Results: We determined that TLN-4601 potently inhibited the anchorage-dependent and -independent growth of KRAS-transformed human pancreatic nestin-positive (HPNE) duct-derived cells. We also found that the growth of KRAS mutation-positive pancreatic carcinoma cell lines was inhibited by TLN-4601. We then assessed the ability of TLN-4601 to antagonize RAS signal transduction. Consistent with the ability to directly antagonize RAS, we found that TLN-4601 treatment caused cell context-dependent reduction in RAS and RAF-1 protein expression and an inhibition of p70 S6 kinase and MEK1/2 phosphorylation.
Conclusions: Our results support the use of TLN-4601 for pancreatic cancer treatment and are consistent with a model where the anti-tumor activity of TLN-4601 is mediated, in part, through antagonism of RAS signaling. The mechanism of action of TLN-4601 is cell context-dependent and is associated with antagonism of multiple facets of Ras signal transduction.

580 POSTER

RhoA and RhoB inversly modulate estrogen receptor alpha expression and transcriptional activities in breast cancer cell lines

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Background: About two thirds of mammary tumors express estrogen receptor alpha (ERα) and hormonotherapy is then recommended. Nevertheless, there are systematically resistances to these treatments that impose the search for new pharmacological targets. Estrogens act mainly through the well-known ERα. However, cross-talks have been clearly demonstrated between ER and growth factors signalling pathways. Ras family proteins, such as Rho proteins, are key elements in those cross-talks. RhoA is frequently overexpressed in breast cancers and has been shown to down-regulate ER-mediated transcription. Our purpose was then to decipher the effect of Rho proteins inhibition on ERα expression and transcriptional activities.
Material and Methods: We specifically abolished the expression of either RhoA or RhoB proteins using two independent sequences of interfering RNA for each protein in MCF-7, MELN, T47D, ZR75 cells (hormonodependent breast cancer cell lines) and in LCC2 and LCC9 cells (hormonoresistant breast cancer cell lines). We then studied the impact of RhoA and RhoB inhibition on the one hand on ER target gene expression (by RTq-PCR or by a luciferase assay) and on the other hand, on ERα expression in cell model. Finally, we analyzed ER expression in RhoB knock out mice.
Results: We first showed in MCF-7 cells that RhoA inhibition increases both the expression of a luciferase reporter gene controlled by the vitellogenine Estrogen Responsive Element and the Progesteron Receptor (PR) mRNA. The inhibition of RhoA also increases ERα expression both at the mRNA and proteins levels. On the contrary, the inhibition of RhoB decreases the expression of the luciferase reporter gene controlled by the vitellogenine ERE and PR mRNA in MCF-7. Besides, RhoB inhibition decreases ERα expression in MCF-7, TR47D, ZR75, LCC2 and LCC9 cells. We also confirmed this result in Mouse Embryonnic Fibroblasts (MEFs) from RhoB knock-out mouse.
Conclusion: In brief, our results evidence RhoA and RhoB participation in the balance of expression of ER and in the individual modulation of the expression of various target genes. Further investigations, especially experiments in hormonoresistant cells, are now necessary for a better understanding of hormonoresistance.

581 POSTER

Inhibition of protein kinase C as the molecular basis of the synergism between safingol and irinotecan in colon cancer treatment

