Abstract 738: Increased Ret signalling and impact of vandetanib in acquired tamoxifen resistant breast cancer

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Abstract

Deregulation of the tyrosine kinase Ret and its coreceptors (GFRα) has been implicated in neoplasia, and Ret is of interest as a therapeutic target in endocrine-treated breast cancer. This study evaluated in vitro impact of vandetanib, a tyrosine kinase inhibitor able to target Ret in addition to EGFR and VEGFR2, in ER+ breast cancer cells that have acquired resistance to tamoxifen treatment.

Tamoxifen resistant TAMR and endocrine responsive MCF7 cells were grown in vitro for 7 days +/- vandetanib (0.5-5μM) +/- exogenous growth factors (10-50ng/ml), and also in continuous culture with vandetanib (1μM), to monitor growth impact and emergence of vandetanib resistance. For Western blotting or immunoprecipitation, log phase cells were transferred for 24hr to serum-free medium and pre-treated for 1hr +/- vandetanib followed by Ret ligands GDNF or artemin for 5mins. Immunohistochemistry for Ret activity was performed on an ER+ tamoxifen-treated clinical breast cancer TMA sample series using Y1062 Ret phospho-antibody with HScore staining assessment.

Growth of TAMR cells was substantially inhibited by vandetanib ($p<0.001$, IC50 0.6μM) with complete cell loss by 17weeks, contrasting rapid emergence of resistance in endocrine responsive MCF7 cells. TAMR cells were more sensitive to vandetanib vs. gefitinib ($p<0.001$; 1μM each agent), indicating mitogenic signalling in addition to EGFR contributed to TAMR growth. TAMR cells had elevated basal Ret expression, activity and interaction with elevated GFRα3 coreceptor expression; mature VEGFR2 was not detected in TAMR cells. Exogenous GFRα3 ligands artemin or GDNF modestly stimulated TAMR cell growth and hyperactivated Ret, downstream kinases (including MAPK, AKT) and ER Ser167, confirming functional GFRα/Ret signalling and its cross-talk with ER. Increased phospho-Ret immunostaining was also associated with shortened DFI ($p=0.036$) and survival ($p=0.011$) in tamoxifen-treated clinical ER+ breast cancers. Vandetanib (0.5-1μM) depleted GFRα3/Ret activity and decreased phospho-EGFR in TAMR cells under basal and Ret ligand-stimulated conditions, inhibited MAPK, p70S6K and S6RP phosphorylation, and partially-reduced levels of phospho-AKT and phospho-ER. However, vandetanib failed to consistently impact on HER2, 3 or 4 activity; moreover, hyperactivation of all erbB receptors by exogenous heregulin B1 (10ng/ml) recovered AKT, MAPK, p70S6K and ER AF-1 phosphorylation in the presence of vandetanib and was able to overcome the basal growth-inhibitory impact of this agent in TAMR cells.

These findings demonstrate a central importance for increased Ret signalling and its cross-talk with ER in tamoxifen resistant breast cancer that can be targeted in vitro by vandetanib. Further studies are required to determine optimal combination treatments with vandetanib to circumvent potential intrinsic resistance in clinical breast cancers that exhibit heregulin B1 enrichment.