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

S.S. Bacus[1], P. Trusk[1], L. Lyass[1], J.E. Hill[1], N.L. Spector[2]. [1]*Targeted Molecular Diagnostics, Westmont, USA;* [2]*GlaxoSmithKline, Research Triangle Park, USA*

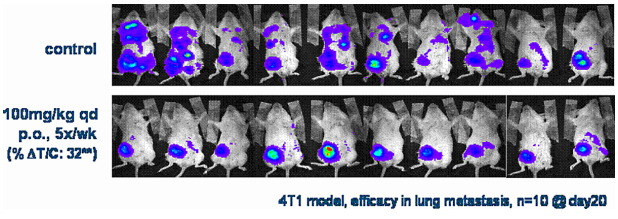
Background: Therapies targeting ErbB2 represent an attractive strategy in breast cancer. Trastuzumab, an anti-ErbB2 monoclonal antibody, is an approved treatment for patients with ErbB2-overexpressing breast cancers. Tykerb is a potent, reversible inhibitor of ErbB2 and ErbB1 tyrosine kinase (TKI) and 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

E. Kawahara[1], T. Yamaura[2], O. Ohmori[1], K. Nonomura[2], Y. Murakami[2], S. Niwa[3], T. Meyer[4], J. Mestan[4], T. Honda[2], S. Hatakeyama[2]. [1]*Novartis Institutes for BioMedical Research, Grobal Discovery Chemistry, Tsukuba Ibaraki, Japan;* [2]*Novartis Institutes for BioMedical Research, Discovery Biology, Tsukuba Ibaraki, Japan;* [3]*Novartis Institutes for BioMedical Research, Autoimmunity & Transplantation, Vienna, Austria;* [4]*Novartis Institutes for BioMedical Research, Expertise Platform Kinase, Basel, Switzerland*

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

M. Beloueche-Babari[1], L.E. Jackson[1], P. Workman[2], M.O. Leach[1]. [1]*Cancer Research UK Clinical Magnetic Resonance Research Group, Institute of Cancer Research, Surrey, United Kingdom;* [2]*Cancer Research UK Centre for Cancer Therapeutics, Institute of Cancer Research, Surrey, United Kingdom*

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.