Namodenoson Inhibits the Growth of Pancreatic Carcinoma via De-regulation of the Wnt/β-catenin Signaling Pathway

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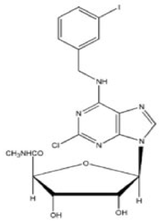
BACKGROUND

- Pancreatic cancer is a very aggressive malignancy with an overall 5-year survival rate of 8%; in 52% of cases, the disease is diagnosed at stage IV.[1]
- Systemic treatments for pancreatic cancer are lacking. The effect of gemcitabine alone, or in combination with other chemotherapies is modest, and checkpoint inhibitors also failed to show a significant clinical benefit.[2]
- Namodenoson (CF102, Cl-IB-MECA; **Fig. 1**) is a selective orally bioavailable A3 adenosine receptor (A3AR) agonist. It is overexpressed in tumor cells (e.g., melanoma, prostate, breast, colon, and liver cancer), but not in the adjacent healthy tissue.[3]
  - Namodenoson is currently being studied in a pivotal phase III trial as a treatment for advanced liver cancer (clinicaltrials.gov identifier, NCT05201404).

OBJECTIVES

The objectives of the current preclinical study were to examine the anti-growth effect of namodenoson on pancreatic carcinoma cells both *in vitro* and *in vivo* and to investigate the molecular mechanisms involved.

Fig. 1. Namodenoson: Chemical structure.



METHODS

Cells and Reagents

- All experiments were performed with the pancreatic adenocarcinoma BxPC-3 cell line; namodenoson was prepared by WuXi. It was dissolved in DMSO to yield a stock solution of 10 mM.

In Vitro and In Vivo Studies

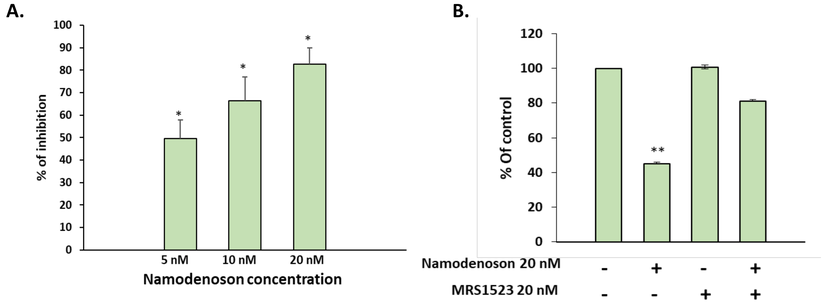
- Presto Blue assay was used to monitor cell growth after incubation of BxPC-3 cells ($15x10^4$/mL) with 5, 10, or 20 nM namodenoson in 96-well plates for 24 h at 37°C.
- Nude male Balb/C mice (Harlan Laboratories, Israel), aged 2 months (mean weight, 25 g), were administered a subcutaneous flank injection of BxPC-3 cells ($2.5x10^6$). When the tumor reached a size of 150-200 mm^3, the animals were randomly assigned to 2 groups each containing 10 animals (namodenoson, 100 μg/kg body weight given orally twice daily for 35 days, and control). Tumor size was measured twice weekly.
- Western blot analyses were performed (using the same conditions as the Presto Blue studies in 10-cm plates) to assess the expression levels of cell growth regulatory proteins using rabbit polyclonal antibodies against phosphorylated-PKB/Akt (p-PKB/Akt), NF-kB, β-Catenin, A3AR, PI3K, GSK-3β, cyclin D1, ERK 1/2, MEK 1/2, Raf, Bad, and Bax (Santa Cruz Biotechnology Inc., USA).
- The *in-vivo* experiments were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at Can-Fite BioPharma (Israel).

RESULTS

In Vitro Effects of Namodenoson on Pancreatic Carcinoma Growth

- *In vitro* analysis using the Presto Blue assay demonstrated a significant dose-dependent inhibition of BxPC-3 cell growth upon treatment with namodenoson, which was mitigated by the A3AR antagonist MRS1523, showing that the effect of namodenoson was A3AR-mediated (**Fig. 2**).

