overexpression and/or mutations have also been reported in acute myeloid leukemia, lung, prostate, breast and colon carcinomas.
Here we describe preclinical studies performed with NMS-P626, an orally available, highly potent and selective small-molecule inhibitor of TrkA.
Proliferation profiling of NMS-P626 against an extended panel revealed that, amongst other lines previously described as being sensitive to TrkA inhibition, the human colorectal cancer cell line KM12 is highly sensitive, suggesting dependence on TrkA signaling: 72 hour proliferation of KM12 was inhibited with an IC50 of 19 nM. Western blot analysis of KM12 cell lysates revealed the presence of a phospho-TrkA immunoreactive band with a molecular weight of ca.70 kDa, consistent with Tropomyosin3 (TPM3)-TrkA, the product of a 1q21/23 inversion previously described as a recurrent chromosomal aberration in papillary thyroid carcinoma and in a single case of colorectal cancer. cDNA sequencing and biochemical analyses confirmed expression in KM12 cells of a TPM3-TrkA fusion protein identical to the previously identified form, in which an N-terminal portion of the TPM3 protein is fused to the kinase domain of TrkA, resulting in constitutive kinase activation. RNA silencing of TrkA confirmed that knockdown of TPM3-TrkA leads to cell growth arrest and inhibition of AKT and MAPK pathways in KM12 cells. Likewise, phospho-TrkA, phospho-AKT and phospho-MAPK signals were inhibited in KM12 treated with NMS-P626. When administered orally to nude mice bearing KM12 tumor xenografts, NMS-P626 induced tumor stabilisation (>90% TGI), with ex vivo analysis confirming sustained target modulation.
Together, these data demonstrate that activated TrkA is a driving mutation in the KM12 colon carcinoma cell line, and that pharmacological modulation with NMS-P626, a selective TrkA inhibitor with an excellent preclinical profile, yields significant therapeutic benefit in this tumor model.

103 POSTER

Combination treatment of targeting Stat3 and HIF-1alpha is a potent strategy for prostate cancer therapy

N. Jing[1], K.R. Reddy[1], Y.L. Guan[1]. [1]*Baylor College of Medicine, Medicine, Houston, TX, USA*

Background: Two pathways which are upregulated in prostate cancer are the signal transducer and activator of transcription 3 (Stat3) pathway and the hypoxia sensing pathway. Stat3 was identified as an important target for cancer therapy since it participates in oncogenesis through the upregulation of genes encoding apoptosis inhibitors (Bcl-xL, Bcl-2, Mcl-1, and survivin), cell-cycle regulators (cyclin D1 and c-*myc*), and inducers of angiogenesis (VEGF). Stat3 is constitutively activated in 80% of prostate cancer. HIF-1alpha (HIF-1a) and HIF-2alpha (HIF-2a), which mediate the cellular response to hypoxia, activate the transcription of many genes crucial for cancer progression, including angiogenesis, cell survival, glucose metabolism, invasion and metastasis. Overexpression of HIF-1a in human cancers associates with poor prognosis and treatment failure in a number of cancers. Moreover, prolonged use of a target drug can result in drug resistance and reducing drug responsibility. Here we developed a combination treatment with targeting both phospho-Stat3 and HIF-1a to increase tumor response and reduce drug resistance and treatment failure.
Methods: We employed western blots, cell cycle analyses, immunohistochemistry, TUNEL and xenograft models to determine the drug efficacy and mechanism of the combination treatment.
Results: We combined two anti-cancer agents: T40214 (a phospho-Stat3 inhibitor) (Jing et al. 2004; PMID:15374974) and JG244 (a HIF-1a inhibitor) (Guan et al, 2010; PMID:19755960) together to evaluate the drug efficacy of the combination treatment in mice bearing human (or murine) prostate tumors. Our results demonstrated that (1) after treatments the mean tumor volumes in mice xenografts treated by placebo, T40214 and JG244 alone were increased 5.8, 3.1 and 2.5 folds, respectively. The mean tumor volume in mice treated by JG244 and T40214 combination was only increased 1.5 ($P < 0.002$) folds. (2) The drug efficacy in inmmuno-competent mice (C57BL/6) bearing murine prostate tumors (TRAMP-C2) showed that comparing with the tumors treated by placebo and T40214 alone, the combination treatment with mixing T40214 and JG244 together significantly suppressed the growth of murine prostate tumors. (3) The mechanism studies indicated that this combination treatment dramatically increased apoptosis of prostate cancer cells in tumor and significantly suppressed prostate tumor growth as well.
Conclusion: Our results provided solid evidence that compared with each agent used alone, the combination treatments dramatically increased apoptosis in tumors and promoted drug efficacy, suggesting that combination treatment including a HIF-1a/2a inhibitor not only has therapeutic efficacy in targeting HIF-1a/2a, but also could reduce the hypoxia-induced drug resistance to other therapies (e.g. T40214) and enhance drug efficacy. This approach could make prostate cancer treatments more effective and improve survival even in patients with metastatic disease.

