

activation of ErbB3 and to sensitize platinum-resistant cells to subsequent chemotherapy.

Materials and Methods: Cisplatin-sensitive (A2780) and -resistant (A2780cis) ovarian cancer cell lines were acquired from ATCC. Primary ascites cells were isolated from advanced stage ovarian cancer patients who had undergone prior therapy. Cells were propagated *in vitro* and cell viability assays were performed in the presence of platinum-based drugs, taxanes, or MM-121. Heregulin stimulation and inhibition of signaling by MM-121 were evaluated by quantitative western blotting and reverse phase protein arrays. Drug sensitivity and signaling were assessed for A2780 and A2780cis using subcutaneous xenografts. Basal heregulin levels were measured by ELISA and mRNA-based methods.

Results: Consistent with our hypothesis that platinum resistance can be mediated by ligand-driven ErbB3 signaling, basal heregulin levels were increased in A2780cis cells relative to A2780 cells, and growth of A2780cis (but not A2780) xenografts was sensitive to MM-121. Interestingly, patient-derived ascites cells exhibited differential sensitivity to paclitaxel and platinum-based drugs, and cells collected serially from the same patient over the course of treatment provided evidence of acquired resistance. Ascites cells responded differently to heregulin stimulation and inhibition by MM-121, suggesting that only a subset of cells develop resistance through this mechanism. In most cases, cells that are responsive to heregulin are also responsive to MM-121, and MM-121 sensitizes some of these cells to paclitaxel treatment.

Conclusion: Platinum resistance is associated with increased heregulin expression or responsiveness to heregulin in cell lines and primary ascites cells. MM-121 could potentially play a role in overcoming platinum resistance or in sensitizing cells to subsequent chemotherapy. As only a subset of cells use this mechanism, a biomarker-based strategy to identify patients could be useful. A Phase 2 randomized open label study of MM-121 in combination with paclitaxel versus paclitaxel alone is currently enrolling patients with advanced ovarian cancer.

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POSTER

TAS-2913 is a Mutant Selective EGFR Inhibitor for NSCLC: Characterization Against EGFR T790M in Cell and Xenograft Models

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Background: The activation of epidermal growth factor receptor (EGFR) pathway with active mutation, L858R or deletion in exon19 results in initiation of cancer proliferation, increased metastasis potential, and neoangiogenesis. Small molecule inhibitors of EGFR kinase, have been found effective NSCLC patients harboring activating mutations. Over time, most tumors develop resistance to EGFR inhibitor by the development of drug-resistant mutations including gatekeeper T790M mutation (more than 50% cases) or C-MET amplification.

TAS-2913 is a potent and unique kinase inhibitor that targets the mutant forms of EGFR by inhibiting the common activating mutations (L858R, del exon 19) and the gatekeeper mutation (T790M) while sparing EGFR wild type signaling.

Methods: *In vitro* enzyme inhibition activity of TAS-2913 for EGFR and its mutants was determined by using TR-FRET assay. For growth inhibition assay, cells were treated with TAS-2913 for 3 days, and living cells were determined by using CellTiter Glo™ which measures cellular ATP. Antitumor activity of TAS-2913 as a single agent was assessed in NSCLC xenograft models harboring different EGFR status. To test whether TAS-2913 inhibits phosphorylation of EGFR with T790M mutation in tumor tissues, we examined pharmacodynamic analysis in H1975 (T790M/L858R), HCC827(del exon 19) and NUGC3(Wt) xenograft model.

Results: In biochemical assays, TAS-2913 inhibits EGFR mutants in sub-nano molar range with more than 10-fold selectivity compared with wild type EGFR. TAS-2913 caused significant tumor growth inhibition in the H1975 (T790M/L858R) xenograft model. Partial tumor regression was observed at over 100 mg/kg, whereas it did not cause body weight loss. TAS-2913 at doses of 100 mg/kg completely inhibited auto-phosphorylation of EGFR and its downstream signal pathways in H1975 and HCC827 but not in NUGC3 xenograft models.

Conclusion: These results suggest that TAS-2913, a potent mutant-selective EGFR inhibitor, may represent a new therapeutic strategy in the treatment of NSCLC resistant to EGFR/TKI.

