Carcinogenicity Assessment of the Pan-Caspase Inhibitor, Emricasan, in Tg.rasH2 Mice.

Elbekai, RH[1], Paranjpe, MG[1], Contreras PC[2].

[1]BioReliance Corporation, Rockville, MD 20850 USA, [2]Conatus Pharmaceuticals, San Diego, CA

Abstract

Emricasan, formerly IDN-6556, is a small molecule that is currently being evaluated in clinical trials to reduce hepatic injury and liver fibrosis. Since emricasan is an irreversible pan caspase inhibitor, it is a potent inhibitor of apoptosis and caspase-mediated inflammation. Thus, it was important to determine whether emricasan promotes tumorigenesis in a humanized mouse model. Tg.rasH2 mice received Lab Diet formulated with 0, 10, 25, and 75 mg/kg/day of emricasan, for 26 weeks. At terminal sacrifice, blood was collected for clinical pathology analysis and tissues were collected, fixed in formalin, processed to slide, and evaluated microscopically. There were no treatment related deaths or overt signs of toxicity for the duration of the study. There was no evidence of a carcinogenic effect in the peripheral blood leukocyte counts. Liver microgranulomas, which are background lesions, were slightly increased, especially in males. Increases in the incidence of the activated germinal centers were seen in the spleens and mesenteric lymph nodes of male and female mice, and in the mandibular lymph nodes of male mice. Atrophy of ovaries and testicular degeneration were also seen in emricasan treated animals. Although several non-neoplastic lesions were observed, there was no evidence of emricasan-related tumor formation in any tissue. In addition, none of the non-neoplastic lesions were considered pre-neoplastic. Thus, the potent pan-caspase inhibitor, emricasan, is not considered carcinogenic.

Introduction

Emricasan (emricasan, ((L-alaninamide, N-[2-(1,1-dimethylethyl)phenyl]-2-oxoglycyl-N-[(1S)-1-(carboxymethyl)-2-oxo-3-(2,3,5,6-tetrafluorophenoxy)propyl]) is a small molecule, irreversible inhibitor of activated caspases currently being evaluated to reduce hepatic injury/fibrosis in man. One of the concerns with chronic administration of a caspase inhibitor is the theoretical potential for tumorigenesis. While there are other programmed cell death pathways in addition to caspase-mediated apoptosis that interact to effect cell death and repair, it was important to evaluate directly whether emricasan could promote tumorigenesis in an accepted model of carcinogenesis. The Tg.rasH2 (CByB6F1-Tg(HRAS)2Jic (+/- hemizygous c-Ha-ras)) mouse is a hemizygous transgenic mouse carrying multiple copies of human c-Ha-ras gene with its own promoter and enhancer. A 26-week carcinogenicity assay using Tg.rasH2 mice is accepted as an alternative to the 2-year traditional mouse bioassay by the international regulatory agencies. Thus, the effect of emricasan on carcinogenesis in the Tg.rasH2 transgenic mouse was evaluated.

Materials and Methods

The 26-week study was conducted in Tg.rasH2 mice (Taconic BioSciences, Inc.). Animals were randomized to five groups (N=25/sex). Animals were dosed with feed only, emricasan in feed at dose levels of 10, 25, and 75 mg/kg/day, or the positive control (urethane). On the first day of treatment, animals were 6-10 weeks of age. A subset of CByB6F1 WT animals were bled after one week of dosing, while the remaining animals were bled after twenty-five consecutive weeks, for bioanalysis and TK analysis. Noncompartmental TK analysis was performed using WinNonlin Professional Edition (Pharsight Corporation). Animals were euthanized 26 weeks after the first day of study. Tissues listed in Table 2 were collected, fixed in 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and evaluated microscopically. The incidence of tumors was analyzed by Peto's mortality-prevalence method, without continuity correction, incorporating the context in which tumors were observed. All tissues were examined in the vehicle and emricasan treated groups. Only the Lungs and spleen were examined in urethane treated animals. there was a statistically significant increase in the incidence of splenic hemangiosarcomas and lung adenomas/carcinomas in the positive control group, proving the validity of the assay.

Results

1. Toxicokinetics

Day	Target Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-12} (ng·hr/mL)
176	10	M	2.55	1.50	12.1
		F	2.41	13.5	19.1
	25	M	5.38	1.50	30.0
		F	4.59	1.50	47.6
	75	M	15.7	1.50	91.4
		F	12.8	13.5	123

Table 1: Toxicokinetic parameters for IDN-6556 in the CByB6F1 mice after 176 days of receiving emricasan in feed. Collection was performed at 0800 hours from the control group and at 0800, 1100, 1400, 1700, 2000 hours from the emricasan treated groups. was detected by a validated method using liquid chromatography (LC) with tandem mass spectrometric detection (MS/MS).

