

disruption of this cascade in GISTs. Thus, unraveling the role of MAPK signaling pathway, we aimed to shed light on the molecular lesions that underlie the development of KIT&PDGFRA wild-type GISTs.

Material and Methods: It were used 26 GISTs, previously identified by us as wild-type for KIT and PDGFRA gene. To evaluate KIT activation and presence of autocrine/paracrine loops we performed immunohistochemistry (IHC) to KIT phosphorylated form and to SCF (KIT ligand). To analyse MAPK signalling alterations/activation we evaluated N, H, K-RAS family and BRAF mutations status by PCR-SSCP, and studied RKIP and phospho-ERK expression by IHC.

Results: Positive SCF expression was observed in 76.9% (20/26) of cases and co-expression of SCF/CD117 was present in 65% of the cases. All phospho-KIT positive GISTs showed co-expression of SCF/CD117. Thus, we showed the presence of autocrine/paracrine mechanisms associated with KIT activation in ~20% of cases. Despite the absence of RAS mutations, we found BRAF mutations in ~4% (1/26) of GISTs. RKIP expression was lost in 8% (2/26). Furthermore, phospho-ERK showed that MAP kinase is activated in ~30% (8/26) of cases.

Conclusions: Based on the low frequency of alterations/activation of the MAPK we concluded that this pathway does not play a pivotal role in the pathogenesis of KIT&PDGFRA wild-type GISTs. Nevertheless, the potential therapeutic role of activated MAP kinase and particularly BRAF mutations warrants further studies in this subset of imatinib-resistant GISTs.

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POSTER

Novel inhibitors of BRAF based on a 2,6-disubstituted pyrazine scaffold

I. Niculescu-Duvaz¹, E. Roman¹, S.R. Whittaker², F. Friedlos¹, R. Kirk², I.J. Scanlon¹, L.C. Davies¹, D. Niculescu-Duvaz¹, R. Marais², C.J. Springer¹. ¹ICR, CR-UK Centre for Cancer Therapeutics, Sutton, Surrey, United Kingdom; ²ICR, CR-UK Centre for Cell and Molecular Biology, London, United Kingdom

BRAF, is a serine/threonine-specific protein kinase, that is mutated in 7% of cancers, with an incidence of 70% in melanoma. The mutant form of BRAF, which has a glutamate for valine substitution at position 600 (V600EBRAF) leads to increased proliferation and survival of malignant melanoma cells. 2-(3,4,5-Trimethoxyphenylamino)-6-(3-acetamidophenyl)-pyrazine, was identified as a low micromolar (IC₅₀ = 3.5 µM) BRAF inhibitor from a high-throughput screen of a library of 23,000 compounds. This compound was chosen as the starting point of a hit-to-lead program aimed at developing inhibitors of mutant V600EBRAF. Here we describe the synthesis of a series of compounds derived from the hit with emphasis on the optimization of the pyrazine ring and phenyl ring in order to increase the potency against V600EBRAF and selectivity compared to CRAF. The biological activity of the new inhibitors was assessed against mutant V600EBRAF in vitro. BRAF inhibitors were identified with IC₅₀s of 300–500 µM for V600EBRAF. Five inhibitors show 5 → 86 fold selectivity for V600EBRAF compared to CRAF.

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POSTER

Phase I study of the safety and pharmacokinetics of an oral, film-coated (FC) tablet of CP-868,596, a PDGFR inhibitor, in patients with advanced cancers

L. Lewis¹, N. Reddy², F. Guo³, K.J. Pierce⁴, A.J. Olszanski⁵, S. Balan⁶. ¹Dartmouth-Hitchcock Medical Center, Clinical Pharmacology, Lebanon, NH, USA; ²Dartmouth-Hitchcock Medical Center, Pharmacology, Lebanon, NH, USA; ³Pfizer Global Research and Development, Clinical Pharmacology, New London, CT, USA; ⁴Pfizer Global Research and Development, Biostatistics and Report, New London, CT, USA; ⁵Pfizer Global Research and Development, Oncology Drug Development, New London, CT, USA; ⁶Dartmouth-Hitchcock Medical Center, Norris Cotton Cancer Center, Lebanon, NH, USA

Background: CP-868,596 is an oral inhibitor of platelet-derived growth factor receptors (PDGFR). PDGF and its receptor play an important role in angiogenesis, and influences cell growth and survival through signal-transduction pathways. As previously reported (N. Lewis et al. ASCO 2007), the tablet formulation was well tolerated with the exception of a high incidence of nausea and vomiting. An extension of the study assessed a pH-dependent (alkaline-labile) FC tablet in an effort to reduce the incidence of nausea and vomiting and to compare safety, tolerability and PK to the tablet formulation.

Materials and Methods: FC CP-868,596 was administered on an empty stomach, without antiemetics, in 4-week cycles to patients with advanced solid malignancies. Four cohorts (100 mg QD, 200 mg QD, 100 mg BID and 140 mg BID) were studied. PK samples were collected after a single dose and at steady state; parameters were estimated by non-compartmental techniques.

