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Treatment of advanced solid tumors with golvatinib (E7050) in combination with lenvatinib (E7080)

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Background: Lenvatinib (L) is an oral multi-targeted tyrosine kinase inhibitor of VEGFR 1−3, FGFR 1−4, PDGFRα, RET and KIT (Phase 1 single-agent MTD 24 mg QD). Golvatinib (G) is a highly potent, small molecule ATP-competitive inhibitor of the MET receptor tyrosine kinase and multiple members of the Eph receptor family as well as KIT and RON, based on isolated kinase assays (Phase 1 single-agent MTD 400 mg QD). Combination therapy with agents that block MET, VEGF, FGF, PDGFα and ephrin signaling constitutes a promising approach to circumvent adaptive or pre-existing tumor resistance pathways, and preclinical models indicate that the combination of L and G could overcome some intrinsic resistance to each agent. This Phase 1b study assessed safety, MTD, and preliminary antitumor activity of L plus G in patients (pts) with advanced solid tumors.
Methods: Pts with advanced solid tumors, ECOG PS 0−1, ⩾18 years (yrs) and adequate organ function were eligible. Starting dose was 12 mg/day L and 200 mg/day G once daily administered continuously in 28-day cycles in a conventional 3+3 dose escalation design (dose range 12−20 mg/day L and 200–400 mg/day G). An expansion cohort confirmed the MTD and established the recommended Phase 2 (RP2) dose.
Results: 28 pts (M/F: 15/13; median age 61.5 yrs [range 34−75]) received combination dosing (median exposure 2.5 cycles [range 1−13], 8 pts ongoing at data cut) of L plus G in escalation cohorts (12 mg + 200 mg [n = 3]; 20 mg + 200 mg [n = 3]; 20 mg +300 mg [n = 6]; 20 mg + 400 mg [n = 8]) and in the expansion cohort 20 mg +300 mg [n = 8]. Two DLTs (at 20 mg + 400 mg) were observed during dose escalation: elevated AST and hyponatremia (1 pt) and inability to achieve at least 75% of planned dose in cycle 1 due to fatigue. The MTD/RP2 dose was determined to be 20 mg L + 300 mg G (4 DLTs in 14 evaluable pts [6 pts escalation cohort and 8 pts expansion cohort] at this dosing level). Frequently (>30%) occurring AEs of any grade and irrespective of relationship were fatigue, diarrhoea, nausea, vomiting, decreased appetite and dehydration. One fatal event of dyspnea, 21 days after discontinuing study drug for progressive disease, was observed. There was 1 possibly related G4 event of bowel perforation in the expansion cohort. Confirmed partial responses were seen in 5 (28%) pts [prostate (2 pts), endometrial (2 pts) and nasopharyngeal cancer]. Stable disease for at least 8 weeks was seen in an additional 13 pts.
Conclusions: The MTD and RP2 dose was lenvatinib 20 mg combined with golvatinib 300 mg. This dose combination toxicity was consistent with the individual agents' toxicity profiles, and there were no new safety signals identified. The observed activity warrants further evaluation of the combination in patients with advanced solid tumors.

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Glutathione S-transferases M1−5 reduce the aggressive behaviour in breast cancer by modulating the PI3K/AKT pathway

