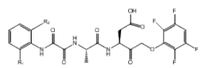
The potent pan-caspase inhibitor IDN-7314 does not affect tumor growth rate nor does it antagonize the efficacy of sorafenib in models of hepatocellular carcinoma

Patricia C. Contreras*, Alfred P. Spada
Conatus Pharmaceuticals, San Diego, California

Introduction:

IDN-7314 is a potent, orally active pan-caspase inhibitor that is a close structural analogue of the pan-caspase inhibitor emricasan, (IDN-6556).



IDN-7314: R_1 = F, R_2 = F
IDN-6556: R_1 = ^{t}Bu, R_2 = H

Emricasan is currently under active clinical development in patients with liver fibrosis due to NASH. In addition, clinical studies are also being conducted with emricasan in patients with liver cirrhosis. The following preclinical studies were carried out to evaluate the effect of the potent pan-caspase inhibitor, IDN-7314 in an animal model of hepatocellular carcinoma (HCC) alone or in combination with sorafenib a chemotherapeutic agent licensed for the treatment of HCC.

Background:

Caspases play a central role in the process of apoptosis, a form of programmed cell death, and 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 independent of 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 chemotherapy[5], there are multiple studies *in vivo* demonstrating that inhibition of caspase activity can facilitate the anti-tumor activity of radiation therapy and chemotherapy[6] possibly via activation of necroptosis or increased release of TNFα[7]. To investigate this potential in preexisting tumors, we studied the effect of a pan-caspase inhibitor with and without sorafenib in *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 IC_{50} values ranging from <0.08 to 7 nM. It also potently inhibited Fas-induced apoptosis of Jurkat cells (IC_{50} = 6.3 nM) and was protective in the mouse model of Fas-induced hepatitis (ED_{50}=0.7 mg/kg, PO)[8]. In other models of liver disease, IDN-7314 was protective in alcohol and monocrotoline models[9]. IDN-7314 was also protective in the bleomycin model of lung fibrosis. In a model of colon carcinoma utilizing cells resistant to 5-FU, IDN-7314 in combination with 5-FU synergistically inhibited tumor growth by activation of necroptosis and increased secretion of TNFα[6]. Therefore, IDN-7314 is suitable for evaluating the effect of caspase inhibition in a model of HCC.

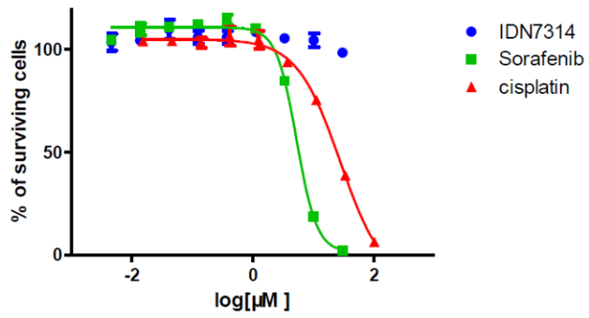
Methods:

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% CO_2. Survival was assessed using CellTiter-Glo® Luminescent 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 10^7) in 0.2 mL of PBS mixed with matrigel (1:1). When the mean tumor size was ~200 mm^3 (Day 14), mice were treated with vehicle, IDN-7314 (10 mg/kg PO), sorafenib (10 mg/kg PO) or 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.

Results:

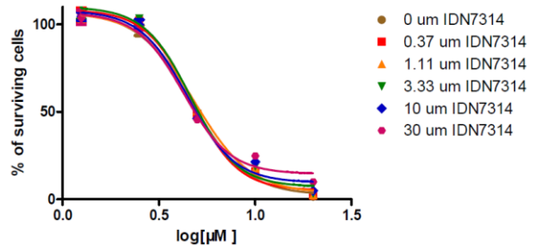
As expected, both sorafenib and cisplatin reduced the viability of PLC/PRF/5 cells in a concentration dependent manner (Figure 1). Treatment with the potent pan caspase inhibitor, IDN-7314 (0.0015-30 μM), alone had no effect on cell survival.



	Sorafenib	Cisplatin
IC_{50} (μM)	5.252	27.57

Figure 1. Survival of PLC/PRF/5 cells incubated with sorafenib, cisplatin or IDN-7314

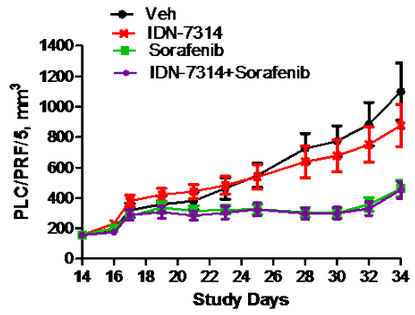
PLC/PRF/5 cells were incubated with sorafenib ± IDN-7314 to assess if caspase inhibition would alter the potency or efficacy of sorafenib. IDN-7314 over a concentration range of 0.37-30 μM had no effect on the potency of sorafenib (Figure 2).



