Results

Objective: The goal of our study was to identify novel therapeutic strategies that exploit the metabolic niche of cross-resistance resistant breast cancer cells in the context of relapse, refractory and metastatic disease. By implementing a novel ex vivo platform that informs the temporal ordering of the molecular events leading to cross-resistance we reasoned that drug schedules could be designed to target the origins of resistance.

Fig. 2 In-vitro validation that a drug-induced hybrid epithelial mesenchymal phenotype correlates with cross therapy resistance and glucose uptake

A) Schematic describes generation of drug tolerant cells (DTC) Breast cancer cells were treated with a high dose (20x IC50) of docetaxel followed by drug washout and acute population growth. B) DTC were analyzed by flow cytometry for epithelial and mesenchymal markers CD24 and CD44, respectively. Right panel shows quantification of the distinct phenotypes. CD24+CD44lo (mesenchymal-like) or CD44+CD24hi (a hybrid phenotype). C) MDA-MB-231 breast cancer cells were treated with docetaxel (DTX) at a non-cytotoxic dose for indicated time points. Glucose uptake was measured by 2-NBDG uptake and CD44 expression was determined by antibody fluorescence using flow cytometry.

Fig. 3 Drug-induced CD44 is required to support PI3K/AKT activity and glucose uptake via GLUT1 translocation

A) Western blot analysis of proteins that co-precipitate with AKT in breast cancer cells treated with low dose of docetaxel or vincristine. siRNA targeting CD44 or scrambled control were transfected prior to drug exposure. B) Glucose uptake determined by 2-NBDG fluorescence in parent cells or docetaxel tolerant breast cancer cells that were treated with AKT or mTOR inhibitors P1103 or everolimus, respective. C) Schematic depicts the mechanism of glucose uptake following exposure to docetaxel or cytotoxic chemotherapy-induced stress.

Fig. 4 Mathematical modeling indicates a temporal order of signaling networks drive drug-induced glucose uptake

A) Systems biology was developed using available literature to loosely connect CD44 expression and glucose uptake. Evidence for an involvement of reactive oxygen species (ROS), hypoxia inducible factor 1 alpha (HIF) and GLUT1 are all implicated in this network. B) Simulated temporal kinetics of molecular dynamics in the case where drug concentration in the system remains constant over time.

Fig. 5 Glucose metabolism inhibition reverses acquired cross-resistance to anthracyclines

A) Cell viability curves show sensitivity of breast cancer cells to the anthracycline, doxorubicin. Cells were either derived from a parent cell line (non drug-treated) or cells that survive high dose treatment with docetaxel (docetaxel-tolerant). Note the acquisition of cross-resistance in the drug tolerant cell population B) Cell viability curves show sensitivity of breast cancer cells to doxorubicin in drug naive or parent cells or docetaxel tolerant cells. Black line indicates doxorubicin alone, red line indicates addition of the AKT kinase inhibitor P1103.

Fig. 6 Clinical explant predicts sequence dependent inhibition of metabolic transition improves combination therapy – validated in vivo TNBC model

A) Ex-vivo CANscript platform was employed to test and predict the outcome of combination regimens that include taxane followed temporally by doxorubicin and an inhibitor of the hexokinase, Lonidamine. Lower panel shows histogram quantification of tumor cell viability (black bars) or glucose uptake in residual population of tumor cells (red bars). B) in vivo tumor volume curves of syngeneic mouse model (4T-1 triple negative breast cancer cells) following treatment with docetaxel and doxorubicin +/- Lonidamine in discrete sequence and temporal order. The temporally-constrained addition of Doc-P1103 overcomes tumor regrowth following cessation of treatment.

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