KX-01, A NOVEL SRC KINASE INHIBITOR DIRECTED TOWARDS THE PEPTIDE SUBSTRATE SITE, INDUCES ROBUST APOPTOSIS AND SYNERGIZES WITH TAMOXIFEN AND CHEMOTHERAPY IN BREAST CANCER.

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New therapeutic regimens that increase efficacy or reduce onset of resistance for endocrine therapy and chemotherapy are needed for better clinical management of breast cancer. c-Src is an oncogenic non-receptor tyrosine kinase that is up-regulated in approximately half of all breast cancer. However the efficacy of existing multi-kinase Src inhibitors in breast cancer has been limited. KX-01 (Kinex Pharmaceuticals) is a novel class of non-ATP Src inhibitor that targets the peptide binding site of Src and is currently completing Phase-1 testing for solid tumors. In a panel of breast cancer cell lines, KX-01 resulted in dose dependent inhibition of growth and induction of apoptosis that was independent of p53 status, and was preceded by rapid inhibition of Src activity. KX-01 induced apoptosis in two cell lines reported to be resistant to multi-kinase Src inhibitors, MDA-MB-468 cells and BT-549. Cell cycle analysis revealed that KX-01 (50 nM, 6 hours) resulted in significant accumulation of MDA-MB-231 cells (ERα/PR/HER2/neu negative) and MCF-7 cells (ERα positive) in G2/M phase. Immunofluorescent staining for mitotic phase marker phospho-histone 3 indicated that cells had arrested in mitotic phase and many of the mitotic arrested cells were undergoing apoptosis (TUNEL), a novel cell death for a small molecule tyrosine kinase inhibitor. KX-01 induced nuclear accumulation of cyclin B1, and activation of CDK1, MPM2 and Cdc25C that is required for progression past the G2/M checkpoint. KX-01 resulted in cytochrome C release and activation of caspases 6, 7, 8 and 9. A matrix design using the median-effect principle to delineate the interaction between two drugs was applied for KX-01 alone and in combination tamoxifen (TAM), paclitaxel (PAC) or doxorubicin (DOX). Combinations of KX-01 (5-75 nM) with each of these agents resulted in synergistic growth inhibition of MCF-7 cells (KX-01 + TAM) and MDA-MB-231 cells (KX-01 + DOX or PAC). In addition, synergistic induction of apoptosis was achieved by combining low doses of KX-01 with DOX, PAC or TAM. c-Src induces phosphorylation of ERα at serines 118 and 167, sites required for full receptor activity. KX-01 combined with TAM resulted in decreased phosphorylation at serine 167 that was associated with reduced transcriptional activity of ERα. In tumor xenograft models, KX-01 resulted in a dose dependent inhibition of MDA-MB-231 and MCF-7 tumor growth after 30 days (1, 2.5 or 5 mg/kg body weight, twice daily by oral gavage). In MDA-MB-231 xenografts, KX-01 reduced metastasis to bone (femur) and lung as measured by PCR for detection of human chromosome 17. These data define KX-01 as a potently active Src kinase inhibitor that induces robust cell death, tumor growth inhibition and anti-metastatic effects. Combinations of KX-01 with endocrine therapy and chemotherapy present a promising new strategy for clinical management of breast cancer.