to generate blood concentration plots. TEMSR C_{max} in BrCA was 53% higher but transient while SIR C_{max} decreased 11.9% compared with other subjects. SIR C_{trough} at 168 hr was unchanged. Effect of nonwhite race on C_{max} in RCC was less than effect for BrCA. No other covariates affected TEMSR or SIR disposition.
Conclusions: Saturable distribution model for TEMSR adequately predicted concentrations through wide (1–250 mg) dosing range. Collectively, data suggest no PK basis for modifying TEMSR dose in pts with RCC receiving 25 mg IV.

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B-RAF mutation is associated with altered patterns of negative feedback of MAPK signaling that correlate with increased output of the pathway and increased sensitivity to MEK inhibition

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Activating mutations of B-RAF occur in melanomas and other human tumors and correlate with sensitivity to MEK inhibitors. Pharmacologic MEK/MAPK inhibition results in reduction of D cyclins, hypophosphorylation of the RB protein and G1 cell cycle arrest in ^{V600E}B-RAF tumor cells, but not in tumor cells in which MAPK activation is driven by mutant or amplified receptor tyrosine kinases (RTKs). Activation of MAPK signaling causes negative feedback regulation of the pathway. We find that tumor cells driven by RTKs have high levels of P-ERK but almost undetectable P-MEK, suggesting feedback inhibition of the pathway upstream of MEK. MEK inhibitors relieve this feedback and cause rapid induction of MEK phosphorylation. In contrast, this feedback is absent in B-RAF mutant cells, in which P-MEK is high and declines after MEK inhibition. These data suggest the possibility of a compensatory increase in feedback downstream of MEK in B-RAF mutant cells. Our data demonstrate that, compared to cells with active RTKs, B-RAF mutant cells have significantly higher expression levels of at least ten critical MEK/MAPK-dependent mRNAs, including *DUSP* and *SPRY* family members, which encode proteins involved in feedback regulation of MAPK signaling, as well as transcription factors previously shown to be downstream effectors of RAS–MAPK signaling (*ETS*, *FOS*). In mutant B-RAF compared to activated RTK tumor cells, we find ten-fold greater expression of mRNA for DUSP6, which encodes a MAPK phosphatase (MKP) which dephosphorylates ERK1/ERK2. Taken together, the increased P-MEK and increased expression of ERK transcriptional targets suggest elevated output of the MAPK signaling pathway in B-RAF mutant compared to RTK-driven tumor cells. The increased output leads to increased MKP levels and increased feedback at the level of MAPK in B-RAF mutant tumors, resulting in levels of P-ERK that are comparable to those found in RTK driven tumors. The implications of this model are that: 1) P-ERK level is a poor measure of pathway output and a poor indicator of tumor cell sensitivity to MEK inhibitors; levels of DUSP6 and other transcriptional targets of MAPK may be better predictors of sensitivity to these compounds. 2) Increased output of the pathway may be due to the insusceptibility of B-RAF mutants to upstream feedback, resulting in the transcription of both downstream feedback and effector proteins, which together are responsible for the transformed phenotype.

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The mTOR effector p70 S6 kinase 1 (S6K1): a specific biomarker for the biological effects of the dual HER1/HER2 kinase inhibitor Lapatinib (GW572016) in HER2-overexpressing breast cancer cells

