Investigating the Role of KS-01 as an Anti-Cancer Agent in Colorectal Cancer

Pillay, R.P, Kaur, M

University of the Witwatersrand, Johannesburg, South Africa

Introduction: Colorectal cancer (CRC) is the third most frequent cancer in the world. Due to its highly heterogeneous nature there are several limitations in treatment strategies for CRC. Various cancers, including CRC, maintain a high cholesterol phenotype for cell proliferation and survival. Thus, targeting cholesterol for the treatment of cancer has become an attractive approach. Additionally, the mutational landscape of CRC has made apparent genetic alterations in the WNT signalling pathway, including downstream activators and inhibitors such as β- catenin and secreted Frizzled-related proteins (sFRP), respectively. This study displayed the use of KS-01, a patented cyclodextrin with cholesterol sequestration properties, in the treatment of CRC as well as proposed KS-01's mechanism of action, linking it to the WNT pathway.

Methodology: Cell viability assays were performed on HT-29 cells, pre- and post- KS-01 treatment at various concentrations for 24 hours. This was followed by cholesterol staining, using Vybrant™ Alexa Fluor™ lipid raft labelling. Subsequently, immunofluorescence and Western blotting was used to determine protein expression of sFRP in HT-29 cells.

Results: A 70% reduction in cell viability was observed in HT-29 at a concentration of 10 mM KS-01. In addition, there was a significant reduction in lipid raft content post KS-01 treatment. These lipid rafts are cholesterol-rich domains in the plasma membrane, which play an essential role in cell signalling. Lastly, both Western blotting and immunofluorescence displayed a significant upregulation in sFRP protein expression in KS-01 treated cells.

Discussion and conclusion: Since cholesterol is important for the maintenance of the cancerous state, KS-01 through cholesterol depletion is a promising drug for the treatment of CRC. Mechanistically it could be proposed that, KS-01 treatment inhibits the WNT signalling pathway, through its stabilization of sFRP. This in turn could inhibit ß- catenin and other downstream proteins of the WNT pathway that lead to tumourigenesis. Itcould thus be said that KS-01 acts dually to inhibit cancer growth.

References: 1. Gu, L., Saha, S. T., Thomas, J., & Kaur, M. (2019). Targeting cellular cholesterol for anticancer therapy. *The FEBS journal*, *286*(21), 4192-4208. 2. Abdulla, N., Vincent, C. T., & Kaur, M. (2021). Mechanistic Insights Delineating the Role of Cholesterol in Epithelial Mesenchymal Transition and Drug Resistance in Cancer. *Frontiers in cell and developmental biology*, *9*, 728325. 3. Guerra, F. S., da Silva Sampaio, L., Konig, S., Bonamino, M., Rossi, M. I. D., Costa, M. L., & Mermelstein, C. (2016). Membrane cholesterol depletion reduces breast tumor cell migration by a mechanism that involves non-canonical Wnt signaling and IL-10 secretion. *Translational Medicine Communications*, *1*(1), 1-10.

Keywords: CRC, cholesterol, KS-01, WNT signalling pathway, sFRP.

Acknowledgements:
This research was financially supported by the National Research Foundation (NRF), South Africa (grant number: 129356) to MK, and NRF Doctoral Scholarship (121437) to RP.