The Anticancer Effects of Supinoxin (RX-5902) in Renal Cell Cancer

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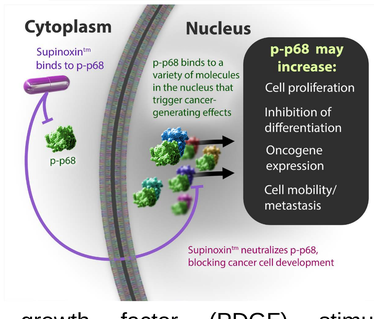
Abstract

DEAD box RNA helicase DDX5/p68 may play several important roles in cancer. In particular, phosphorylated p68 has been shown to be associated with cell transformation, epithelial mesenchymal transition (EMT), and cell migration, deeming it a promising target for novel anti-cancer therapeutics. We have previously shown that Supinoxin (RX-5902) interacts with phosphorylated p68 on Tyr593, interfering with the phosphorylated p68-β-catenin signaling pathway. In this study, we first demonstrated anti-proliferative effects in ten renal cancer cell lines with a high level of sensitivity to Supinoxin (IC50 of 39 nM); TK-10 was the only resistant cell line in this study. We also sought to examine the tumor growth inhibition (TGI), tumor growth delay (TGD), and survival benefits of Supinoxin in the human renal cell carcinoma tumor (Caki-1) xenograft mouse model, using two different dosing schemas: weekly dosing at 20-160 mg/kg for 4 weeks, or 50-70 mg/kg daily (5 days on/2 days off) for 3 weeks. Weekly dosing of Supinoxin at 160 mg/kg resulted in a 75% TGD (P<0.001). Daily administration of Supinoxin resulted in dose-dependent TGI (80 and 96%; Day 21) and TGD (68 and 104%, P<0.001), and extended the overall survival of the animals at both doses (P<0.0001). At the dose of 70 mg/kg daily, 6/10 animals demonstrated partial tumor regressions and 1/10 a complete tumor regressions. Supinoxin did not result in a reduction in body weight gain, treatment related deaths, or clinical observations in either of the dosing schemas. Sunitinib (60 mg/kg; daily for 21 days) resulted in TGD for both in vivo studies validating the Caki-1 model herein. These data support the potential therapeutic activity of Supinoxin in renal cell cancers. A Phase 1 study of Supinoxin on relapse/refractory solid tumors is ongoing (NCT02003092).

Introduction

- Supinoxin (RX-5902) is an orally administered, potential first-in-class, small molecule inhibitor of phosphorylated-p68 (P-p68)
- P-p68, which is selectively overexpressed in cancer cells and is absent in normal tissue, increases the activity of multiple cancer related genes including cyclin D1, c-jun and c-myc, and plays a role in tumor progression and metastasis
- RX-5902 is currently being evaluated in a Phase I dose-escalation clinical trial in cancer patients with relapsed/refractory solid tumors (NCT02003092)

Mechanism of Action



- Platelet-derived growth factor (PDGF) stimulation leads to phosphorylation of p68 at Y593 in the cell nucleus
- The Y593-phosphorylated p68 (p-p68) promotes β-catenin nuclear translocation; studies to better elucidate p-p68-Wnt interaction are needed
- p-p68 facilitates β-catenin nuclear translocation by blocking phosphorylation of β-catenin by GSK-3β and displacing Axin from β-catenin
- The β-catenin nuclear translocation and subsequent interaction with transcription factors is required for the epithelial–mesenchymal transition process

Methods

In vitro

Renal cancer cells were plated in 96-well plates. After 24 hours, the cells were treated with various concentrations of RX-5902 for 96 hours. Cell growth inhibition was measured and IC50s were obtained.

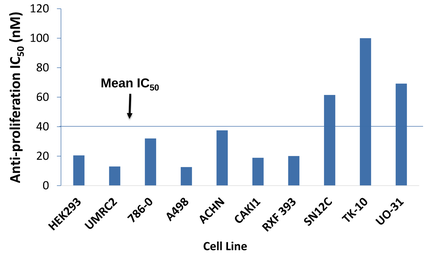
In vivo Studies:

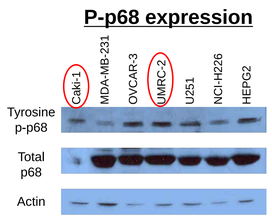
Tumor growth inhibition (TGI), tumor growth delay (TGD), and survival benefits of RX-5902 was evaluated in the human renal cell carcinoma tumor (Caki-1) xenograft mouse model. Tumor cells were injected subcutaneously in female athymic nude mice (*nu/nu*). Drug administration started at group mean starting tumor volume of ~100 mm^3. Tumor volumes and body mass were measured twice weekly until the study ended. RX-5902 oral dosing was performed using two different dosing schemas:

