**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 cancer progression, emerging as 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 schemes: 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 RX-5902 was evaluated in the human renal cell carcinoma tumor (Caki-1) xenograft mouse model, using two different dosing schemes: 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<0.0001). 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 schemes. 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).

**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 by IC50 values across all cell lines.

**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. Drug administration started at group mean starting tumor volume of ~100 mm³. Tumor volumes and body mass were measured twice weekly until the study ended. RX-5902 oral dosing was performed using two different dosing schemes:

1. Weekly oral dosing (QWK) (oral gavage) at 20-160 mg/kg for 4 weeks
2. 50-70 mg/kg daily (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 vivo**

<table>
<thead>
<tr>
<th>Tumor Volume (mm³)</th>
<th>RX-5902 (N=10) (60 mg/kg; QD)</th>
<th>RX-5902 (N=10) (50 mg/kg; 5on/2off)</th>
<th>Sunitinib (N=10) (60 mg/kg; QD)</th>
<th>Sunitinib (N=10) (50 mg/kg; 5on/2off)</th>
</tr>
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<tbody>
<tr>
<td>Day 21</td>
<td>36**</td>
<td>68***</td>
<td>63</td>
<td>81</td>
</tr>
<tr>
<td>Mean TGD%</td>
<td>63**</td>
<td>97</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

%TGD TGI% Regression PR CR

Sunitinib (N=10) (60 mg/kg; QD) 63 0 0
Sunitinib (N=10) (50 mg/kg; 5on/2off) 81 0 0
RX-5902 (N=10) (60 mg/kg; QD) 36** 63 0 0
RX-5902 (N=10) (50 mg/kg; 5on/2off) 68*** 97 0/10

**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)