2. Survival

Dose	Sex	Cause of Death
0	F	Thyroid Sarcoma
	F	Multicentric Mesothelioma
	F	Spleen Hemangiosarcoma Lung Carcinoma
	F	Mandible Hemangiosarcoma
10	M	Undetermined
	F	Mesothelial Proliferation
	F	Skeletal Muscle Hemangiosarcoma
25	M	Spleen Hemangiosarcoma
75	F	Mesothelial Proliferation
	F	Skin Hemangiosarcoma

Table 2:
Animals found dead or sacrificed in moribund condition and their cause of death. Mortality in the vehicle control and emricasan treated groups consisted of 4 females in the vehicle control, 1 male and 2 females at 10 mg/kg/day of emricasan, 1 male at 25 mg/kg/day, and 2 females at 75 mg/kg/day. The cause of death for these animals was mainly tumor related, although the cause of death could not be established for 1 male animal. None of the deaths were caused by overt toxicity and none were directly related to treatment with emricasan.

3. Non-Neoplastic Lesions

Dose (mg/kg/day)					Dose (mg/kg/day)				
	0	10	25	75		0	10	25	75
Liver Microgranulomas					**Liver Infiltration, Inflammatory cells**				
Males	12	18	24	25	Males	0	6	20	25
Females	19	24	24	25	Females	1	7	20	24
Spleen, Activated Germinal Centers					**Cecum, Submucosal Lymphoid Aggregates**				
Males	3	12	13	7	Males	2	3	2	5
Females	2	5	10	11					
Mesenteric LN, Activated Germinal Centers					**Cecum, Inflammatory Lesions**				
Males	0	6	7	19	Males	0	6	9	6
Females	5	10	15	17	Females	0	4	4	5
Mandibular LN, Activated Germinal Centers					**Colon, Submucosal Lymphoid Aggregates**				
Males	0	2	1	3	Males	6	6	9	14
Ovarian Atrophy					**Testicular Degeneration**				
Females	0	12	22	23	Males	3	3	4	13

Table 3: Emricasan induced a variety of inflammatory and non-inflammatory lesions in Tg.rasH2 mice. However, none of the non-neoplastic lesions were considered preneoplastic.

Results

3. Lung Tumors

	Dose (mg/kg/day)				HCD
	0	10	25	75	
Lung, Single Adenoma					
Males	1	1	2	2	0-6
Females	1	1	2	1	0-6
Lung, Multiple Adenoma					
Males	0	1	0	0	0-1
Females	0	0	0	0	0-1
Lung, Carcinoma					
Males	0	0	0	0	0-2
Females	1	1	0	0	0-1

Table 4: The incidence of lung tumors in control and emricasan-treated Tg.rasH2 mice. *Historical control data data. N=25 animals/sex/dose group. Statistical analysis did not reveal any significant increase in the incidence of lung of spleen tumors in emricasan treated groups (Peto's method, p≥0.05)

4. Hemangiosarcomas

	Dose (mg/kg/day)				
MALES	0	10	25	75	HCD
Spleen	0	1	2	1	0-4
Multicentric	1	0	0	0	NR
Nasal Cavity[1]	0	1	0	1	0-1
Total	1	2	2	2	0-4
	Dose (mg/kg/day)				
FEMALES	0	10	25	75	HCD
Spleen	1	0	1	1	0-4
Skeletal Muscle	0	1	0	0	NR
Ovary[1]	1	0	0	0	0-1
Salivary Gland	0	1	0	0	NR
Skin	0	0	1	1	0-1
Mandible	1	0	0	0	0-1
Total	3	2	2	2	0-5

Table 5: The incidence of vascular tumors in control and emricasan-treated Tg.rasH2 mice. NR: Not recorded in BioReliance Historical Control Range. N=25 animals/sex/dose group. Statistical analysis did not reveal any significant increase in the individual or combined incidence of all vascular tumors, in emricasan treated groups (Peto's method, p≥0.05).[1]A hemangioma was diagnosed in this tissue. In all other tissues, a hemangiosarcoma was diagnosed

5. Rare, Non-Vascular , Non-Pulmonary Tumors

There was a limited number of non-vascular, non-pulmonary tumors in vehicle and emricasan treated groups. The incidence was limited to only one animal in a group. These tumors involved isolated organs and/or falls within the historical control ranges established at BioReliance.

Conclusion

Emricasan did not increase the incidence of neoplastic lesions in Tg.rasH2 mice and is thus considered to have no carcinogenic potential in human.

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