

METABOLISM AND PHARMACOKINETICS OF ENMD-1198, AN ORALLY BIOAVAILABLE MICROTUBULE DESTABILIZING AGENT, IN RATS, DOGS AND HUMANS

Theresa M. LaVallee¹, Glenn M. Swartz¹, Gizachew Kifle¹, William E. Fogler¹, Anthony M. Treston¹, Daniel L. Gustafson², Carolyn F. Sidor¹ and D. Ross Camidge³

¹EntreMed, Inc., Rockville, Maryland; ²Colorado State University, Fort Collins, CO; ³University of Colorado Cancer Center, Denver, CO

ABSTRACT

Background: Clinical studies with 2-methoxyestradiol (2ME2, Panzeno[®]), a microtubule destabilizing agent, demonstrate an excellent safety profile for 2ME2 with evidence of clinical benefit, including prolonged stable disease, and complete and partial responses across a range of patients with different advanced cancers. Studies have demonstrated that 2ME2 is metabolized by conjugation at the 3- and 17-positions, as well as oxidation at the 17-position. A series of analogs of 2ME2 modified at positions 3 and 17 was generated. ENMD-1198 (2-methoxyestra-1, 3, 5, (10), 16-tetraene-3-carboxamide) was selected as the lead molecule for further clinical study due to good metabolic stability and potent antiproliferative properties in preclinical models. Preclinical toxicity studies evaluating daily, oral dosing of ENMD-1198 for 28 days have shown that there is a 10-fold difference in the maximum tolerated dose (MTD) between rats and dogs. In female rats, the MTD is 60 mg/m²/d, which results in a C_{max} of 927 ng/mL and an AUC of 3724 ng/mL·h, whereas the MTD in female dogs is 600 mg/m²/d, which results in a C_{max} of 4395 ng/mL and an AUC of 29235 ng/mL·h. The organs affected by ENMD-1198 in both species were those with cell populations of high mitotic potential including bone marrow, lymph nodes, spleen, thymus, GI tract, and testes, consistent with its mechanism of action.

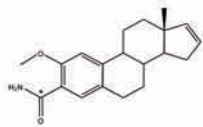
Methods: To assess metabolism differences between rats and dogs, radiolabeled compound was synthesized and metabolic profiles were evaluated. A first-in-man Phase 1 clinical trial of ENMD-1198 in patients with advanced cancers is ongoing with a starting dose based on data from the most sensitive species (rats). The metabolic profiles from the ENMD-1198 dosed rats and dogs were compared to the plasma samples from patients dosed with 30 mg/m² of ENMD-1198.

Results: While there were clear differences between rats and dogs, overall, the common metabolism of ENMD-1198 appears to be the formation of metabolites via hydroxylation of the B or D ring or des-methylation of the O-methyl group followed by conjugation with glucuronide or sulfate. Minimal amounts of the conjugates were found in plasma of rats. Consistent with preclinical data from both the rat and dog, once daily, oral dosing of ENMD-1198 in humans demonstrates dose proportionality across the dose range 5-30 mg/m². At 5, 10, 20 and 30 mg/m², mean peak plasma exposures are 68.8, 141.2, 418.7 and 677.8 ng/mL, respectively. The plasma concentration time curves have resulted in AUCs of 1312, 1074, 2138 and 3914 ng/mL·h, respectively and a half life of approximately 13 h (range 10.6 to 19.5).

Similar to dogs, ENMD-1198 was the major component in the human plasma at all time points. There were no dominant metabolites (>20% of total area of the identified peak) found in human, rat or dog plasma. M7a/b and M10, hydroxylated ENMD-1198, are the most abundant plasma metabolites in all three species.

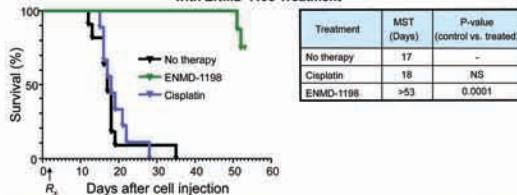
Conclusions: ENMD-1198 is a 2ME2 analog with good pharmacokinetic parameters and significant preclinical antitumor activity. There is a species difference in sensitivity, but no differences in dominant metabolites. A Phase 1 dose escalation study in humans is ongoing, with dose proportional PK exposures across the range 5-30mg/m², and a metabolite profile in humans that appears similar to both rats and dogs.

Figure 1. Structure of ENMD-1198



2-Methoxyestra-1, 3, 5, (10), 16-tetraene-3-carboxamide, MW 311.42
Site of ¹⁴C-label indicated by the *.