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Background: The human GSTM (GSTμ) gene family encodes five closely related enzymes, GSTM1–GSTM5 (GSTMs) that glutathionylate and thereby detoxify electrophilic compounds including carcinogens, environmental toxins and products of oxidative stress. Further, GSTMs regulate a number of protein kinases by either direct binding or by glutathionylation. Remarkably, six many independent gene expression profiling studies have demonstrated that increased GSTM expression strongly correlates with favorable clinical outcome of primary ER+ breast cancers. To understand whether this correlation is a causal one, we have explored this question *via* breast cancer genomic data analyses and *in vitro* modulation of these enzymes.
Methods: RNA-Seq analysis of 136 breast cancer patients was used to identify a gene signature associated with GSTM levels. *In vitro* up or down modulation of GSTMs was conducted in a panel of 10 breast cancer cell lines. Cell viability was measured using Cell-titer GLO and invasion assay by Boyden chamber. Protein expression of GSTMs and newly discovered targets were investigated by protein arrays, ELISA and Western blotting.
Results: Functional classification of the gene signature associated with GSTM RNA levels found enrichment of genes involved in the PI3K/AKT pathway, indicating an inverse correlation between PI3K/AKT pathway activation and GSTM expression. This GSTM signature was independently validated in publicly available genome-wide expression datasets (~1000 patients) and found to associate with poor clinical outcome. To explore the role that GSTMs play in breast cancer we transiently modulated the expression of all 5 isoforms *in vitro*. GSTMs downregulation by siRNA significantly increased cell viability, intracellular reactive-oxygen species (ROS) levels and invasion potential. Interestingly, these changes were mainly observed in ER+ cell lines carrying a PIK3CA activating mutation, reinforcing the hypothesis that the GSTM-associated phenotype acts through the PI3K signaling pathway. We next profiled gene expression after the modulation of GSTMs and again linked GSTM levels to PI3K pathway activity. Remarkably, downregulation of GSTMs was correlated with a significant increase in the activation of MAPK, AKT and the transcription factor STAT5. Transcription factor motif analysis shows that STAT5 is significantly enriched in the promoter region of genes belonging to the GSTM signature.
Conclusions: Our findings indicate that GSTMs expression directly limits the aggressive behavior of the large subclass of ER+ breast cancer cells that have constitutively activated PI3K/AKT pathway.

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Development of small molecule activators of protein phosphatase 2A for the treatment of lung cancer

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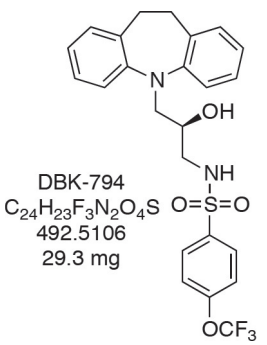
Background: KRAS is the most common recurrent oncogenomic mutations driving the growth of NSCLC and accounting for ~25% of patients with advanced NSCLC. Patients with KRAS mutations respond poorly to current therapies driving the pursuit of new treatment strategies to improve the lives of patients suffering from KRAS driven lung cancers. While oncogenic kinases have proven to be successful targets for cancer treatment, the therapeutic targeting of phosphatases, the key negative regulators of these same pathways, has remained largely unexplored. Through reverse engineering of tricyclic neuroleptic drugs, we developed a first-in-class series of molecules, as represented by TRC-794 and DT-1154, that have favorable pharmaceutic properties, directly bind and activate the serine/threonine phosphatase 2A (PP2A). A critical role for PP2A as a tumor suppressor has previously been established, and inhibition and loss-of-function changes in PP2A occur in human lung cancers, and its dominant targets are protein kinases and oncogenic proteins including ERK and AKT.
Methods: A panel of lung cancer cell lines was used to understand the functional and biological effects of TRC-794 and DT-1154. To understand the effects of these small molecule activators of PP2A (SMAPs) on cell viability and survival, we used MTT and colony formation assays. Apoptosis was evaluated through annexin V staining and cell cycle profile analysis. Additionally, global phosphoproteomic profiling was performed using TiO2 enriched chromatography coupled with MS/MS analysis. Effects of TRC-794 and DT-1154 in vivo was assessed using A549 and H358 xenograft and *Kras* transgenic mouse models.



Results: Treatment of lung cancer cell lines with SMAPs resulted in decreased cell viability, decreased colony formation, and an increase in apoptosis. Global phosphoproteomic analysis of TRC-794 treated KRAS lung cancer cell lines revealed ERK signaling as the only commonly perturbed pathway in drug treated cell lines which was confirmed by western blotting. Single agent TRC-794 and DT-1154 treatment of KRAS GEMM and xenograft mouse models of lung cancer resulted in tumor stasis, induction of tumor cell apoptosis and cell cycle arrest to comparable levels seen with a combination of AKT and MEK inhibitors. SMAP treatment was associated with significant AKT and ERK dephosphorylation in vivo. Additionally, the compounds demonstrate favorable pharmacokinetics and show no overt toxicity in mice or rats.