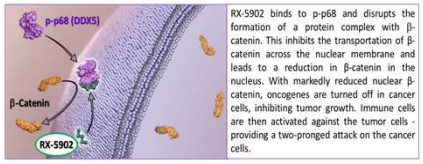
The anticancer effects of RX-5902 through phosphorylated p68-nuclear β-catenin signaling

Young Bok Lee, Christina George and Deog Joong Kim
Rexahn Pharmaceuticals, Inc.

#1298

Background & Methods

Background: RX-5902 is an orally available novel inhibitor targeting phosphorylated p68 RNA helicase (p-p68), a member of the DEAD (Asp-Glu-Ala-Asp) box family of RNA helicases. Phosphorylated p68 has been shown to be associated with cell transformation, epithelial mesenchymal transition (EMT) and cell migration and thus may play a vital role in cell proliferation and tumor/cancer progression by blocking the nuclear translocation of β-catenin. We have previously shown that RX-5902 inhibits the growth of cancer cells at low nanomolar IC50 (10 to 24nM) and interacts with p-p68, interfering with the p-p68-β-catenin signaling pathway. In this study, we have expanded our investigation to see the effect of RX-5902 on nuclear β-catenin in several types of cancer cell lines and the breast tumor bearing mice.

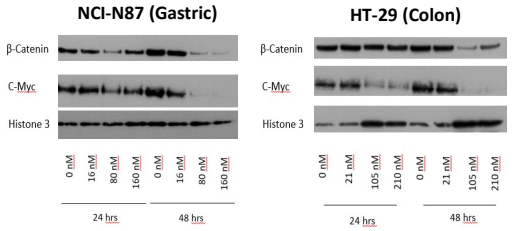


RX-5902 binds to p-p68 and disrupts the formation of a protein complex with β-catenin. This inhibits the transportation of β-catenin across the nuclear membrane and leads to a reduction in β-catenin in the nucleus. With markedly reduced nuclear β-catenin, oncogenes are turned off in cancer cells, inhibiting tumor growth. Immune cells are then activated against the tumor cells - providing a two-pronged attack on the cancer cells.

Methods: Human cancer cells were plated onto 6-well plates and treated with RX-5902 at various concentrations for 24 hrs and 48 hrs. Nuclear fraction was purified from collected cancer cells using the nuclear extract kit. The proteins in the nuclear cell lysates were separated by SDS-PAGE for western blotting. Tumor samples were collected at 1 hr and 24 hrs after RX-5902 1st dosing of the MDA-MB-231 tumor bearing mice. The proteins in the tumor lysates were separated by SDS-PAGE for western blotting.

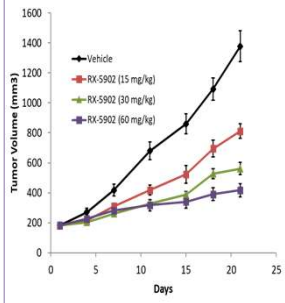
Results

Effect of RX-5902 on nuclear β-catenin and c-Myc in cancer cell lines (Western Blot)



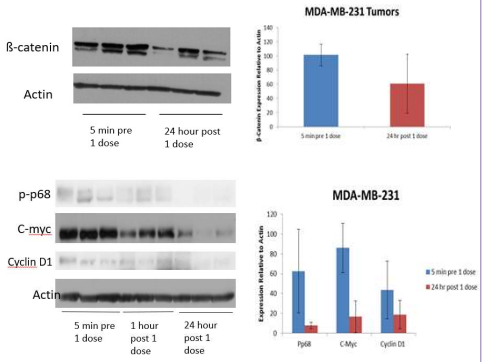
Indication	Cell Line	IC_{50}	Nuclear β-Catenin upon RX-5902 Treatment
Colon	HCT-116	19 nM	Decrease
	HT-29	21 nM	Decrease
Gastric	MKN-45	20 nM	Decrease
	NCI-N87	16 nM	Decrease
Hepatic	HepG2	19 nM	Decrease
	SK-Hep1	20 nM	Decrease
Head and Neck	FaDu	12 nM	Decrease
Melanoma	SK-MEL-28	20 nM	Decrease
Breast	MDA-MB-231	20 nM	Decrease
Pancreatic	MiaPaca-2	8 nM	Decrease
Ovarian	OVCAR-3	12 nM	Decrease
	A2780	22 nM	Decrease
Prostate	PC-3	21 nM	Decrease
	DU-145	21 nM	Decrease

Dose-related inhibition of TNBC tumor growth by RX-5902



- Human breast cancer cells (MDA-MB-231)
- Dosed orally 5 days on, 2 days off
- Results: Dose dependent TGI%
  - RX-5902 at 15 mg/kg: 45%
  - RX-5902 at 30 mg/kg: 58%
  - RX-5902 at 60 mg/kg: 72%
- No effect on body weight
- N=11

Effect of RX-5902 on β-catenin, p-p68, c-Myc and cyclin D1 in TNBC tumors (Western Blot)



Conclusions

- Western blot data showed a decrease of nuclear β-catenin protein as well as p-p68 and c-myc in a concentration-dependent manner in tested cancer cell lines.
- *In vivo* study in MDA-MD-231 xenograft model indicated the tumor growth inhibition by RX-5902 may be related to decreased p-p68 and β-catenin as well as cyclin D1 and c-myc.
- These studies may support the use of RX-5902 as a therapy in multi cancer indications through the disruption of the phospho-p68/β-catenin interaction and blocking the nuclear translocation of β-catenin.

For further information about RX-5902 and Rexahn Pharmaceuticals, please contact:
Dr. DJ Kim: kimdj@rexahn.com, 240-268-5300 X306

rexahn PHARMACEUTICALS