104 POSTER

Plasma metabolomic analysis of genetic and pharmacological manipulation of PI 3-kinase pathway activation in mice using liquid chromatography coupled to mass spectrometry (LC-MS)

R. Pandher[1], C. Ducruix[1], G. Box[1], A. Henley[1], M. Valenti[1], A. De-Haven Brandon[1], B. Vanhaesebroeck[2], P. Workman[1], S.A. Eccles[1], F.I. Raynaud[1]. [1]*The Institute of Cancer Research, CRUK Centre for Cancer Therapeutics, Sutton Surrey, United Kingdom;* [2]*Queen Mary University of London, Centre for Cell Signalling, London, United Kingdom*

Background: This study evaluated the plasma metabolome of mice in which the PI3K pathway in the host (or an implanted tumour) was activated by loss of the upstream suppressor, PTEN. Plasma samples were collected from PTEN knockout (+/−) mice and their wild-type littermates as well as from normal athymic mice and those bearing PTEN null human tumour xenografts (U87MG glioblastoma or PC3 prostate adenocarcinoma). The effects of the PI3K inhibitor GDC-0941 were also evaluated in mice bearing U87MG xenografts and compared with the effects of the cytotoxic agent BCNU.
Materials and Methods: Protein was removed from plasma samples using Whatman protein precipitation plates. The extracted plasma samples were analysed on an LC-MS system with chromatographic separation achieved on a 1.8 μm particle column with a 13 minute water/acetonitrile gradient containing 0.1% formic acid.
Results: Seventeen plasma metabolites were significantly different in *PTEN* KO (+/−) mice compared with their wild-type littermates. These metabolites included amino acids (proline, citrulline, tyrosine and tryptophan), glycerophospholipids (glycerophosphocholine and ethanolamines), acylcarnitines (palmitoylcarnitine, linoleyl carnitine and stearoylcarnitine) and osmoregulators (proline betaine). Similar changes were identified in animals bearing PTEN null tumours: proline betaine, m/z 160.13, carnitine and indoxyl sulphate were increased in the case of U87MG tumour and m/z 160.13 and indoxyl sulphate in PC3 tumour-bearing animals. A single treatment of the pan-class I PI3K inhibitor GDC-0941 gave opposite effects to that observed in PTEN KO mice with changes observed in six metabolites including proline, proline betaine, *m/z* 160.13, carnitine, tyrosine and glycerophosphocholine. Chronic GDC-0941 treatment affected proline betaine, acetylcarnitine, citrulline and carnitine in a dose-dependent manner. The metabolomic signature following cytotoxic treatment of U87MG tumour bearing animals with BCNU showed different changes in several metabolites when compared with GDC-0941 treatment including proline betaine, *m/z* 160.13, phenylalanine, carnitine, glycerophosphoethanolamine.
Conclusions: LC-MS based metabolomics has successfully identified distinct exo- metabolomic signatures in *PTEN* KO mice and in PTEN null human tumour xenograft models following PI3K inhibitor and BCNU treatment.

105 POSTER

BIIB024, a potent pan-Raf kinase inhibitor for melanoma and solid tumors

B. Elenbaas[1], L. Singh[1], A. Boccia[2], P. Cullen[3], H. Peng[4], E. Rohde[5], B. Raimundo[6], G. Kumaravel[4], I. Joseph[2]. [1]*Biogen IDEC, Discovery Cancer Therapeutics, Cambridge Massachusetts, USA;* [2]*Biogen IDEC, Oncopharmacology, San Diego California, USA;* [3]*Biogen IDEC, Research Assays, Cambridge Massachusetts, USA;* [4]*Biogen IDEC, Medicinal Chemistry, Cambridge Massachusetts, USA;* [5]*Biogen IDEC, Drug Metabolism and Pharmacokinetics, Cambridge Massachusetts, USA;* [6]*Sunesis Pharmaceuticals, Medicinal Chemistry, South San Francisco California, USA*

The Raf kinases (A-Raf, B-Raf and C-Raf) are key regulators of cell proliferation and survival that control signaling through the MAPK pathway, composed of Ras, Raf, MEK and ERK. This pathway is frequently deregulated in cancer by mutations, leading to increased cancer cell proliferation and survival. In particular, Ras oncogenes are mutated in 25% of all cancers and B-Raf is mutated in 7% of all cancers, including 60% of melanomas. B-Raf is an attractive therapeutic target because most tumors with B-Raf mutations and some tumors with Ras mutations are sensitive to inhibition of Raf or MEK in pre-clinical models. In addition, clinical efficacy has been observed in B-Raf mutant melanomas with the PLX4032 and GSK2118436 B-Raf inhibitors. BIIB024 is a potent, oral pan-Raf kinase inhibitor that is being developed for the treatment of melanoma and solid tumors. BIIB024 potently inhibits oncogenic B-Raf^{V600E} mutant kinase and the wild-type B- and C-Raf kinases in biochemical assays. In a large biochemical kinase screening panel containing 222 unique human kinases, BIIB024 inhibited a small subset of kinases in a similar potency range as Raf kinases. To determine which cancer cell types are sensitive to BIIB024, in vitro pERK signaling and proliferation assays were conducted