inhibitor ARRY-142886 (AZD6244)*. 41st Annu Meet Am Soc Clin Oncol (ASCO) (May 13-17, Orlando) 2005, Abst 3075.

21. Chow, L., Eckhardt, S., Reid, J. et al. *A first in human dose-ranging study to assess the pharmacokinetics, pharmacodynamics, and toxicities of the MEK inhibitor, ARRY-142886 (AZD6244), in patients with advanced solid malignancies*. 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther 2005, Abst C162.

22. *Array BioPharma receives phase 2 milestone payment from AstraZeneca*. Array BioPharma Press Release September 14, 2006.

23. *Randomised study to compare the efficacy of AZD6244 vs TMZ (NCT00338130)*. ClinicalTrials.gov Web site 2006.

24. *AZD6244 versus pemetrexed (Alimta®) in patients with non-small cell lung cancer, who have failed one or two prior chemotherapy regimen (NCT00372788)*. ClinicalTrials.gov Web site 2006.

25. *AZD6244 vs. capecitabine (Xeloda®) in patients with advanced or metastatic pancreatic cancer, who have failed first line gemcitabine therapy (NCT00372944)*. ClinicalTrials.gov Web site 2006.

Additional Reference

Friday, B., Yu, C., Smith, P., Adjei, A. *A potential role for modulation of a negative feedback loop between Erk and Raf in mediating sensitivity to the MEK inhibitor AZD6244 (ARRY-142886) in human lung cancer cell lines*. Proc Am Assoc Cancer Res (AACR) 2006, 47: Abst 4868.