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Evaluation of pan-HER and c-MET inhibitors tested *ex vivo* in live primary HER2- breast cancer cells with hyperactive c-MET and ErbB family signaling

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Background: To elucidate the role of c-Met signaling and its involvement with ErbB signaling as a cancer driver, a new assay using an impedance biosensor, the CELx multi-pathway signaling function (CELx MP) test, was developed. The CELx MP Test measures *ex vivo* real-time live cell response to specific ErbB and c-Met agonists to diagnose breast tumors with hyperactive HER1, HER2, HER3, HER4, and c-MET signaling activity. A recent study quantified c-MET and ErbB-driven signaling activity in epithelial cell samples derived from fresh breast tumor specimens obtained from 74 HER2- breast cancer patients. Of the cell samples tested, 20 of 74, (27.0%; 95% CI=18%-38%) had both hyperactive c-MET signaling and at least one hyperactive ErbB-family receptor signaling. Using primary breast cancer cells with hyperactive c-MET and ErbB signaling and the CELx MP test, the current study set out to: 1) determine the IC_{50} values of six pan-HER and five c-MET inhibitors; and 2) characterize the efficacy of combinations of each pan-HER inhibitor with each c-MET inhibitor.

Methods: Epithelial cells from six HER2- tumor specimens with hyperactive c-MET and ErbB-driven signaling were obtained. Real-time live cell response to specific ErbB and c-Met agonists (NRG1b, EGF, or HGF) alone and in combination, with or without one of six pan-HER antagonists (neratinib, lapatinib, ibrutinib, dacomitinib, sapitinib, poziotinib) or one of five c-MET antagonists (tepotinib, cabozantinib, crizotinib, capmatinib, or savolitinib) was quantified using an xCELLigence RTCA impedance biosensor. Each individual drug IC_{50} was determined using a 1000-fold, 5-point, dose response curve with a single fixed concentration of a corresponding agonist. For the drug combination efficacy studies, fixed concentrations of the agonist mixture and clinically relevant concentrations of combinations of the antagonists were used to determine the percentage inhibition of the ErbB and c-MET signaling.

Results: The IC_{50} values for the individual c-MET and pan-HER inhibitors ranged from 3.10nM - 28nM and 2.67nM – 137.27nM, respectively. In the drug efficacy studies, an average of at least 80% of the ErbB and c-MET signaling activated by NRG1, EGF, and HGF co-stimulation was inhibited by each combination of c-MET and pan-HER inhibitors.

c-MET inhibitors	IC_{50} (nM)	Average Inhibition (%) w/different ErbBi's
Capmatinib	3.10	94
Savolitinib	3.56	98
Tepotinib	14.70	96
Cabozantinib	27.36	99
Crizotinib	28.21	100

Pan-HER inhibitors	IC_{50} (nM)	Average Inhibition (%) w/different c-METi's
Poziotinib	2.67	100

Neratinib	4.81	100
Ibrutinib	13.10	99
Dacomitinib	22.06	100
Sapitinib	41.28	98
Lapatinib	137.27	80

Conclusions: The CELx MP test using live cells measures IC_{50} values comparable to those derived using cell-free methods. Every combination of pan-HER and c-MET inhibitors provided comparably high (at least 80%) levels of inhibitory effect *ex vivo*. This suggests the sub-group of HER2- breast cancer patients diagnosed with coincident hyperactive c-MET and ErbB signaling by the CELx Test may respond to virtually any pan-HER and c-Met inhibitor combination. Studying combinations designed to minimize drug toxicities without sacrificing efficacy should thus be possible.