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POSTER

MicroRNA-224 and -375 in Trastuzumab and Lapatinib Acquired and Innate Resistant HER2 Positive Breast Cancer Cells

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Background: HER2 positive breast cancer accounts for approximately 25% of all breast cancer cases. Trastuzumab (T), a humanised monoclonal antibody, and lapatinib, a small molecule tyrosine kinase inhibitor are approved established treatments for HER2 positive breast cancer; however, patients that initially respond frequently develop resistance.

Aim: The aim of this study is to investigate microRNAs in cell line models of acquired trastuzumab and lapatinib resistance.

Methods: MicroRNA was extracted from the HER2 positive cells; SKBR3 and BT474 and the acquired trastuzumab and lapatinib resistant variants SKBR3-T, SKBR3-L and BT474-Tr, in triplicate. MicroRNA profiling was performed on SKBR3, SKBR3-T and SKBR3-L using Taqman Low Density Arrays (TLDA). Differentially regulated miRNAs were selected using >2-fold change and a P-value of <0.05. Individual quantitative RT-PCR (qRT-PCR) was performed to confirm alterations in miRNAs. Further validation was carried out on other models of acquired resistance, BT474-T, HCC-1954-L and EFM-192A-L compared to their parents. Functional studies were carried out using Ambion® Pre-miR miRNA Precursors and Anti-miR miRNA Inhibitors for miR-224 in SKBR3 cells.

Results: TLDA analysis identified nine and six differentially regulated microRNAs in the SKBR3-T and SKBR3-L cells, respectively. Individual qRT-PCR assays confirmed that miR-9 was 2.2-fold up-regulated ($p = 0.04$), while miR-224 was 1.6-fold down-regulated ($p = 0.01$) in SKBR3-T compared to the SKBR3 cells. In the SKBR3-L cells, miR-224 was 5.2-fold down-regulated ($p = 0.0002$) and miR-375 was 1.5-fold down-regulated ($p = 0.01$) compared to the SKBR3 cells. Further validation of these targets in the other models of acquired resistance showed that miR-224 expression is lost in the BT474-Tr compared to the BT474 cells however, is not significantly altered in the HCC-1954 L and follows the opposite trend in the EFM-192A-L. MiR-375 is significantly altered in the HCC-1954-L and EFM-192A-L cells. MiR-9 is not significantly altered in the BT474-T cells. Transfection of SKBR3 cells with pre-miR-224 significantly decreases cell proliferation by 23.5% ($p = 0.04$).

Conclusions: This is the first report of the involvement of microRNAs – miR-9, miR-224 and miR-375 in trastuzumab and lapatinib resistance in HER2 positive breast cancer.

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POSTER

Alterations in Apoptosis-related Genes in Cell Line Models of Acquired Lapatinib Resistance, Identifies Potential Therapeutic Targets for the Treatment of Lapatinib Resistant HER2-positive Breast Cancer

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Background: Acquired resistance to lapatinib in the treatment of HER2-positive breast cancer limits its clinical efficacy. We examined alterations in apoptosis in cell line models of acquired lapatinib resistance, in order to identify potential mechanisms to overcome acquired lapatinib resistance.

Materials and Methods: Three models of acquired lapatinib resistance were established by continuous exposure of the SKBR3, HCC1954 and EFM192A cells to 250nM, 1250nM and 1000nM lapatinib respectively for 6 months to establish the SKBR3-L, HCC1954-L and EFM192A-L cells. TUNEL assays and PARP cleavage were used to identify changes in apoptosis in the SKBR3 and SKBR3-L cells. Microarray analysis of the SKBR3 and SKBR3-L cells was performed to identify changes in the expression of apoptosis-related genes, which were subsequently confirmed by western blotting and qRT-PCR. The effects of TRAIL and obatoclax were determined in the lapatinib resistant cell lines using proliferation and TUNEL assays.