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Background: Safingol is a synthetic sphinganine which has been developed as a protein kinase C (PKC) inhibitor, and is currently evaluated in Phase I clinical trials. As PKC has been found in elevated levels in colon cancer cells, the aim of this study was to investigate the effects of safingol on colon cancer cell viability and its potential to enhance the cytotoxic effect of irinotecan for colon cancer therapy.
Materials and Methods: The anti-cancer effects of safingol as single agent or in combination with irinotecan in HT-29 and LS-174T colon cancer cells were determined using MTT assay. The combination index (C.I.), based on the median effect principle by Chou and Talalay, was computed to determine drug synergism. Treated cells were stained with annexin-V/7AAD to determine the extent of apoptosis. The expression levels of phosphorylated PKC and its downstream substrate, MARCKS, were determined using Western blot.
Results: As a single agent, safingol reduced colon cancer cell viability in a concentration-dependent manner, with IC_{50} values of $2.5\pm1.1\,\mu M$ and $3.4\pm1.0\,\mu M$ in HT-29 and LS-174T, respectively. Over 50% of treated HT-29 cells underwent apoptosis after a 48-h exposure to $10\,\mu M$ safingol. Interestingly, 24.9% of treated cells were annexin-V^- but $7AAD^+$, suggesting the possibility of necrosis or other death mechanisms. Further studies with the pan-caspase inhibitor, Z-VAD-FMK, indicated that cell death was not prevented in safingol-treated cells, indicating that safingol exerted its cytotoxicity via a caspase-independent mechanism. A 1:1 (mol/mol) combination of safingol/irinotecan was synergistic in both HT-29 and LS-174T, with C.I. values <1.0. This combination enabled significant dose reduction of irinotecan, with 4-fold and 250-fold reduction in HT-29 and LS-174T, respectively. Although safingol was developed as a PKC inhibitor, no decrease was observed in the expression of p-PKC or the downstream substrate p-MARCKS with $10\,\mu M$ safingol. However, treatment with safingol/irinotecan combination was associated with decreased expression of p-PKC and p-MARCKS, suggesting a possible molecular basis for the observed synergistic effect.
Conclusions: Our results show that inhibition of PKC by safingol/irinotecan combination could be a potentially effective strategy for colon cancer treatment. Future *in vivo* studies are warranted to further explore the therapeutic potential of this drug combination.

582 POSTER

An acquired point mutation in MEK2 causes resistance to allosteric MEK inhibitors

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Mutations in the ATP binding site are emerging as a common acquired resistance mechanism for ATP competitive kinase inhibitors, however potential resistance mechanisms for allosteric kinase inhibitors are poorly understood. We studied drug resistance mechanisms for an allosteric MEK inhibitor by generating a drug-resistant cell line in vitro. Exposure of the k-ras mutant colon cell line HCT116 (gIC50=2 nM) to increasing concentrations of the MEK inhibitor GSK1120212 led to isolation of a drug resistant population capable of growing under high concentration ($1\,\mu M$) of drug (gIC50 > $7\,\mu M$). The drug resistant population was also resistant to other allosteric MEK inhibitors while remaining sensitive to inhibitors of other targets (KSP and PI3K). Clones were isolated and several MAPK pathway related genes were sequenced. A single point mutation in MEK2 resulting in the amino acid change L119P was identified. MEK2-L119 is located within the allosteric binding site for GSK1120212 as well as other reported MEK inhibitors (PD0325901 and AZD6244). siRNA to MEK2 reduced levels of MEK2-L119P but not MEK1 and re-sensitized these cells to GSK1120212. The homologous MEK1 L115P mutant construct was engineered to test whether it could similarly confer resistance to GSK1120212. Exogenous expression of the MEK1-L115P mutant but not MEK1-wt was demonstrated to confer drug-resistance to tumor cell lines sensitive to GSK1120212. Finally, HCT116 (MEK2-L119P) formed tumors in mice that were relatively resistant to GSK1120212 compared to wild type HCT116 tumors. These data demonstrate that resistance to MEK inhibitors including GSK1120212 can be caused by a mutation of MEK2-L119P or MEK1-L115P permitting phosphorylation of ERK in presence of drug. To date, these mutations or polymorphisms have not been identified in clinical tumor samples.

583 POSTER

Analysis of MAP kinase signalling pathway in KIT & PDGFRA wild-type GISTs

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Background: Gastrointestinal stromal tumours (GISTs) are commonly driven by oncogenic mutations in KIT and PDGFRA genes, which are important molecular targets to specific kinase inhibitors, such as imatinib mesylate. However, 10−40% of GISTs patients are wild-type for KIT and PDGFRA genes. The prognostic significance of wild-type GISTs is controversial, and they rarely respond to imatinib mesylate. MAPK pathway is implicated in some tumor types through alterations in RAS, RAF or RKIP (Raf Kinase Inhibitor protein) molecules. Few studies have investigated the

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.

585 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

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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.

586 POSTER

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