Polo-like Kinase 1 Inhibitor Onvansertib Synergizes with Paclitaxel in Breast Cancer Carrying p53 Mutation

Antonio Giordano, MD 1; Yueying Liu 1; Christiana Kappler 2; Yeonhee Park 3; Elizabeth Yeh 3; Mark Erlander, PhD 4; Stephen Etheredge 2

1. Department of Medicine, Division of Hematology & Oncology, Medical University of South Carolina, Charleston SC; 2. Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston SC; 3. Department of Public Health Sciences, Hollings Cancer Center, Medical University of South Carolina, Charleston SC; 4. Department of Cell and Molecular Pharmacology & Experimental Therapeutics, Medical University of South Carolina, Charleston SC; 5. Tirovagene Oncology, San Diego CA.

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Somatic mutation in TP53 gene (mutp53) is a strong prognostic marker in breast cancer. We hypothesize that mutp53 in the context of breast cancer can predict synergy to paclitaxel plus onvansertib, an orally available highly selective PLK1 inhibitor.

Methods

Growth Assay: cells were plated in 6-well plates at a density of 100,000 cells per well and treated in triplicate with a wide range of drug concentrations or DMSO. After 3 days exposure to drug, cell number was determined using a Caliper Imaging Cytometer (Neocam, USA).

Immunoblotting, Apoptosis and DNA Content Analysis: cells were synchronized with double thymidine block. For synchronization, SUM149 and SUM159, were treated with 2mM thymidine, incubated for 24 hours, then washed with PBS before adding fresh media. After an 18-hour release, cells were again treated with thymidine for an additional 24 hours of incubation. Finally, cells were released for 3 hours in fresh media and treated with GS641634 (0.12365) or DMSO. In Vivo: five million SUM159 cells were injected in the mammary fat pad of NOD-SCID IL2 receptor gamma null female mice (Jackson Laboratory). When tumors reached a volume greater than 40 mm3, 35 mice were randomized to receive onvansertib 120 mg/kg day 1-2 every week by oral gavage (P.O.), paclitaxel 10 mg/kg day 1 every week by intraperitoneal injection (I.P.), or the combination of the two drugs at the same dose of single agents, or control vehicle. In each arm, P.O. and I.P. vehicle was given as per the control arm.

Objectives

• Somatic mutation in TP53 gene (mutp53) is a strong prognostic marker in breast cancer. Triple negative breast cancer (TNBC) is characterized by up to 80% mutp53 and carry the worse prognosis among all subtypes of breast cancer.

• In SUM149, a basal-like 2 (BL2) TNBC line isolated from an African American patient with Inflammatory Breast Cancer (IBC), PtK1 is a gene necessary for growth and survival.

• Polo-like kinase 1 (PLK1) regulates progression of cells through the G2 phase of the cell cycle.

• In the early pre-clinical development of Ptk1 targeted drugs, it was observed that cancer cells with mutp53 were more responsive and had lower IC50 than cancer cells with wild-type p53.

• Our central hypothesis is that onvansertib will increase the sensitivity to paclitaxel in metastatic breast cancer affecting the ability of cancer cells with mutp53 to progress through mitosis (Fig. 1).

Conclusions and Perspective

• Onvansertib, an orally highly-selective PLK1 inhibitor, and paclitaxel showed synergy in cell models of breast cancer with p53 mutations

• PLK1 inhibitor GS641634 induced mitotic arrest (Cyclin B1 and PLK1 accumulation followed by pH3 accumulation) earlier in mutp53 to paclitaxel combination treated cells.

• This difference could be explained by the faster doubling time and earlier mitoses entry by SUM149 compared to SUM159.

• Treatment with single PLK1 inhibitor induced apoptosis after 24 hours in both cell lines (Fig 6A). In the DNA content analysis with propidium iodide, the percentage of aneuploid SUM149 (Fig 6B) and SUM159 cells (Fig 6C) increased over time (24-48 hours) after treatment with GS641634 and after combination of GS641634 with doxilcetin. Indeed, aberrant mitotic exit was observed when SUM149 and SUM159 cells were treated with single agent and the combination of PLK1 inhibitor plus doxilcetin (Fig 6D). In Vivo: efficacy of onvansertib alone or in combination with paclitaxel against SUM159 xenografts

Correspondence: email: antonio.giordano@musc.edu