1. Weekly oral dosing (QWK) (oral gavage) at 20-160 mg/kg for 4 weeks
2. 50-70 mg/kg oral daily (oral gavage) (5 days on/2 days off) for 3 weeks

In both studies Sunitinib (tyrosine kinase inhibitor) was administered as a reference agent at 60 mg/kg, po, once daily (QD) for 21 days

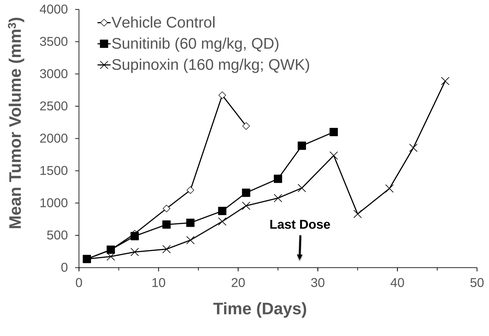
Anti-tumor Activity – In Vitro





- Ten renal cancer cell lines were tested
- Except one cell line (TK-10), all seem to be sensitive to RX-5902
- Overall IC_{50} ~ 39 nM across all cell lines
- High level of p-p68 expression in renal cancel cell lines (Western blots)
- p-p68 may serve as a potential predictive biomarker

Anti-tumor activity – In vivo

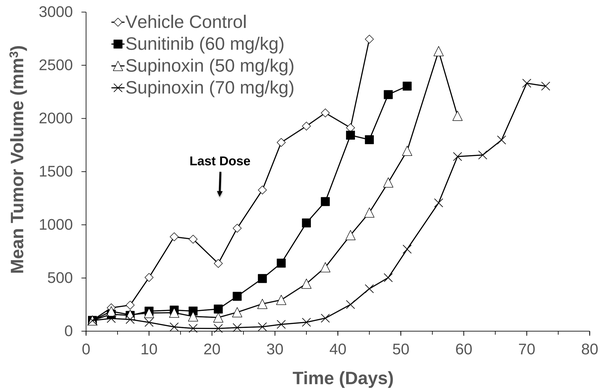


	%TGD	TGI%
Sunitinib (N=7) (60 mg/kg)	48***	60
RX-5902 (N=7) (160 mg/kg, weekly)	60***	75

TGI at Day 21; ***, P<0.001 vs. vehicle

Antitumor activity of RX-5902 in Caki-1 tumor xenograft model. RX-5902, at 160 mg/kg when given orally once weekly for 4 weeks, inhibited tumor growth (both reduction in TGI% and %TGD), and was well tolerated.

Anti-tumor Activity – In vivo



	%TGD	TGI%	Regression PR	Regression CR
Sunitinib (N=10) (60 mg/kg; QD)	36**	63	0	0
RX-5902 (N=10) (50 mg/kg; 5on/2off)	68***	81	0	0
RX-5902 (N=10) (70 mg/kg; 5on/2off)	104***	97	**6/10**	**1/10**

TGI on Day 28. **, P<0.01; ***, P<0.001 Compared to vehicle

- Oral administration of RX-5902, when given on a 5 days ON/2 days OFF schedule for 21 days, dose-dependently inhibited tumor growth in Caki-1 xenograft model
- At a higher daily oral dose of RX-5902 (70 mg/kg), 6 of 10 (60%) mice showed a partial response (PR) and 1 of 10 (10%) mice showed complete response (CR)
- Both doses were well tolerated based on body mass measurements
- Doses of 50 and 70 mg/kg in mice correspond to approximately 250 to 340 mg daily dose in human patients (assuming a 60 kg person)

Summary and Conclusions

- RX-5902 demonstrates antiproliferative effects in various renal cancer cell lines
- When given orally on a weekly basis, or 5 days a week (current clinical dosing paradigm), RX-5902 inhibits tumor growth with clinically meaningful TGI% (>60%)
- These data support the potential therapeutic benefits of RX-5902 in renal cell cancers
- A Phase 1 study of RX-5902 on relapsed/refractory solid tumors is ongoing (NCT02003092)

Investigator Disclosures

All authors are employees of Rexahn Pharmaceuticals, Inc.

For further information about RX-5902 and Rexahn Pharmaceuticals please contact Reza Mazhari (mazharir@rexahn.com) or DJ Kim (kimdj@rexahn.com)