For MRS analyses, cells were extracted using a dual phase method and ^{31}P and ^{13}C spectra of the aqueous fractions recorded on a 500 MHz Bruker spectrometer. Metabolite concentrations were normalised relative to cell number and internal standards.
Results: Inhibition with CI-1040 for 24h caused a marked decline in levels of P-ERK1/2 that was visible at 0.2 μM, 0.5 μM and 1 μM as detected by Western blotting. Cyclin D1 levels also decreased at 0.2 μM CI-1040 and were reduced further as the drug concentration increased. ^{31}P MRS analysis showed that this treatment was associated with a dose-dependent reduction in PC levels to 85±6% ($p = 0.07$), 57±8% ($p = 0.02$) and 65±7% ($p = 0.03$) of controls at 0.2 μM, 0.5 μM and 1 μM CI-1040 respectively. Time course analysis with 1 μM CI-1040 showed that the reduction in P-ERK1/2 levels seen at 24h was also present at 3h, 6h and 16h. ^{31}P MRS showed that PC levels remained unchanged at 3h (108±10%, $n = 2$) and 6h (99±10%; $n = 3$, $p = 0.9$) but decreased later at 16h reaching 64±7% of control ($n = 2$). ^{13}C MRS analysis of extracts from cells incubated in ^{13}C-choline showed that the levels of ^{13}C-labelled PC formed from choline decreased to 64±7% following exposure to 1 μM CI-1040 ($n = 3$, $p = 0.02$) suggesting reduction of de novo PC synthesis via inhibition of choline transport and/or phosphorylation.
Conclusions: Our results show that inhibition with CI-1040 in human melanoma cells is associated with a time- and concentration-dependent reduction in PC levels that results from decreased choline transport and/or phosphorylation. Thus, PC could have potential as a biomarker for monitoring the action of MEK inhibitors in melanomas during clinical trials.
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References

[1] M Beloueche-Babari et al. Cancer Res 2005; 65(8): 3356.

563 POSTER

In vitro activity of the multi-targeted kinase inhibitor sorafenib (BAY43–9006) against gastrointestinal stromal tumor (GIST) mutants refractory to imatinib mesylate

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Although most GIST patients show good response to imatinib, many of them develop resistance during further treatment. The development of resistance to small molecule kinase inhibitors has emerged an important problem for targeted therapy in cancer. Acquired resistance commonly occurs via secondary gene mutation in the KIT kinase domain. Sorafenib was initially identified as a potent RAF and VEGFR inhibitor and was subsequently shown to also inhibit the related receptor tyrosine kinases FLT3, KIT and PDGFR. Sorafenib was recently approved for the treatment of advanced renal cell carcinoma.
We tested the ability of sorafenib to inhibit imatinib-resistant mutants. Primary imatinib resistant tumors cells and/or murine Ba/F3 cells, expressing imatinib-resistant KIT-V654A, KIT-T670I or PDGFRA-D842 mutations were evaluated for sensitivity to sorafenib by Western blotting and proliferation assays. Sorafenib inhibited the KIT kinase activity of V654A and T670I mutants as measured by Western blots at concentrations ranging from 1 to 5 μM. Sorafenib also suppressed proliferation of the cells expressing these mutations. In contrast, sorafenib did not inhibit the PDGFRA-D842V mutant. Clinical studies with sorafenib have shown that serum concentrations up to 13.3 μM could be safely achieved in patients receiving the standard dose of 400 mg twice daily. Therefore, our findings suggest that sorafenib can be an efficient therapy for patients with GIST that carry the acquired KIT-V654A or KIT-T670I mutations.
In conclusion, our in *vitro and ex vivo* findings indicate that sorafenib has good inhibitory activity against the V654A and T670I mutations in KIT that confer resistance to imatinib, in contrast to PDGFRA-D842V that confers resistance to both agents.

