maximum tolerated dose in mice resulted in significant tumor regression. Erlotinib is also active in these tumors, which is consistent with clinical findings. In contrast, engineered lung tumors driven by Kras do not exhibit regression even at much higher dosing levels. More importantly, AV-412 is active against engineered tumors driven by the drug resistant mutant $EGFR^{L858R\&T790M}$ derived by *in vivo* functional complementation. In addition, AV-412 is also active against chimeric breast tumors driven by HER2.
Conclusion: These results provide direct evidence in a genetically defined system for determining the specific genetic context of AV-412 response, which in turn provide insights for AV-412 clinical activity and is strongly suggestive of the utility of the platform for human drug response prediction.

560 POSTER

Inhibition of erbB1/2 by small molecule tyrosine kinase inhibitors, but not trastuzumab, affects metabolic pathways: implications to cardiac toxicity

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Background: Therapies targeting ErbB2 represent an attractive strategy in breast cancer. Trastuzumab, an anti-ErbB2 monoclonal antibody, is an approved treatment for patients with ErbB2-overexpressing breast cancers. Tykerb is a potent, reversible inhibitor of ErbB2 and ErbB1 tyrosine kinase (TKI) and is currently in Phase III clinical trials in breast and other carcinomas. The principal adverse event attributable to trastuzumab is cardiac toxicity. This study was conducted to elucidate mechanisms that affect metabolic pathways by a TKI and an antibody directed to ErbB2 and their effects on breast cancer cells, primary human adipocytes and cardiomyocytes.
Material and Methods: Western blotting was used for pAkt, pErk1/2, pAMPKα and peEF2 (Cell Signaling, Beverly, MA); ERRα, ERRγ (R&D Systems Minneapolis, MN); PGC-1 (Chemicon International, Temecula, CA); MCAD (Cayman Chemicals, Ann Arbor, MI) and Actin (Sigma, St. Louis, MO). Lipid staining: cells were fixed in NBF and stained with Oil Red O (Sigma). Cells: AU565, breast cancer cells, primary cardiomyocytes, and adipocytes were grown in RPMI supplemented with 15% BFS and treated with BAPTA/AM (Calbiochem): 5–30 μM; GW-2974 (Sigma): 1–25 μM; trastuzumab (Genentech): 5–50 mg/ml; Heregulin (LabVision, Fremont, CA): 5–100 ng/ml.
Results: Our results show that treatment with GW-2974 (or with Tykerb) directed to ErbB1/2 alter fatty acid metabolic pathways through activation of adenosine monophosphate kinase (AMPK), a key regulator in mitochondrial energy producing pathways in human cardiac cells, adipocytes and breast cancer cells. The changes include phosphorylation of AMPK and eEF2, upregulation of ERRα and PGC-1, activators of fatty acid oxidation, in cardiomyocytes, and downregulation of lipid expression in human cardiomyocytes, adipocytes, breast cancer cells, as well as downregulation of fatty acid synthase (FAS), increased lipid oxidation and changes in ion and calcium channels. The metabolic changes were reversed by calcium chelation. Trastuzumab, which downregulated FAS and changed ion channels, failed to activate AMPK, but downregulated survival pathways such as AKT and Heregulin.
Conclusions: Our results show that treatment using TKI to inhibit ErbB1/ErbB2 pathways in breast cancer cells, in human cardiomyocytes, and adipocytes results in activation of AMPK through changes in calcium channels. AMPK regulates cellular energy homeostasis. Activation of AMPK after stress is associated with protection of cells against injury such as ischemia and nutrient depletion, thereby helping to preserve the levels of cellular ATP. Thus, activation of AMPK will likely provide protection against cellular damage by ErbB targeted therapy alone or when given in combination with chemotherapy. The failure of trastuzumab to activate AMPK together with downregulation of survival pathways may point to the source of its cardiac toxicity.

561 POSTER

Discovery of a novel FAK inhibitor, NVP-TAE226, and its activities on in vivo and in vitro models

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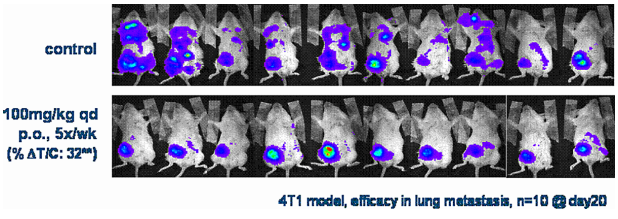
Background: Focal Adhesion Kinase (FAK) is an attractive anti-cancer drug targets because FAK is a key molecule of tumor cell proliferation, migration, and survival. FAK is generally overexpressed in various types of tumor cells and is closely correlated with invasive potential. FAK levels are greatest in highly metastatic tumors. Activation of integrins and the growth factor receptors result in FAK autophosphorylation at Y397 and the presentation of suitable binding sites for proteins containing either SH2 or phosphotyrosine binding domains. Recent evidences indicate that FAK plays important roles in cancer cell proliferation and survival. A selective FAK inhibitor would be expected to halt or kill invasive tumor cells, and potentially interfere with normal cell migration (e.g. endothelial cells).
Methods: We have discovered NVP-TAE226, a novel small molecule inhibitor of FAK. The compound was evaluated in kinase enzymatic assays, cell-based kinase assays and *in vivo* models. Anti-metastasis effect was evaluated by applying *in vivo* imaging. All procedures in this study were in compliance with the regulations of Animal Welfare Committee in Novartis Institutes for BioMedical Research Tsukuba.
Results: NVP-TAE226 inhibits FAK with low nanomolar IC50 values in a purified kinase enzymatic assay. In cell-based kinase assays, FAK was inhibited with an IC50 range of 100 to 300 nM compared to the other kinases tested which were >10-fold less sensitive. Oral administration of NVP-TAE226 showed potent inhibition of orthotopic tumor growth and spontaneous metastasis in a dose-dependent manner. The compound was well tolerated in mice in terms of body weight changes. Inhibition of FAK autophosphorylation at Y397 and Akt phosphorylation at Serine473 was observed in a dose-dependent manner in 4T1 breast carcinoma.



Conclusion: NVP-TAE226 represents a novel class of selective and small molecule kinase inhibitors that have potential clinical applications with a potent *in vivo* activity.

562 POSTER

Inhibition of MEK1/2 signalling with CI-1040 in human melanoma cells leads to alterations in phosphocholine metabolism

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Background: RAS-RAF-MEK-ERK (or MAPK) signaling is deregulated in many cancers, especially melanomas, and inhibitors of this pathway are now in clinical trials. Detecting biomarkers of MAPK signaling inhibition could facilitate the clinical evaluation of this novel therapy. Using magnetic resonance spectroscopy (MRS), we have previously shown that treatment with the early prototype MEK inhibitor U0126 correlated with a drop in phosphocholine (PC) levels in human breast and colon cancer cells (1). Here we investigate: a) whether inhibition with the MEK1/2 selective inhibitor CI-1040 in human melanoma cells could trigger similar metabolic effects as U0126; and (b) the mechanistic basis for any observed changes.
Materials and Methods: WM266.4 human malignant melanoma cells were treated with 0.2 μM, 0.5 μM or 1 μM CI-1040 for 24h or with 1 μM CI-1040 for 3h, 6h, 16h and 24h. For mechanistic studies, cells were also treated with 1 μM CI-1040 for 24h followed by a 3h incubation in fresh medium containing 1 μM CI-1040 and 100% 100 μM [1, 2]-^{13}C-choline. Inhibitor action was verified by Western blotting for P-ERK1/2 and cyclin D1 levels.

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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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.

564 POSTER

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*.

565 POSTER

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