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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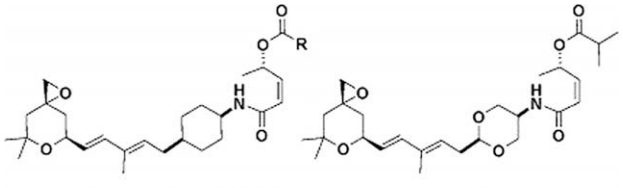
Background: The spliceosome is a large macromolecular complex present in cells that is required for the accurate and efficient editing of pre-RNAs into mRNA. Recently evidence has been presented that this complex may be a suitable target for cancer chemotherapy. We have developed a series of lead compounds (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.
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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. PI3Kα 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 PI3Kα was used for molecular design and the binding modes of several compounds were confirmed by X-ray crystal structure of PI3Kγ.
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 PI3Kα (IC50 = 0.014 μM) 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 PI3Kγ. In human tumor cell lines with PI3K pathway activation, CH showed potent antiproliferative activity [HCT116(CRC): IC50 = 0.20 μM, KPL-4(BC): IC50 = 0.032 μM, T-47D(BC): IC50 = 0.056 μM, SK-OV-3(Ovarian): IC50 = 0.12 μM]. 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.