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Small molecule inhibitor BMS-536924 completely reverses IGF-IR-mediated transformation of immortalized mammary epithelial cells

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Type I insulin-like growth factor receptor (IGF-IR) is overexpressed in a number of cancers and contributes to tumor invasion and metastasis. We have previously reported that a dominant active IGF-IR (CD8-IGF-IR) causes rapid mammary tumorigenesis when overexpressed in the mouse mammary gland. To elucidate the molecular mechanisms of tumor formation, we stably overexpressed CD8-IGF-IR in immortalized, but non-transformed, mammary epithelial cells (MCF-10A). MCF-10A-CD8-IGF-IR cells showed constitutive IGF-IR phosphorylation in the absence of any IGF stimulation. MCF-10A-CD8-IGF-IR showed numerous features of transformation including growth in the absence of serum, lack of contact inhibition in monolayer and foci formation, anchorage-independent growth in soft-agar, and invasion through matrigel. Interestingly, MCF-10A-CD8-IGF-IR cells were also able to grow as xenografts in immunocomprimised mice (when injected with or without matrigel) an uncommon feature following transformation of MCF-10A cells with a single oncogene. BMS-536924 was effective at blocking both IGF-I stimulated wild-type IGF-IR and also CD8-IGF-IR activity. Inhibition was observed at 10–100 nM and was maximal at 1 microM, a concentration which didn't affect epidermal growth factor (EGF)-mediated activation of EGFR signaling. Monolayer growth assays showed that BMS 536924 induced a dose dependent inhibition of proliferation with an IC_{50} of 0.4–0.8 μM, whereas the IC_{50} in anchorage independent growth was nearly a log-fold lower. Flow cytometry indicated that BMS-536924 caused a G0/G1 block in the cell cycle. BMS-536924 was also able to completely reverse the CD8-IGF-IR induced invasion. Finally BMS-536924 at 100 mg/kg/day caused a 70% reduction in MCF-10A-CD8-IGF-IR xenograft volume. These results demonstrate that the new small molecule, BMS 536924 is an effective inhibitor of IGF-IR, causing complete reversion of an IGF-IR-mediated transformed phenotype *in vitro* and blocking growth *in vivo*.

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Growth-inhibitory and anti-angiogenic effects of the novel MEK inhibitor PD0325901 in preclinical models of human malignant melanoma

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The Raf/MEK/ERK signaling module is a central mediator of tumor cell proliferation, survival, and angiogenesis. *BRAF* mutations may sensitize cancer cells to the growth-inhibitory effects of small-molecule MEK inhibitors; we therefore tested the effects of PD0325901, a novel MEK inhibitor, in a panel of human melanoma cell lines, with or without *BRAF* mutations. Cells were exposed to increasing concentrations of PD0325901 and analyzed for ERK phosphorylation, cell growth/proliferation, and apoptosis. VEGF and IL-8 production were also assessed, under normoxic and hypoxic conditions. PD0325901 strongly inhibited ERK phosphorylation in a dose- and time-dependent manner; inhibition was already evident at 1 nM and almost complete at ⩾10 nM, was detectable after 15 min, and persisted for at least 48h. PD0325901 potently inhibited cell growth (IC_{50}: 10−40 nM) in human melanoma cells harboring either mutant (M14, A375P, ME10538, ME4686) or wild-type (ME4405, ME13923) *BRAF*; the wild-type *BRAF* cell lines ME1007 and ME8959, conversely, proved relatively resistant ($IC_{50} > 100$ nM). Cell growth inhibition was due to inhibition of cell cycle progression, with depletion of S-phase cells and accumulation in G_0/G_1, and subsequent induction of apoptosis, both of which were further enhanced by decreasing the concentration of serum in the culture medium. We also investigated the anti-angiogenic potential of PD0325901 in the mutant *BRAF* cell line M14; in this model, PD0325901 significantly decreased VEGF protein secretion under both normoxic and hypoxic conditions. Inhibition of VEGF production took place at the transcriptional level, as demonstrated by the PD0325901-induced, dose-dependent decrease of HIF-1a protein expression and transcriptional activity. In addition, PD0325901 also strikingly decreased the production