Figure 2. Significantly Increased Survival of H2122 Tumor Bearing Mice with ENMD-1198 Treatment



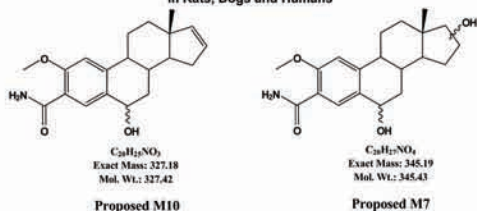
Female SCID mice were inoculated in pleural cavity with 1x10⁶ H2122 (NSCLC) cells. After 3 days, mice were treated once daily with vehicle or ENMD-1198 (200 mg/kg) administered by oral gavage or i.p. q3dx7 Cisplatin (6 mg/kg) until dead or sacrificed at the end of the study. All no therapy and Cisplatin treated mice were dead on day 35 and 28, respectively. The ENMD-1198 treated mice were sacrificed on day 53 to assess the dissemination of tumor in the thoracic cavity. The necropsy result indicated there was tumor, although few tumor nodules were found in the lung, diaphragm, or ribcages. Results demonstrate significantly increased survival with ENMD-1198 as compared to Cisplatin.

Table 1. The NOEL and MTD of ENMD-1198 Has a 10-Fold Difference in Rats and Dogs

	Sprague Dawley Rats			Beagle Dogs		
	Oral Dose (mg/m ²)	C _{max} (ng/mL)	AUC (ng·hr/mL)	Oral Dose (mg/m ²)	C _{max} (ng/mL)	AUC (ng·hr/mL)
NOEL	30	392	1041	400	3111	25485
MTD	60	927	3724	600	5092	40093

No observed effect levels (NOEL) and maximum tolerated doses (MTD) of ENMD-1198 were determined after repeated daily oral administration for 28 days. Toxicokinetic parameters of ENMD-1198 were determined after daily oral administration on study day 1 using LC/MS/MS analysis. The mean values for male and female beagle dogs and female rats are shown (plasma levels in male rats are not shown and are lower than the female by approximately five fold).

Figure 3. Putative Structures for the Major Metabolites Identified in Rats, Dogs and Humans



The structures of metabolites were proposed based on available mass spectral data.

Table 2. Plasma Metabolic Profiles from Orally Dosed ¹⁴C-ENMD-1198 in Rats

Time Post Dose	Total # of Peaks	Peak #	% HPLC Distribution of ¹⁴ C-ENMD-1198 and Its Metabolites (300 mg/m ² , po), ≥5.0% in Any of the Samples			
			3h	6h	12h	24h
Sprague Dawley Rats (Male/Female)	33	9	ND	ND	ND	7.96
		M4a	1.84	1.42	2.03	14.00
			2.08	2.35	1.00	13.14
		M7a	7.00	5.60	8.78	18.8
			2.94	3.64	10.21	10.98
		M7b	7.18	7.29	1.5	0.78
			1.04	1.06	0.60	0.70
		M9a	4.87	4.66	3.96	5.75
			0.80	0.70	3.39	11.65
		M9b	5.85	7.52	7.62	2.36
	0.88	0.53	1.77	0.87		
M10a	0.23	0.19	1.06	1.33		
	0.78	0.77	2.86	5.49		
M11	4.03	6.61	12.8	8.43		
	0.49	ND	ND	ND		
M10b	10.58	10.03	6.36	1.84		
	2.00	1.93	1.91	1.84		
M12	7.04	7.04	0.34	ND		
	3.82	3.39	1.48	2.87		
50	0.69	1.01	1.75	0.87		
	1.97	1.21	2.81	5.49		
ENMD-1198	18.28	15.30	4.97	2.08		
	75.02	75.95	43.27	2.50		

Sprague Dawley rats were orally dosed with ¹⁴C-ENMD-1198 and determination of metabolites in plasma samples was accomplished using LC/MS/MS and LC/MS/MRM (multiple reaction monitoring) detection, where ion transition from molecular ion to specific fragment ion(s) are detected. The major metabolites found in all matrices of rat were hydroxylation product of ¹⁴C-ENMD-1198. The Phase 1 hydroxylation metabolites includes isomers of mono-hydroxylation (MW 327), di-hydroxylation (MW 345 and MW 343), desmethyl (phenol, MW 297), and possible tri-hydroxylation (MW 359) product of ¹⁴C-ENMD-1198. The Phase 2 major metabolites include conjugates of hydroxylation metabolites. The conjugates include, glucuronide (all less than 5% in plasma) and sulfate (M4a). There were 33 peaks detected. ND, Not detected.

