

Concentration of Milataxel (MAC-321, TL00139) and its Major Metabolite (M-10) in Xenograft Tumors from Mice Dosed Orally and Intravenously

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Abstract

Milataxel is a novel taxane that has shown substantial anti-tumor activity versus taxane-sensitive and taxane-resistant human tumors in mouse xenograft models. Milataxel has entered phase II clinical studies. Mice implanted with human xenograft tumors (SKMES-lung, HCT-15-colon and A-375 melanoma) were treated with oral and a non-cremophor milataxel IV formulation. Concentrations of milataxel and its major metabolite, M-10, were measured using HPLC-UV- β RAM and LC/MS. Levels for parent and metabolite were found to be different when measuring 14 C labeled species in tumor. M-10 levels were typically 50% higher than parent in tumors from non-Pgp cell lines when milataxel was administered either orally or IV. Measurable levels of parent drug and metabolite persisted for 10 days post dosing. In-vitro IC50 evaluation of milataxel and M-10 show both to be potent cytotoxics, but M-10 was shown to be a substrate for Pgp. Oral milataxel has completed a phase I study and plasma levels of M-10 and parent were measured.

Introduction

Milataxel, a potent taxane in clinical studies, has shown curative activity in SKMES (human lung) tumors implanted in nude mice.¹ SKMES tumors² harvested from nude mice post dosing with 14 C labeled milataxel (73 mg/kg QDX1—IV and 104 mg/kg QDX1 PO) were processed and drug and metabolite concentrations were determined by LC/MS.³ The concentration of the primary mouse and human metabolite (M-10) was higher than parent in SKMES and A375 (melanoma)² at the time-points measured and M-10 was present up to 10 days in most xenograft tumors studied. Drug and metabolite concentrations in HCT-15 (human colon)⁴ were also measured and showed significant levels of the active metabolite, M-10.

Methods & Materials

- HPLC-UV- β RAM, Model: LC-10, Shimadzu/Model: (IN/US Systems)—Used to chromatographically separate and detect analytes by UV absorbance and radioactive 14 C level.
- LC/MS/MS System, Model API 300 and API 3000 (Sciex) or Model: LC Quattro (Micromass).
- HPLC Columns—Chromolith Performance RP-18e, 100X4.6 (Merck), Discovery C18, 250 X 4.6 X 5 um (Supelco), Genesis C-18, 50 x 2.1 mm x 4 um (Jones Chromatography), Curosil-G, 250 x 4.6 x 4 um (Phenomenex).
- 14 C milataxel—Provided by Wyeth Pharmaceuticals, Radio Synthesis Department.
- M-10 Standard—Synthesized by Taxolog Inc. Fairfield NJ.

Tumor Xenografts

- SKMES Human non-small cell lung carcinoma (Piedmont Research Center, Morrisville, NC).
- HCT-15 Human colon carcinoma (Wyeth Research, Pearl River, NY).
- A375 Human melanoma (Piedmont Research Center, Morrisville, NC).

Formulation

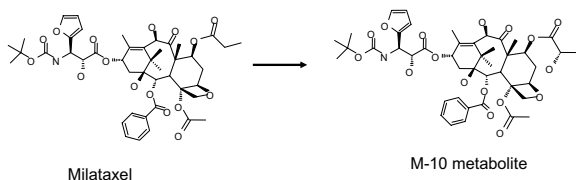
- IV dosing solutions-5% ethanol, 95% Liposyn II.
- PO dosing solutions-5% ethanol, 5% Cremophor, 90 saline.

Tumor Extraction Preparation

- Tumor tissue homogenized, and extracted with organic solvent.

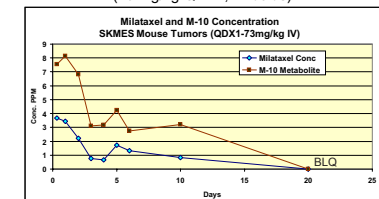
Structures and Metabolism

- Primary mouse metabolism of milataxel observed in-vitro.