Results: Fourteen patients enrolled in this portion of the trial [(11 male/3 female); median age (range): 63 (41–80)]. The most common treatment-related AEs were nausea (72%), vomiting (43%), dehydration and diarrhea (21%) and peripheral edema (14%). One DLT of nausea and vomiting occurred in the 140 mg BID cohort and was considered the MTD of FC CP-868,596 on an empty stomach. Main PK characteristics of the FC tablet were similar to the non-coated tablet: CP-868,596 was rapidly absorbed orally: median T_{max} 2 to 6 hours; moderate accumulation (mean AUC accumulation of 1.36–1.81 when given QD, and 2.91–4.46-fold when given BID). The mean terminal t_{1/2} ranged from 12.9 to 18.5 hours and was similar across all dose levels. No objective responses were seen.

Conclusion: The use of FC CP-868,596 appeared moderately to reduce the emetogenic potential of CP-868,596 compared to the non-coated tablet. However, the mitigation of nausea and vomiting was most effectively accomplished via administration of CP-868,596 with food. The FC cohorts support the development of an enteric coated formulation which could be given with food to further improve tolerability. The safety and PK characteristics of the FC tablet were similar to the non-coated tablet of CP-868,596.

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POSTER

Modulation of signaling through SEK1 and MKK7 differentially affects oxaliplatin sensitivity in hypoxic colon cancer cell lines

I. Vasilevskaya¹, D. Roberts¹, M. Selvakumaran¹, S. Johnson¹, P. O'Dwyer¹. ¹University of Pennsylvania, Abramson Family Cancer Center, Philadelphia, USA

Transcriptional changes in response to hypoxia are regulated in part through MAP kinase signaling to AP-1, thus contributing to resistance of cancer cells to platinum compounds. Recently we demonstrated that the inhibition of either SEK1 or MKK7 in HT29 cell line diminished hypoxia-induced AP-1 activation, with a more pronounced effect in MKK7-deficient cells. Inhibition of SEK1 rendered HT29 cells more sensitive to oxaliplatin, while the opposite effect was observed for MKK7, both in vitro and in vivo. These results prompted us to further investigate the role of hypoxia-induced signaling in oxaliplatin cytotoxicity. Using siRNAs targeting JNK1 and JNK2 in SEK1- and MKK7-deficient HT29 cells, we show that inhibition of JNK2 leads to increased oxaliplatin resistance, especially in the MKK7-deficient line. Accordingly, HT29 cells stably expressing dnJNK2 demonstrate the highest resistance to oxaliplatin under hypoxia, whereas expression of dnJNK1 enhances sensitivity to oxaliplatin as compared to parental cell line. Increase in oxaliplatin resistance upon MKK7 and/or JNK2 down-regulation is accompanied by dramatic inhibition of c-Jun phosphorylation during hypoxia, while in oxaliplatin-sensitive SEK1- and JNK1-deficient cells it is not affected, suggesting the critical role for MKK7/JNK2/c-Jun module in hypoxic activation of AP-1. The effects of down-regulating SEK1 and MKK7 on gene expression in HT29 cell line under oxic and hypoxic conditions were also assessed by microarray analysis. Our data demonstrate that induction of HIF-1-regulated genes is not affected by modulation of signaling through either kinase. Genes differentially repressed in hypoxic SEK1-deficient cells include several aldo-keto reductase 1 family members (B10, C1 and C2), calretinin and aldehyde dehydrogenase 3A1; whereas metallothionein-1F, TGF alpha and ribonuclease P RNA component H1 genes demonstrate the highest induction ratios.

Finally, we show that in a panel of colon cancer cell lines, down-regulation of SEK1 by dominant-negative or shRNA constructs results in increased sensitivity to oxaliplatin. Inhibition of SEK1 also leads to partial reversal of acquired oxaliplatin resistance in cells derived from HCT116 and HCT116 p53^{-/-} cell lines. Taken together, these data further support a positive contribution of MKK7/JNK2 to oxaliplatin cytotoxicity, and identify SEK1 as a potential target for reversal of hypoxic resistance to oxaliplatin in colon cancer cell lines.

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POSTER

Understanding the role of Raf signaling in B-Raf V600E mutant versus wildtype tumors

G. Hatzivassiliou¹, S. Sideris², I. Yen², S. Gloor³, M. Callejo³, B. Brandhuber³, B. Liu¹, M. Belvin¹, S. Malek². ¹Genentech Inc., Cancer Signaling, South San Francisco, CA, USA; ²Genentech Inc., Biochemical Pharmacology, South San Francisco, CA, USA; ³Array BioPharma, Biochemistry, Boulder, CO, USA

The B-Raf serine/threonine kinase and its closely related homolog c-Raf are downstream effectors of Ras and provide survival, growth and proliferation signals by activating the MEK/ERK kinase cascade. Activating mutations in B-Raf, predominantly V600E amino acid substitutions occur in several tumor types and lead to constitutive activation of the Raf/MEK/ERK pathway. Targeting B-Raf activity in human tumors is a promising strategy for cancer therapy; however, it remains unclear whether better