# 29P PNU-74654 enhances the antiproliferative effects of 5-FU in breast cancer and antagonizes thrombin induced cell growth via the Wnt pathway

S. Shahid Saleh1, A. Avan2, F. Rahmani3, S.M. Hassanian4, M. Hashemzehi3, F. Amerizadeh5, G. A. Ferns6, M. Khazaei3

1Clinical Oncology, Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, 2Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, 3Division of Medical Education, Brighton & Sussex Medical School, Falmer, UK, 4Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Background: The Wnt/beta-catenin pathway is one of the main pathways that are dysregulated in several malignancies, including breast cancer, and may therefore be a potential therapeutic target. We have investigated the anticancer activity of PNU-74654 in breast cancer, as a Wnt/ beta-catenin inhibitor, either alone or in combination with 5-FU in vitro and in vivo.

Methods: Cell viability was assessed in 2 and 3-dimensional (D) cell culture models. The ability of PNU-74654 to inhibit the chemotaxis of cells was investigated using an in vitro migration assay, and the expression of several candidate genes involved in the cell cycle, migration, as well as the markers of Wnt/b-catenin pathway were investigated by qRT-PCR and/or Western blotting as well as cell cycle analysis by flow cytometry. The effect of PNU-74654 on oxidative balance was evaluated by determining the malondialdehyde (MDA) and concentration of total thiols (T-SH), and the activity of catalase (CAT) and superoxide dismutase (SOD). We reconstructed a Boolean model in order to understand dynamic behavior of genes, while the robustness of this model was assessed by Hamming distance.

Results: PNU-74654 suppressed cell growth at an IC50 of 122 ± 0.4 umol/L and synergistically enhanced the antiproliferative activity of gemcitabine by modulating the Wnt pathway. The 3-D cell culture model showed that PNU-74654 caused tumor shrinkage. It reduced the migration of MCF-7 cells (by an 18% reduction in invasive behavior). Treatment with PNU-74654 through perturbation of E-cadherin and MMP3/9, PNU-74654/5-FU combination enhanced the percentages of cells in S-phase, and significantly increased apoptosis. Moreover, our data showed that this agent was able to inhibit the growth of tumor in a xenograft model, although this effect was more pronounced in the animals treated with PNU-74654 plus 5-FU.

Conclusions: The antitumor activity of PNU-74654 were shown in breast cancer.

Legal entity responsible for the study: Mashhad University of Medical Sciences, Mashhad, Iran.

Funding: Mashhad University of Medical Sciences, Mashhad, Iran.

Disclosure: All authors have declared no conflicts of interest.