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**

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**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)**

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**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**

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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

in a panel of cancer cell lines from different tumor types that differed in their Ras and Raf mutational status. BIIB024 potently inhibited signaling and proliferation in B-Raf mutant cell lines and a subset of B-Raf wild-type lines. To evaluate the ability of BIIB024 to inhibit the MAPK pathway in vivo, pharmacodynamic studies were performed in mice bearing B-Raf mutant or wild-type tumors. Following a single, oral dose of BIIB024 at 50 mg/kg, strong p-ERK suppression (>80%) was observed in both B-Raf mutant and wild-type tumor models up to at least 16 hours. In tumor xenograft efficacy studies, BIIB024 showed dose-dependent efficacy in the B-Raf mutant melanoma model WM-266-4 with daily, oral dosing. In addition, BIIB024 caused rapid regressions of large, established tumors in 2 B-Raf mutant models, WM-266-4 and A-375. Once dosing was terminated, the tumors that re-grew remained sensitive to BIIB024 in a 2nd dosing cycle. BIIB024 also showed efficacy in some Ras mutant/B-Raf wild-type models, further demonstrating its in vivo pan-Raf activity. A phase I clinical trial of BIIB024 is planned.

**106** POSTER

**Preclinical studies and characterization of BMS-794833, a small molecule inhibitor of Met and VEGFR-2 kinases**

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The receptor tyrosine kinase Met, is the only known high affinity receptor for hepatocyte growth factor (HGF) ligand, also known as scatter factor. Met activation can occur through HGF binding to the Met extracellular ligand binding domain, Met over-expression, and/or activating mutations in the receptor. Met receptor activation subsequently results in a variety of pleiotropic responses critical to carcinogenesis, including tumor cell motility, migration, proliferation, invasion and survival. Clinically, Met expression has been shown to be an independent prognostic factor in breast cancer, and increased levels of circulating HGF and/or Met expression have been detected in patients with tumors of diverse histological origins. Furthermore, elevated levels of Met and/or HGF strongly correlate with poor patient prognosis. These findings suggest that Met and HGF are viable candidates for targeted cancer therapies. Here, we describe the small molecule aminopyridine, BMS-794833, which exhibits potent inhibition of Met activity as demonstrated in a variety of *in vitro*assays including cell migration and cell scattering, phosphorylation of downstream signaling pathways, and immunohistochemical analysis. In addition, BMS-794833 also demonstrated inhibition of VEGFR enzymatic activity in biochemical kinase assays, indicating its dual potential as an anti-angiogenic agent. *In vivo*, BMS-794833 exhibited dose-dependent anti-tumor activity against multiple tumor types without overt toxicities at efficacious dose levels. Taken together, these findings support the utility of BMS-794833 as an anti-cancer therapeutic agent which has been nominated for clinical development.

**107** POSTER

**Spliceosome-targeting agents modulate alternative mRNA splicing in vitro and in vivo**

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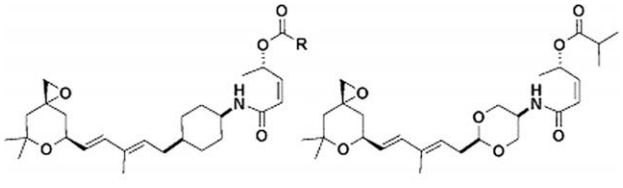
**Background:** The spliceosome is a large macromolecular complex present in cells that is required for the accurate and efficient editing of pre-RNAs into mRNA. Recently evidence has been presented that this complex may be a suitable target for cancer chemotherapy. We have developed a series of lead compounds (Judemycins) that interact with the spliceosome and demonstrated that these molecules are selectively toxic to human tumor cell lines.