Results: We established three separate models of acquired lapatinib resistance with lapatinib IC₅₀ values greater than 1 μM. Using TUNEL assays and PARP cleavage we showed that SKBR3 cells undergo apoptosis in response to lapatinib whilst the SKBR3-L cells do not. While no significant difference in apoptosis induction was observed when the cells

were treated with a chemotherapy agent to activate the intrinsic pathway of apoptosis, the SKBR3-L cells displayed significantly increased sensitivity to activation of the extrinsic pathway of apoptosis using either TRAIL (12.5 ng/ml $p=0.008$) or TNF α (125 ng/ml $p=0.008$). The EFM192A-L cells also displayed enhanced sensitivity to TRAIL, whereas the HCC1954-L cells did not. Using microarray analysis, we identified alterations in expression of members of the BCL2 family. We observed increased expression of MCL-1 and BID, and decreased expression of BAX in the SKBR3-L cells. We also examined expression of transcription factors which regulate expression of BCL2 genes and TRAIL receptors. FOXO3a levels were increased in the SKBR3-L cells whereas c-JUN was reduced in the SKBR3-L cells. FOXO3a is regulated by Akt and we found that the increased sensitivity to TRAIL in the SKBR3-L and EFM192A-L cells was associated with decreased pAkt. In addition to the enhanced sensitivity to TRAIL, the three resistant cell lines also showed increased sensitivity to the BCL2 inhibitor obatoclax.

Conclusions: Acquired lapatinib resistance results in alterations of apoptosis-related proteins, which are associated with acquired sensitivity to obatoclax and TRAIL. Thus obatoclax and/or TRAIL may represent novel therapeutic strategies for lapatinib resistant HER2-positive breast cancer.

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POSTER

PP2A – a Novel Target for the Treatment of Lapatinib-resistant Breast Cancer

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Background: Mechanisms of acquired lapatinib resistance are poorly understood. We developed two novel cell line models of acquired lapatinib resistance and used proteomic profiling to identify proteins and pathways associated with resistance.

Methods: SKBR3 and HCC1954 cells were conditioned with 0.25 and 1 μ M lapatinib respectively for 6 months, resulting in lapatinib-resistant SKBR3-L and HCC1954-L cells. The phospho-proteome of lapatinib-sensitive and resistant SKBR3 cells was compared. Immunoblotting was performed for phospho- and total eEF2, eEF2k, S6k and mTOR. Analysis of mTOR, eEF2k and PP2A mediated regulation of p-eEF2 was performed using specific inhibitors (rapamycin, NH125 and okadaic acid). Levels of p-eEF2 were determined following 24 hours lapatinib treatment in a panel of 15 HER2+ve cell lines.

Results: SKBR3-L cells are resistant to lapatinib (IC_{50} of $6.5 \pm 0.4 \mu$ M versus 0.1 ± 0.01 in SKBR3 cells) and proteomic analysis of phospho-enriched samples identified eukaryotic elongation factor 2 (eEF2) as a phosphoprotein which was significantly lower in the SKBR3-L cells compared to SKBR3 cells. Examining p-eEF2 in SKBR3-L cells revealed that: (i) lapatinib increases the levels of p-eEF2 in SKBR3-par cells only; (ii) SKBR3-L cells do not display increased phosphorylation of mTOR or S6k, nor increased sensitivity to mTOR inhibition; (iii) SKBR3-L cells are significantly more sensitive than SKBR3 cells to PP2A inhibition ($p=0.007$) and (iv) PP2A inhibition restores p-eEF2 in SKBR3-L cells. In addition, HCC1954-L cells which are resistant to lapatinib (IC_{50} $2.67 \pm 0.08 \mu$ M versus $0.42 \pm 0.02 \mu$ M in HCC1954-par cells) also display reduced levels of p-eEF2 compared to parental cells, are more sensitive to PP2A inhibition and display increased p-eEF2 in response to PP2A inhibition. Using a panel of HER2+ve breast cancer cell lines (10 lapatinib-sensitive, 5 innate lapatinib-resistant) we also found that, following lapatinib treatment, there was a greater increase in p-eEF2 in lapatinib-sensitive compared to lapatinib-resistant cell lines ($p=0.01$).

Conclusion: Increased sensitivity to okadaic acid, in two novel models of lapatinib resistance, suggests PP2A may be a rational target for the treatment of lapatinib-resistant breast cancer. In addition, a significant correlation between lapatinib sensitivity and increased p-eEF2 and suggests that p-eEF2 may be a potential pharmacodynamic biomarker of lapatinib response.

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POSTER

Identification of Tyrosine Kinase Inhibitors as Modulators of OCT2 Function

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Background: Recent studies have highlighted the importance of OCT2-mediated transport of various substrates, including oxaliplatin, metformin,

and ifosfamide, as a key mechanism of drug elimination and an initiating event in organ-specific side effects. We hypothesized that identification of specific OCT2 inhibitors could be beneficial in decreasing adverse events associated with this transporter while maintaining antitumor efficacy.

