Hepatoma cells, PLC/PRF/5, were incubated with sorafenib, cisplatin, or IDN-7314 to assess the efficacy of sorafenib alone or in combination with cisplatin or IDN-7314 to inhibit tumor growth by activation of necroptosis and increased secretion of TNFα.

Background:

Caspases play a central role in the process of apoptosis, a form of programmed cell death, and in inflammation. Excessive caspase-mediated apoptosis and inflammation are key drivers of pathology in chronic and acute liver disease. As such, caspases are attractive targets for the treatment of a variety of liver diseases.1 However, there has been a longstanding theoretical concern regarding caspase inhibition and its potential to either induce tumor formation or promote tumor growth of preexisting tumors.2 However, there is a growing recognition that there are multiple programmed pathways that can be induced in response to caspases making cell death significantly more complex than originally believed.3 In light of the current understanding of these pathways, it is important to critically reconsider the role of caspases in the setting of cancer.4 While there are in vitro studies showing that inhibition of caspases may increase survival of transformed cells and resistance to chemotherapy5, there are multiple studies in vivo demonstrating that inhibition of caspase activity can facilitate the anti-tumor activity of radiation therapy and chemotherapy6 possibly via activation of necroptosis or increased release of TNFα. To investigate this potential in preexisting tumors, we studied the effect of a pan-caspase inhibitor with and without sorafenib in vitro and in vivo models of HCC.

IDN-7314 is an irreversible pan-caspase inhibitor that inhibits activated caspase 3, 6 and 8 with IC50 values ranging from <0.08 to 7 nM. It also potently inhibited Fox-induced apoptosis of Jurkat cells (CC50 = 6.3 μM) and was protective in the mouse model of Fox-induced hepatic (ED50=0.7 mg/kg, PO).7 In other models of liver disease, IDN-7314 was protective in alcoholic and monocrotaline models.8 IDN-7314 was also protective in the mouse model of viral hepatitis, demonstrating that inhibition of caspases by IDN-7314 affected tumor growth.9 To investigate this potential in preexisting tumors, we studied the effect of a pan-caspase inhibitor with and without sorafenib in vitro and in vivo models of HCC.

IDN-7314 does not affect tumor growth rate nor does it antagonize the efficacy of sorafenib in models of hepatocellular carcinoma

Methods:

**In Vitro Studies:** Hepatoma cells, PLC/PRF/5, were incubated with sorafenib, cisplatin, IDN-7314 or a combination of IDN-7314 plus sorafenib for 72 hr at 37°C with 5% CO2. Survival was assessed using CellTiter-Glo Luminometric Cell Viability Assay.

**PLC/PRF/5 Xenograft Model:** Female Balb/c nude mice were inoculated SC in the right flank region with PLC/PRF/5 tumor cells (1 x 106) in 0.2 mL of PBS with matrigel (1:1). When the mean tumor size was ~200 mm3 (Day 14), mice were treated with vehicle, IDN-7314 (10 mg/kg PO), sorafenib (10 mg/kg PO) and IDN-7314 plus sorafenib (10 mg/kg each PO). N=10 mice/group. Plasma and tumors were collected 0.5 hr after the last dose and kept frozen until the concentration of IDN-7314 was determined by LC/MS/qqMSE.

**Results:**

As expected, both sorafenib and cisplatin reduced the viability of PLC/PRF/5 cells in a concentration dependent manner (Figure 1). Treatment with the potent pan caspase inhibitor, IDN-7314 (0.0015-30 μM) alone had no effect on cell survival.

**Conclusions:**

There were significant levels of IDN-7314 in plasma and tumor (Table 1). Plasma and tumor levels of IDN-7314 were higher in the IDN-7314 plus sorafenib group vs only IDN-7314 group (Table 1). Plasma and tumor levels of IDN-7314 ranged from 0.020-200 nM and 25-40 nM, respectively.

References:

9. Conatus Pharmaceuticals, San Diego, California.

Table 1: Plasma and tumor levels of IDN-7314 0.5 hr after the last dose in female Balb/c nude mice bearing PLC/PRF/5 xenografts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma (ng/mL)</th>
<th>Tumor (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDN-7314 ± Veh</td>
<td>164 ± 9.3</td>
<td>4.1 ± 1.6</td>
</tr>
<tr>
<td>IDN-7314 + Soraf</td>
<td>270 ± 2.1</td>
<td>4.7 ± 0.5</td>
</tr>
</tbody>
</table>

Mouse = 5/4, IDN-7314 only treatment

Figure 2: Potency of sorafenib to reduce cell viability was determined in the presence and absence of IDN-7314. To determine if inhibition of caspases by IDN-7314 affected tumor growth rate nor does it block the efficacy of sorafenib to inhibit tumor growth.

Figure 3: PLC/PRF/5 xenograft growth in female Balb/c mice treated with sorafenib, IDN-7314 or the IDN-7314 plus sorafenib.