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Background: Regardless of the effects of the mono-HER1 inhibitor gefitinib, the mono-HER2 inhibitor trastuzumab or the dual-HER1/HER2 inhibitor lapatinib on the activation status of HER1 and/or HER2, it is the repercussions of HER inhibitors on downstream signaling pathways that correlate with tumor growth inhibition. Identification of these pathways and whether they are operative or not in the presence of HER inhibitors may enable individual therapeutic decisions to be based on tumor biology rather than histology alone.
Materials and Methods: The purpose of this study was to molecularly profile the effects of trastuzumab and lapatinib on the intracellular oncogenic kinase signaling using paired control- and HER2-transfected breast cancer cells (MCF-7 and MCF-7/Her2−18 clone, respectively). To simultaneously analyze the activation status of all three major families of Mitogen-Activated Protein Kinases (MAPKs), the Extracellular Signal-Regulated Kinases (ERK1/2), c-Jun N-terminal Kinases (JNK 1–3), and different p38 isoforms (/), and other intracellular kinases, such as AKT, GSK-3, RSK1/2, MSK1/2, HSP27 and p70 S6 kinase 1 (p70S6K1), we took advantage of the recently developed Human Phospho-MAPK Array (Proteome Profiler^{TM}), a semi-quantitative protein array technology allowing the parallel screening of the relative levels of phosphorylation of multiple intracellular kinases.
Results: Treatment with either trastuzumab or lapatinib identically affected the HER2-regulated activation status of the MAPKs ERK1/2, JNK 1–3, and p38 and of the serine/threonine kinases AKT, GSK-3, RSK1/2, MSK1/2 and HSP27. Interestingly, trastuzumab failed to deactivate p70S6K1 in MCF-7/Her2−18 cells, whereas lapatinib drastically inhibited HER2-enhanced p70S6K1 activation to levels even lower than those observed in MCF-7 control cells, which constitutively exhibit high levels of phospho-p70S6K1 as they naturally bear a genomic amplification of the p70S6K1 gene on chromosome 17q23.
Conclusions: Considering that elevated levels of p70S6K1 have been associated with clinical response to lapatinib while linked to resistance to trastuzumab in patients with metastatic cancers overexpressing HER2 and/or expressing HER1, our current findings strongly suggest that the serine-threonine kinase p70S6K1, a marker for mTOR activity that regulates protein translation, should be considered a specific biomarker for the biological effects of lapatinib in HER2-overexpressing breast cancer.

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Small-molecule inhibitors of HSP90 in IM-resistant gastrointestinal stromal tumors

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Background: Inhibition of the KIT-oncoprotein by imatinib-mesylate (IM) induces clinical responses in the majority of patients with gastrointestinal stromal tumors (GIST). However, most patients develop resistance to IM due to seconday mutations of KIT and the lack of potent salvage therapies represents a major clinical challenge. Oncogenic KIT has recently been shown to be protected from proteasomal degradation by HSP90. Inhibitors of HSP90 may therefore be a promising class of inhibitors in the treatment of IM-resistant GIST. However, geldanamycin-based HSP90 inhibitors show unfavourable pharmacological properties (e.g. solubility, substrates for multi-drug-resistance proteins).
Material and Methods: We therefore characterized the effects of novel, orally-available small molecule inhibitors of HSP90 in GIST in vitro. Antiproliferative effects were screened in IM-resistant GIST cell lines using luminescence-based proliferation assays. Effects on KIT signaling pathways were analysed by western blotting.
Results: HSP90 inhibitors (EC89, EC82, EC137, EC138, EC141, EC144 and EC151) showed strong antiproliferative effects in IM-resistant GIST48 (IC50's 22 nM to 220 nM) comparing well with the effects seen with 17-AAG (IC50: 65 nM). Upon treatment with EC82 and EC141 (6h) complete inhibition of KIT-phosphorylation was observed at doses of 250 nM (EC82, IC50: 82 nM) and 50 nM (EC141, EC50: 20 nM). IC50's for KIT degradation were 170 nM for EC82 and 31 nM for EC141. Complete inhibition (>95%) of AKT and partial inhibition of MAPK (75 and 85%) phosphorylation was observed at equal doses as seen for pKIT. Little antiproliferative effects and no inhibitory effects on pAKT (1.5-fold increase) and pMAPK (6-fold increase) were seen in KIT-negative IM-resistant cell line GIST62.
Conclusions: Novel, orally-available small molecule inhibitors of HSP90 exhibit potent antiproliferative effects in IM-resistant, KIT-positive GIST. These effects are mainly caused by inhibition and degradation of KIT and subsequent inhibition of oncogenic KIT-signaling pathways. Thus, these compounds may represent a promising alternative to ansamycin-based drugs in the development of therapeutic strategies targeting HSP90 in GIST.