Materials and Methods: A high-throughput screen for OCT2 inhibitors was performed using the SJCRH bioactive compound library, consisting of FDA-approved drugs and other chemicals with known biological activity. The test compounds (28 μ M) were assessed initially for their ability to inhibit OCT2-mediated transport of 5.6 μ M ASP (4-[4-(dimethylamino)styryl]-N-methylpyridinium-iodide) using transfected HEK293 cells on an integrated screening system including an automated fluorescence plate reader. All compounds with more than 90% inhibitory activity were further examined in a dose-response analysis in transfected HEK293 and HeLa cells. In addition, select chemicals were tested for their ability to inhibit TEA (¹⁴C-tetraethylammonium) and oxaliplatin (2 μ M) transport by OCT2 and the orthologue mouse transporter, mOCT2 in transfected HEK293 cells. Imipramine (140 μ M) was used as a positive control inhibitor.

Results: Among 8086 bioactive compounds tested, 934 molecules (11%) decreased OCT2-dependent uptake of ASP in HEK293 cells by >80%, and 352 molecules (4.4%) decreased uptake by >90%. Interestingly, various tyrosine kinase inhibitors (TKIs) evaluated in the screen were identified as potent, sub-micromolar inhibitors of OCT2-mediated uptake of ASP, including vandetanib, dasatinib, and crizotinib (IC_{50} <0.020 μ M). Additionally, all TKIs that are currently FDA-approved decreased OCT2-mediated uptake of TEA by >80% ($P < 0.0001$). Dasatinib also completely blocked uptake of oxaliplatin in HEK293 cells transfected with OCT2 or mOCT2 ($P < 0.0001$; IC_{50} <0.1 μ M) by a non-competitive inhibitory mechanism.

Conclusions: These findings indicate that multiple, chemically unrelated TKIs, including dasatinib, can potentially inhibit OCT2 function at clinically achievable concentrations. We are currently exploring the *in vivo* potential of dasatinib as a modulator of chemotherapy toxicities associated with OCT2 transport.

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POSTER

Targeting Mechanism of Cell Fusion as a Novel Approach to Abrogate Multi-Drug Resistance of Metastatic Colon Cancer

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We recently discovered a mechanism by which metastatic colon cancer cells become resistant to different cytotoxic agents such as 5-fluorouracil, oxaliplatin, irinotecan, *in vitro* and *in vivo*. The mechanism is based on cell-to-cell fusion. Fusion between tumor cells with distinct phenotypes can generate cells with new properties at a rate exceeding that achievable by random mutagenesis thereby allowing a better adaptation to survive to the therapeutic treatments. Along this line, we identified that in a context of fusogenic cancer cells, the tetraspanin CD81/CD9 multi-protein complexes may initiate a combination of events that lead to local disruption of interactions between plasma membrane and cortical actin and the consequent plasma membrane fusion. Our data unveiled a key role of GTP-binding protein alpha13 and RhoA activated by the tetraspanin multi-protein complexes in cell fusion events. Therefore, we aim to antagonize cell-to-cell fusion synthesizing peptides targeting Galpha13 and RhoA functions. The peptides were encapsulated into nanoparticles of poly-(lactic-co-glycolic acid) (PLGA) polymer coated with cell-penetrating peptide TAT. Several peptides were assessed and one was found to antagonize cancer cell fusion in a syngeneic model of colon cancer. These findings could have clinical implications with respect to therapy setting and highlight the importance of identifying and using drugs targeting cell fusion in combination to conventional therapy as a new approach to abrogate multi-drug resistance in metastatic cancer.

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POSTER

Establishment of PI3K Inhibitor-resistant Cancer Cell Lines and the Therapeutic Strategies for Overcoming the Acquired Resistance

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Background: Acquired resistance is a major obstacle for conventional cancer chemotherapy, and also for some of the targeted therapies approved to date. Long-term administration of protein tyrosine kinase inhibitors (TKIs), such as gefitinib and imatinib, gives rise to resistant cancer cells carrying a drug-resistant gatekeeper mutation in the kinase domain of the respective target genes, EGFR and BCR-ABL. As for the