**Materials and Methods:** To determine the mechanisms of action and/or toxicity afforded by these agents, we have examined the ability of these compounds to modulate alternate splicing of a series of candidate genes. Briefly, tumor cell lines were exposed to drug for time intervals up to 72 hours and following RNA extraction, the presence of alternative mRNA transcripts was detected by PCR. Oligonucleotide primers designed amplify MDM2, caspase 2 and 9, bcl-$X_L$ and ubiquitin were used to evaluate splicing changes. Similar studies using MDM2 plasmid minigenes following transfection into tumor cell lines have also been undertaken. Finally, the effect of drug exposure on gene splicing in human rhabdomyosarcoma xenografts in vivo was determined following i.v. dosing of Judemycin E in animals bearing Rh18 tumors.
**Results:** Our results indicate that Judemycin can alter the splicing pattern of MDM2, a protein known to be involved in the negative regulation of the p53 tumor suppressor. Effects were observed in both the endogenous MDM2 transcript, as well as from a minigene plasmid construct containing selected exons from this gene. In addition, cells exposed to Judemycin demonstrated alternate splicing of genes encoding proteins involved in the apoptotic cascade. Furthermore, in mice bearing human tumor xenografts, evidence of modulation of RNA splicing has been observed in tumor cells following drug exposure. In contrast, no evidence of alternate transcripts was seen in normal tissues isolated from these animals.
**Conclusions:** These studies indicate that Judemycin targets the spliceosome, resulting in the generation of aberrant mRNAs, and that these transcripts encode modified proteins that may play a role in tumor cell-specific cytotoxicity.
Supported, in part, by an NIH Cancer Center core grant CA-21675 and the American Lebanese Syrian Associated Charities.

**108** POSTER

**Discovery of CH5132799, a novel class I PI3K inhibitor**

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**Background:** Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase and plays crucial roles in tumor progression. PI3K$\alpha$ is reported to be frequently mutated in various human cancers, resulting in constitutive activation. Moreover, the PI3K/Akt pathway is frequently activated by overexpression of growth factor receptor tyrosine kinases or inactivation of the PTEN gene. Therefore PI3K is a promising therapeutic target for cancer.
**Materials and Methods:** We identified dihydropyrrolopyrimidine derivatives as new PI3K inhibitors using structure-based drug design (SBDD). A homology model of PI3K$\alpha$ was used for molecular design and the binding modes of several compounds were confirmed by X-ray crystal structure of PI3K$\gamma$.
**Results:** The first lead compound with a phenolic moiety was generated on the basis of a docking study of known PI3K inhibitors such as PI103 with a 3D structure of PI3K. The phenol moiety played an important role in binding to the enzyme, but it turned out to be metabolically unstable due to glucuronidation. To improve the metabolic stability, we searched for a bioisostere of phenol by computational docking tools and found the aminopyrimidine moiety (Ap) as an alternative. Our second lead compound with Ap showed good antitumor activity in vivo as a result of metabolic stabilization. Further chemical modification to improve the physicochemical and ADME profiles led us to identify a clinical candidate, CH5132799 (CH). We herein disclose the structure and profiles of CH, a novel class I PI3K inhibitor. CH exhibited a strong inhibitory activity against class I PI3Ks, especially against PI3K$\alpha$ (IC50 = 0.014 $\mu$M) but showed less inhibition of class II PI3Ks, class III PI3K and mTOR. We confirmed that CH binds ATP binding sites of the enzyme by the X-ray crystal structure of CH complex with PI3K$\gamma$. In human tumor cell lines with PI3K pathway activation, CH showed potent antiproliferative activity [HCT116(CRC): IC50 = 0.20 $\mu$M, KPL-4(BC): IC50 = 0.032 $\mu$M, T-47D(BC): IC50 = 0.056 $\mu$M, SK-OV-3(Ovarian): IC50 = 0.12 $\mu$M]. CH exhibited good oral bioavailability in mouse, rat, monkey and dog (F: 54.2–101%). In a human breast cancer (KPL-4: PI3K H1047R) xenograft model in mice, oral treatment with CH (25 mg/kg, 12 *q.d.*) showed strong tumor regression (tumor growth inhibition = 179%). More detailed biological profiles of CH will be presented in accompanying posters.
**Conclusions:** We designed and discovered novel PI3K inhibitors by SBDD. CH5132799 is an orally available, potent class I PI3K inhibitor and showed significant antitumor activity in PI3K pathway-activated human cancer xenograft models in mice. CH5132799 is progressing toward phase I clinical trials.