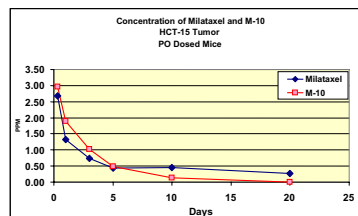
Results

Figure 1. Concentration of 14 C Milataxel and 14 C M-10 in SKMES Mice Tumors (73 mg/kg QDX1, IV bolus)



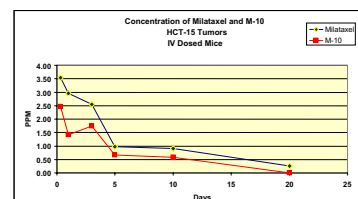
Summary: Table of Levels of milataxel and M-10 Metabolite in Female Harlan Nude Mice With SKMES Tumors; Mice Treated with milataxel Via a Single Intravenous (IV) Injection (73mg/kg); Vehicle: 5% Ethanol-95% Liposyn II. Drug and metabolite detected with beta ram and LC/MS. BLQ-Below Limit Of Quantitation

Fig 2. Concentration of Orally Dosed Milataxel and M-10 in HCT-15 Mice Tumors from 8 hours to 20 days.



Summary: Tumors analyzed post dosing, 104mg/kg QDX1, by extraction of tissue and LC/MS analysis.

Fig 3 Concentration of IV Dosed Milataxel and M-10 in HCT-15 Tumors from 8 hours to 20 Days



Summary: Tumors analyzed post dosing, 73mg/kg QDX1, by extraction of tissue and LC/MS analysis.

Table 1. MTT IC50 Values for Milataxel⁵

MTT IC50 Values in Multiple Cell Lines (nM)		
Cell Line	Milataxel	M10
DLD1	1.0	3.3
HT29	0.7	0.7
Panc1	1.4	1.2
786-0	0.6	1.8
A375	0.6	0.9
A549	0.4	0.4
HOP18	1.2	1.3
MSTO	1.0	2.1
OVCAR4	0.9	1.3
OVCAR5	0.5	0.6
SkeMel28	0.9	1.0
SNB19	1.2	1.5
TK10	0.3	0.5
HCT116	0.5	0.5

Table 2. A-375

14 C Milataxel and 14 C M-10 Concentration in A375 Mice Tumors				
Time-point	Dosing QDX1	Tumor Weight (g)	Milataxel Conc. (ppm)	M-10 Conc. (ppm)
24 Hours Post Dosing	IV (73mg/kg)	0.46	0.44	1.57
24 Hours Post Dosing	PO (104 mg/kg)	0.38	0.55	1.81

Summary: Mice tumor source: (n=1), Athymic female nude Harlan mice, Piedmont Research study PK-e18. LC/MS detection was used for parent and metabolite.

Conclusions

- In tumor lines with low MDR-1 expression, M-10 accumulation in tumors is greater than parent when dosed IV or PO.
- In tumor lines with higher MDR-1 expression, IV dosage shows higher level of parent than M-10, but is reversed with PO dosing.
- The mechanism for higher metabolite concentration in IV dosed mice is not understood.
- M-10 is an active metabolite that shows absorption and retention in several mice tumors and may contribute to the exceptional activity observed in mice implanted with human xenograft tumors.
- Milataxel and M-10 have been observed in human plasma.⁶

References

- TL00139 (MAC-321), A New Taxane With Curative Antitumor Activity Against Human Tumor Xenografts, B. Hollister, et al; Abst. 2734, Annual 2003 AACR Meeting.
- Tumors obtained from implanted mice, Piedmont Research Center Inc., Morrisville, NC.
- LC/MS analysis conducted at Exygen Research, State College, PA
- Tumors obtained from implanted mice, Wyeth Research, Pearl River, NY.
- MTT data generated at Taxolog Research, Tallahassee FL (Hae Lim, Ph.D.).
- Human phase one study-102 conducted by Wyeth-Oral dosing every three weeks.