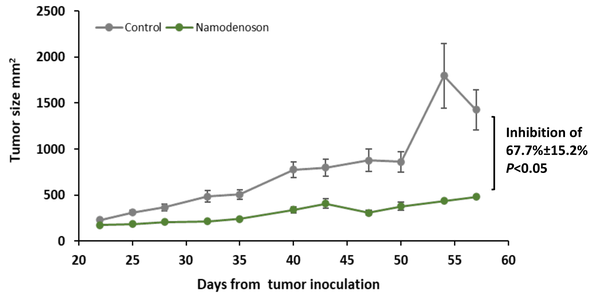
Fig. 2. Inhibition of BxPC-3 cell growth by namodenoson (A) was mitigated by the A3AR antagonist MRS1523 (B). Error bars represent SE. **P* <0.001, ***P* <0.01 (t-test vs control).



In Vivo Effects of Namodenoson on Pancreatic Carcinoma Growth

- Analysis of the inhibitory effect of namodenoson (10 μg/kg) given twice daily for 35 days to nude mice inoculated with BX-PC3 cells demonstrated a significant inhibitory effect of namodenoson on tumor growth (inhibition of 67.7%±15.2% by day 57 vs controls, $P<0.05$, t-test) (**Fig. 3**).

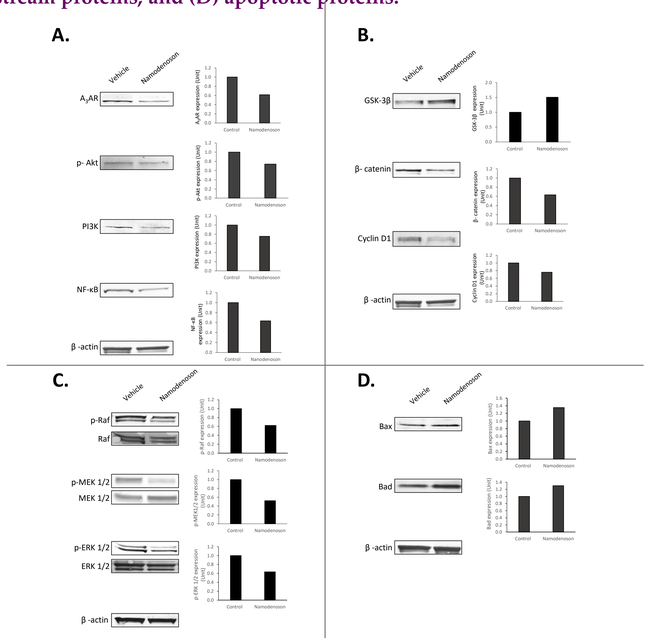
Fig. 3. Inhibition of BxPC-3 cell growth by namodenoson in nude mice. Error bars represent SE.



In Vitro Effects of Namodenoson on Signal Transduction

- Western blot analysis using BxPC-3 cells demonstrated that namodenoson induced a decrease in A3AR expression level, and that the downstream regulatory proteins p-Akt, PI3K, and NF-kB were all down-regulated (**Fig. 4A**). Analysis of proteins within the Wnt signal transduction pathway revealed upregulation of GSK-3β, and a decrease in the expression levels of β-catenin, and cyclin D1 (**Fig. 4B**). A decrease in the expression levels of proteins downstream to the RAS signaling pathway (Raf, MEK 1/2, and ERK 1/2) was also observed (**Fig. 4C**). Additionally, the 2 apoptotic proteins Bad and Bax were up-regulated, suggesting that apoptosis of the BX-PC3 cells was induced (**Fig. 4D**).

Fig. 4. The effect of namodenoson treatment (20 nM, 24 h) on protein expression in BxPC-3 cells as observed in Western blot analyses. (A) cell growth regulatory proteins downstream of A3AR; (B) Wnt/β-catenin signaling pathway proteins; (C) KRAS downstream proteins; and (D) apoptotic proteins.



CONCLUSIONS

- **Namodenoson inhibits the growth of pancreatic carcinoma *in vivo* and *in vitro* in a mechanism that involves A3AR activation, de-regulation of the Wnt/β-catenin and the Ras signaling pathways, and activation of apoptosis.**
- **Our finding supports the continued development of namodenoson as a treatment for pancreatic cancer.**

References: 1. Rawla, et al. World J Oncol 10:10-27, 2019; 2. Ducreux et al. Sem Oncol 46:28-38, 2019; 3. Fishman et al. Purinergic Signal 2023, Epub ahead of print.
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