[IDN-7314] (μM)	0	0.37	1.11	3.33	10	30
Sorafenib IC_{50} (μM)	4.720	4.688	4.878	4.621	4.455	4.252

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 or the efficacy of sorafenib, IDN-7314 ± sorafenib was administered daily starting on Day 14 to mice bearing PLC/PRF/5 xenografts. Tumor growth curves and tumor growth inhibition are shown in Figure 3. As expected, sorafenib reduced the size and weight of the tumors. In contrast, there was no effect of IDN-7314 alone on tumor growth. IDN-7314 also did not affect the efficacy of sorafenib as measured by tumor size or weight.



Treatment	Tumor Size[a]		Tumor Weight[a]	
	mm^3	TGI (%)	mg	TGI (%)
Veh/Veh	1100 ± 189	-	1180 ± 626	-
IDN-7314	877 ± 139	20	1040 ± 475	11
Sorafenib	461 ± 54.5*	58	457 ± 180*	61
IDN-7314 + Sorafenib	452 ± 55.1*	59	429 ± 158*	63

TGI=Tumor growth inhibition
[a]Mean ± SEM

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

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 90-200 nM and 29-40 nM, respectively.

Treatment	Plasma (ng/mL)[a]	Tumor (ng/g)[a]
IDN-7314	144 ± 9.98	34.4 ± 1.26
IDN-7314 + Sorafenib	270 ± 21.4*	47.7 ± 5.12*

[a] Mean ± SEM
*p<0.05 vs. IDN-7314 only treatment

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.

Conclusions:

- IDN-7314 alone did not affect the growth of PLC/PRF/5 cells *in vitro*
- IDN-7314 did not affect the potency of sorafenib to kill PLC/PRF/5 cells *in vitro*
- IDN-7314 alone did not affect the growth of PLC/PRF/5 xenograft *in vivo*
- IDN-7314 did not alter the potency of sorafenib to reduce tumor growth rate
- There were significant levels of IDN-7314 in the xenograft

To our knowledge, this is the first report on the effect of a potent pan-caspase inhibitor in a model of HCC. These results indicate potent pan-caspase inhibition does not increase tumor burden of pre-existing tumors nor does it block the efficacy of sorafenib to inhibit tumor growth. These results are consistent with the hypothesis that non-caspase mediated programmed cell death pathways can be activated/utilized when caspase-mediated apoptosis is blocked.

References:

1. Guicciardi ME, Gores GJ. Apoptosis as a mechanism for liver disease progression. Semin Liver Dis 2010;30:402-410.
2. Hanahan D, Weinberg R. Hallmarks of cancer, Cell 2000;100:57-70.
3. Ludde T, Kaplowitz N, Schwabe, R. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. Gastroenterology 2014;147:765-783.
4. Labi V, Erlacher M. How cell death shapes cancer. Cell death and disease. 2015;6:e1675.
5. Fischer U, Janssen K, Schulze-Osthoff K. Does caspase inhibition promote clonogenic tumor growth? Cell Cycle (2007) 6L 3048-3053.
6. Metzig MO, Fuchs D, Tagscherer KE, Gröne H-J, Schirmacher P and Roth P, Inhibition of caspases primes colon cancer cells for r-fluorouracil-induced TNF-α-dependent necroptosis. *Oncogene* (2015); 1-11.
7. Vandenabeele T, Vanden Berghe T, Festjens N. Caspase inhibitors promote alternative cell death pathways. Sci. STKE. 2006;pe44.
8. Ueno H, Kawa M, Shimokawa H, Hirota M, Ohmi M et al., Synthesis and structure-activity relationships of oxamyl dipeptide caspase inhibitors developed for the treatment of liver disease. Bioorganic & Med Chem Lett (2009) 19: 199-202.
9. Copple, BL, Rondelli, CM, Maddox JF, Hoglen NH, Ganey PC and Roth RA, Modes of cell death in rat liver after monocrotaline exposure. *Tox Sci* (2004) 77: 172-182.

Acknowledgements: The PLC/PRF/5 studies were carried out by CrownBio and the analysis of tissues for IDN-7314 were carried out by MicroConstants.

* pcontreras@conatuspharma.com