disruption of this cascade in GISTs. Thus, unraveling the role of MAPK signaling pathway, we aimed to shed light on the molecular lesions that underline 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 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.

584 POSTER

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

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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 (IIC50 = 3.5 uM) 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 uM for V600EBRAF. Five inhibitors show 5 → 86 fold selectivity for V600EBRAF compared to CRAF.

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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

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

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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.

587 POSTER

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

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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

efficacy can be achieved by inhibiting all Raf isoforms or by selectively inhibiting B-RafV600E. To gain better insight into the role of Raf signaling in tumors and design more effective therapeutic strategies, we have characterized the biochemical and cellular properties of two small molecule Raf inhibitors (Rafi) with different selectivity patterns against wildtype B-Raf and c-Raf versus mutant B-RafV600E. In biochemical kinase assays, Rafi A effectively inhibited only the B-RafV600E protein, while Rafi B was uniformly effective against c-Raf, B-Raf, and B-RafV600E under physiological ATP concentrations. In accordance with these biochemical data, only Rafi B could block basal phospho-ERK levels in wildtype B-Raf melanoma tumor lines, while both were equally effective in B-RafV600E lines. This observation was further supported by the ability of only Rafi B to block phorbol ester-stimulated phospho-Erk levels in human peripheral blood monocytes in vitro and to block epidermal growth factor (EGF)-stimulated pERK levels in EGF receptor-expressing melanoma and colon tumor lines. Despite their distinct abilities to knock down phospho-Erk levels downstream of wildtype Raf versus B-RafV600E signaling, the two inhibitors displayed similar cellular efficacy profiles in in vitro viability studies, being highly selective in blocking the proliferation and survival of B-RafV600E but not wildtype tumor lines. These data suggest that the cellular selectivity of Raf inhibitors against B-Raf mutant tumors is not a function of their biochemical properties but may rather reflect a unique signaling network in B-RafV600E mutant tumors, which renders them dependent on constitutive B-Raf signaling for the maintenance of their transformed phenotype. B-Raf wildtype lines on the other hand may not rely on Raf signaling but rather utilize additional and/or redundant signaling pathways for their survival and proliferation.

588 POSTER

Bench to bedside – Bedside to bench: Preclinical determination of the potential pharmacological activities of vandetanib in the clinic

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Background: Vandetanib (ZACTIMA™) is an orally available inhibitor of VEGFR2, EGFR and RET signalling that has recently completed phase III evaluation in NSCLC and medullary thyroid cancer. In the phase III studies, vandetanib was dosed at 300 mg/day as a monotherapy compared with erlotinib (ZEST) or placebo (ZETA), and at 100 mg in combination with docetaxel (ZODIAC) or pemetrexed (ZEAL) compared with chemotherapy alone. A challenge for multi-targeted agents is to understand the contribution of each pharmacological activity of the agent to the anti-tumour effects observed in the clinic.
Methods: In our current work, we have performed a detailed preclinical evaluation of the relative effects of vandetanib on VEGFR2 and EGFR activity in vivo in mouse models. Importantly, we have used drug doses selected to reflect the vandetanib plasma levels observed in the clinic.
Results: In mice, we demonstrated a substantial reduction in both pVEGFR2 and pEGFR in human tumour xenografts and a surrogate normal tissue (lung) at vandetanib plasma concentrations similar to the steady-state drug levels achieved in patients receiving vandetanib at 300 mg/day, with little additional effect when vandetanib doses were markedly increased. At lower doses, where vandetanib plasma concentrations were broadly similar to those achieved in patients receiving 100 mg/day, there was a significant reduction in both pVEGFR2 and pEGFR, but this was not maximal.
Conclusions: Based on these preclinical data, we consider that the vandetanib plasma exposures achieved in patients at 300 mg/day dosing could produce near-maximal reduction of both pVEGFR2 and pEGFR in tumours, with little additional benefit of increasing vandetanib exposure further. The vandetanib plasma levels in patients receiving vandetanib at 100 mg/day would be expected to substantially reduce both pEGFR and pVEGFR2, though this would be less than maximal. Sub-maximal inhibition of pEGFR and pVEGFR2 may have benefits in terms of increased potential to combine with established or novel therapies. Additionally, the preclinical data suggest that the steady-state drug plasma levels in patients receiving vandetanib 100 mg/day could be sufficient to induce regressions of tumours bearing activating EGFR mutations, though the anti-tumour effects may be more sustained at higher doses, consistent with increased inhibition of both VEGFR2 and EGFR signalling pathways.