Table 3. Plasma Metabolic Profiles from Orally Dosed ¹⁴C-ENMD-1198 in Dogs

Time Post Dose	Total # of Peaks	Peak #	% HPLC Distribution of ¹⁴ C-ENMD-1198 and Its Metabolites (600 mg/m ² , po) ≥5.0% in Any of the Samples			
			0.5h	1h	6h	24h
Beagle Dog (Male/Female)	17	M7a	2.08	3.22	5.53	5.72
			2.45	4.54	8.43	10.87
		M7b	0.59	1.44	2.15	2.62
			0.73	0.99	2.68	4.12
		M9a	ND	ND	0.61	1.05
			ND	0.18	0.86	1.72
		M10b	1.48	ND	ND	1.31
			0.68	0.83	0.93	1.26
		ENMD-1198	92.06	89.14	79.75	80.23
			88.75	85.97	75.71	72.14

Beagle dogs were orally dosed with ¹⁴C-ENMD-1198 and determination of metabolites in plasma samples was accomplished using LC/MS/MS and LC/MS/MRM (multiple reaction monitoring) detection, where ion transition from molecular ion to specific fragment ion(s) are detected. The major metabolites found in all matrices of dog were hydroxylation product of ¹⁴C-ENMD-1198. The Phase 1 hydroxylation metabolites includes isomers of mono-hydroxylation (MW 327), di-hydroxylation (MW 345 and MW 343), desmethyl (phenol, MW 297) product of ¹⁴C-ENMD-1198. The Phase 2 metabolites are glucuronide conjugates of hydroxylation metabolites. There were 17 peaks detected. ND, Not detected.

Table 4. Human Metabolic Profile (30 mg/m² Cohort; ng/mL)

Metabolite (MRM)	Patient 1			Patient 2			Patient 3		
	0.5-2h	4-8h	24h	0.5-2h	4-8h	24h	0.5-2h	4-8h	24h
M7a: (m/z 346→329)	15.6	7.3	3.5	9.9	5.2	2.4	13.8	7.9	2.1
M7b: (m/z 346→329)	20.0	11.5	4.0	12.8	8.2	3.0	15.8	10.6	2.8
M10b: (m/z 328→311)	7.3	5.8	6.3	6.2	5.9	5.4	5.9	8.0	6.2
ENMD-1198 (m/z 312→295)	424.9	143.0	49.6	312.4	87.7	34.9	277.5	154.0	40.9

The day 1 human plasma samples from the cancer patients in the 30 mg/m² cohort of orally administered ENMD-1198 were pooled into 3 samples/pl. Samples were subjected to LC/MS/MS analysis and metabolites monitored were focused on those metabolites found in rat plasma, urine, feces, and bile and in dog plasma and urine. A total of 13 components, including parent drug, were detected in human plasma samples. Samples with compound >5 ng/ml are listed. Sulfate conjugate (M3 and M4) and dihydroxylation metabolites (M9) found in rat and dog samples were either not present or under detection limit in human plasma.

Table 5. Human Pharmacokinetics From Once Daily Orally Administered ENMD-1198

Dose (mg/m ²)	5	10	20	30
n	4	3	3	3
C _{max} (ng/mL)	68.8	141.2	418.7	677.8
T _{max} (h)	1.3	1.7	1.3	1.0
AUC _{0-∞} (ng·hr/mL)	312.4	1073.8	2138.1	3914.4
Half-life (h)	12.2	10.6	11.0	19.5
CL/F (L/h/m ²)	16.5	7.7	8.4	5.5
Vd/F (L/m ²)	291.5	108.5	134.5	153.0
AUC _{0-∞} /dose	62.5	107.4	106.9	130.5

Day 1, cycle 1 mean pharmacokinetic values for cancer patients after oral dosing with ENMD-1198. ENMD-1198 in human plasma was measured using a validated LC/MS/MS based-assay following liquid-liquid extraction. PK data was modeled using non-compartmental analysis. Dose escalation is on-going.

CONCLUSIONS

- Orally administered ENMD-1198 demonstrates good pharmacokinetic parameters in rats, dogs and humans.
- ENMD-1198 was the major component in plasma and the metabolic profiles were similar in rats, dogs and humans.
- In human plasma, there were no major metabolites (i.e. >20% of the total area of the identifiable peaks) found.
- The most abundant metabolite (M7) in human plasma was also the most abundant metabolite in rat and dog plasma. The second most abundant metabolite (M10b) was a minor metabolite in rat plasma and a trace metabolite in dog plasma. Compounds are being synthesized to verify structural identity.
- The major metabolic pathways for ENMD-1198 appear to be Phase 1 (oxidation and hydroxylation of the ring and O-demethylation). Phase 2 conjugates were found in rats and at very low levels in human plasma.
- Despite the metabolic analyses, no dominant metabolite was found that explains the species difference in sensitivity to ENMD-1198.
- ENMD-1198's Phase 1 study continues in refractory solid tumor patients with the current dose level approaching the MTD in rats without any ENMD-1198-related toxicity.