Breast Cancer, Metastatic

**Preclinical Evaluation of Novel Pak4 Allosteric Modulators Against Triple Negative Breast Cancer**

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**Aim:** Triple Negative Breast Cancer (TNBC) is characterized by a lack of receptors for estrogen (ER), progesterone (PR), and hormone epidermal growth factor 2 (HER-2). TNBC occurs in ~20% of diagnosed breast cancers. Hormone targeted therapies are ineffective thus making TNBC more aggressive and difficult to treat. Studies have shown that P21-Activated Kinase 4 (PAK4) is elevated in TNBC. PAK4 regulates cell survival, cell division and apoptosis. PAK4 overexpression in mammary epithelial cells leads to abnormal acini formation and drives tumorigenesis in mice.

**Methods:** Tissue microarrays were used to evaluate Pak4 expression in normal and cancerous breast tissue. We identified and tested selective, orally bioavailable, small molecule Pak4 allosteric modulators (PAMs) against breast cancer cell lines. MTS assay was used to determine effects on proliferation and viability of 30 breast cancer (BC) cell lines including 13 TNBCs. mRNA was analyzed using the Genome Atlas (TCGA) and deep sequencing. Immunoblots were used to measure protein steady state levels and phosphorylation. TNBC xenograft models were used to test efficacy.

**Results:** PAMs demonstrated anti-tumor activity against BC cell lines (IC50 0.04 - 10 µM) of which TNBC were the most sensitive: ~80% of TNBC lines had IC50 < 0.54 µM with minimal cytotoxicity to normal cells. PAM treatment of TNBC cells resulted in the reduction of Pak4 steady state levels and reduced phosphorylation of key growth signaling such as Akt, ERK1/2, β-catenin, cofilin, p21, and cyclin D1. PAMs induced apoptosis through caspas 3 and 8 activation and subsequent cleavage of PARP. Anti-tumor activity was observed against TNBC xenografts in mice at a daily oral dose (QD) of 60 mg/kg of PAMs with no clinical signs of toxicity up to 200 mg/kg.

**Conclusions:** Oral PAMs inactivate Pak4 by directly inducing Pak4 destabilization. This represents a novel mechanism of the protein kinase inactivation involving degradation of Pak4 rather than direct inhibition of the kinase activity. Oral PAMs have potent anti-tumor activity against BC cells including TNBC both in vitro and in vivo where they induce tumor cell growth arrest and apoptosis with excellent tolerability. Based on this anti-tumor activity and high tolerability, PAMs show promise for the treatment of TNBC.

**Disclosure:** W. Senapedis, Y. Landesman, J. Ellis, R. Carlson and E. Baloglu: I am a current employee of Karyopharm Therapeutics, Inc.; O. Kald: I currently am a consultant with Karyopharm Therapeutics, Inc.; D. McCauley: I am a current employee of Karyopharm Therapeutics, Inc.; O. Kald: I currently am a consultant with Karyopharm Therapeutics, Inc.; S. Shacham: I am a current employee of Karyopharm Therapeutics, Inc.; S. Shacham: I am a current employee of Karyopharm Therapeutics, Inc.; S. Shacham: I am a current employee of Karyopharm Therapeutics, Inc.; S. Shacham: I am a current employee of Karyopharm Therapeutics, Inc.; S. Shacham: I am a current employee of Karyopharm Therapeutics, Inc.