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Functional evaluation of members of the LIV-1 family of proteins and their role in breast cancer

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All nine members of the LIV-1 family of proteins belongs to the ZIP superfamily of zinc transporters. Our Affymetrix Human Genome U133A GeneChips® analysis of ZIP7/HKE4/LZT-Hs1, LIV-1, ZIP14/LZT-Hs4, ZIP4/LZT-Hs5 and pS2 in MCF-7 breast cancer cells confirmed that ZIP7/HKE4/LZT-Hs1, LIV-1 and ZIP14/LZT-Hs4 were regulated by estradiol. This study was extended to investigate the expression of all nine LIV-1 family members in breast cancer cells treated with oestradiol, tamoxifen and faslodex. Additionally, we investigated the expression of the LIV-1 family members in our MCF-7 based models of tamoxifen and faslodex resistance using semi-quantitative PCR. Differential expression of these family members was seen with some members constitutively expressed whilst others were either elevated or reduced in the different conditions. This analysis demonstrated that ZIP7/HKE4/LZT-Hs1 was considerably elevated in tamoxifen resistance. In an effort to investigate a possible role for ZIP7/HKE4/LZT-Hs1 in tamoxifen resistant cells siRNA was used to reduced the expression of ZIP7/HKE4/LZT-Hs1. Interestingly, in the presence of siRNA for LZT-Hs1, it was not possible to demonstrate the activation of EGFR or Src as previously observed in the tamoxifen resistant phenotype using Western blotting analysis. This is an exciting result, which suggests a role for ZIP7/HKE4/LZT-Hs1 in driving the growth of tamoxifen resistant breast cancer cells.

590 POSTER

Molecular and antiproliferative effects of inhibitors of fatty acid synthase and of ErbB receptors in ovarian cancer cells

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Treatment of ovarian cancer (OC) is still suboptimal necessitating the search for novel therapies. In normal tissue, the key lipogenic enzyme fatty acid synthase (FASN) converts dietary carbohydrates to triglycerides, whereas in cancer, FASN represents a metabolic oncogene and produces phospholipids for membrane microdomains (lipid rafts) that accommodate clusters of receptor tyrosine kinases including Epidermal Growth Factor Receptor (EGFR, ErbB1) and ErbB2 (HER-2/neu) thus setting the stage for signal initiation. Importantly, both FASN and ErbBs are overexpressed in tumors including OC and represent drugable targets. Recent data suggest a link between FAS and ErbB2 in breast cancer. In OC, the relationship between FAS and ErbB is still elusive. Therefore, we examined the effect of FAS and ErbB inhibition on A2780 ovarian cancer cells (OCC). A FASN inhibitor (C75) and 2 irreversible ErbB inhibitors (EKB-569, Wyeth; CI-1033, Pfizer) inhibit growth of OCC (MTT assay – IC_{50}: C75 = 22 μM; EKB-569 = 5.1 μM; CI-1033 = 3.7 μM). Interestingly, C75 synergizes with EKB-569 or CI-1033 in cell growth inhibition ($p < 0.01$) suggesting cooperation between FAS and ErbB pathways during OCC growth. RT-PCR, real-time analysis and Western blotting revealed that C75 slowly and concordantly reduces EGFR mRNA, protein and activity in OCC. Thus, C75 silences EGFR gene expression at transcriptional levels without directly affecting EGFR signaling. C75 caused deprivation of overall and phosphorylated ErbB2 protein, but failed to diminish ErbB2 mRNA. Although C75 post-transcriptionally represses ErbB2, it does not directly disrupt ErbB2 activity. C75 also caused shut-down of FAS mRNA and protein. On the other hand, EKB-569 abolishes EGFR and ErbB2 protein expression and phosphorylation, but only weakly depresses mRNA levels. Strikingly, EKB-569 also represses FAS mRNA and protein. CI-1033 also failed to affect EGFR and ErbB2 transcript levels, but compromised EGFR activity (but not EGFR protein expression) and ErbB2 protein expression and function. Generally, CI-1033 reduced ErbB function rather than ErbB protein expression. Moreover, CI-1033 correspondingly down-regulated FAS mRNA and protein. Our data indicate that ErbB and FAS pathways mutually interact with each other in OCC. Thus, interference with the FAS and the ErbB systems effectively abrogates their oncogenic activities and may be exploited for OCC treatment.
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