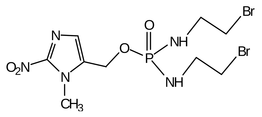
PHASE 1/2 STUDY OF TH-302 COMBINED WITH DOXORUBICIN IN SOFT TISSUE SARCOMA

Chawla SP[1], Cranmer LD[2], Rushing D[3], Butrynski JE[4], Okuno SH[5], Adkins D[6], Reed D[7], Langmuir VK[8], Lorente GA[8], Kroll S[8], Ganjoo KN[9]

[1]Sarcoma Oncology Center, Santa Monica, CA; [2]Arizona Cancer Center, Tucson, AZ; [3]Indiana University Simon Cancer Center, Indianapolis, IN; [4]Dana-Farber Cancer Institute, Boston, MA; [5]Mayo Clinic, Rochester, MN; [6]Washington University, St. Louis, MO; [7]H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL [8]Threshold Pharmaceuticals, Redwood City, CA; [9]Stanford University Medical Center, Stanford, CA.

INTRODUCTION

- TH-302 is a nitroimidazole prodrug of the cytotoxin, bromo-isophosphoramide mustard (Br-IPM).



TH-302

- Under normoxic conditions, TH-302 is essentially inactive but in hypoxic conditions, the nitroimidazole is reduced and Br-IPM is released to alkylate DNA.
- TH-302 has been designed to be selectively activated in highly hypoxic regions that are unlikely to occur in normal tissues. These hypoxic regions are known to be associated with chemotherapy and radiotherapy resistance.
- Preclinical data suggest that after activation, the active moiety may diffuse to areas outside the hypoxic region, a "bystander" effect which may provide additional anti-tumor activity.
- In xenograft sarcoma models, TH-302 was active alone and enhanced the activity of doxorubicin.
- The current study is designed to investigate the safety and activity of TH-302 in combination with doxorubicin in soft tissue sarcoma (STS).

METHODS

Primary Objectives

- Dose escalation: To determine the maximum tolerated dose (MTD) and dose limiting toxicities (DLTs) of TH-302 when used in combination with doxorubicin and prophylactic growth factor support in subjects with relapsed STS
- To make a preliminary assessment of the efficacy of TH-302 in combination with doxorubicin as determined by progression-free rate at 6 months in subjects with advanced soft tissue sarcoma previously untreated with chemotherapy (neoadjuvant and adjuvant chemotherapy permitted) treated at the MTD
- To assess the safety of TH-302 in combination with doxorubicin

Secondary Objectives

- To make a preliminary assessment of the efficacy of TH-302 in combination with doxorubicin as determined by progression-free rate at 3 months, progression-free survival, response rate, duration of response, and overall survival in subjects with advanced soft tissue sarcoma treated at the MTD
- To establish the pharmacokinetics of TH-302 when used in combination with doxorubicin

Study Design

- Open-label, multi-center, dose-escalation study
- Classic dose escalation design
  - Starting dose 240 mg/m^2
  - 40% dose escalations with 3-6 subjects per cohort (smaller dose escalations allowable)
  - MTD defined as highest dose level at which 0 or 1 of 6 subjects experiences a DLT
- Procedures/Assessments
  - TH-302 administered IV over 30-60 minutes
  - Doxorubicin 75 mg/m^2 administered as IV bolus 2 hours after completion of TH-302 for a maximum of 6 cycles
  - Planned enrollment: up to 24 patients in dose escalation followed by 75 patients treated at MTD as part of the dose expansion cohort
  - Prophylactic growth factor support (G-CSF) was added after dosing on Day 8 for all patients after the first cohort at 240 mg/m^2
  - ECG and LVEF measurement at baseline, end of Cycle 4 and study termination
  - Response evaluated according to RECIST criteria after every 6 weeks of treatment
  - Patients with stable or responding disease and acceptable toxicity could receive TH-302 alone after 6 cycles
  - PK sampling for analysis of TH-302 and Br-IPM (Day 1, Day 8) and doxorubicin and doxorubicinol (Day 1)

RESULTS

57 patients (16 patients in dose escalation cohort and 41 patients in the dose expansion) were treated with TH-302 + bolus doxorubicin at 8 US sites from 01 January 2009 to 09 August 2010 (**Table 1**).

- Twenty-nine patients completed 6 cycles
  - 21 patients received single agent TH-302 after 6 cycles
  - 8 patients are ongoing receiving single agent TH-302
- Twenty-one patients discontinued prior to 6 cycles from study:
  - Progressive disease - 9
  - Adverse event - 5
  - Subject decision – 5
  - Investigator decision – 1
  - Clinical deterioration – 1
- Seven patients are ongoing on-study receiving TH-302 and doxorubicin

Table 1: Demographics and Baseline Characteristics

Characteristic		
Gender (N)	Female	33
	Male	24
Age (years)	Median	59
	Range	19-85
ECOG (N)	0	19
	1	38
Prior systemic therapies (N)	0	47
	1	3
	2	0
	3	1
	Neoadjuvant	1
	Adjuvant	5
Histology	Leiomyosarcoma	19
	Liposarcoma	14
	Synovial sarcoma	3
	Malignant fibrous histiocytoma (MFH)	15
	Angiosarcoma	2
	Fibrosarcoma	1
	Unclassified/undifferentiated	3
Disease Status	Locally Advanced Unresectable	10
	Metastatic	47

Safety

Study Drug Exposure and DLT

- Because of grade 4 neutropenia (not meeting 5-day duration definition for DLT) in 3 of 3 patients at 240 mg/m^2, prophylactic G-CSF was added at Day 8 of each cycle for subsequent patients
- Two patients had DLT at 340 mg/m^2: Grade 4 thrombocytopenia and grade 3 infection (cellulitis) with grade 4 neutropenia
- One patient of the first 6 patients had a DLT at 300 mg/m^2: Grade 3 febrile neutropenia
- Two additional DLTs in the next 41 patients at 300 mg/m^2: Grade 3 fatigue, Grade 4 Thrombocytopenia
- The maximum tolerated dose was established at 300 mg/m^2.

Table 2: Study Drug Exposure and DLTs

Dose (mg/m^2)	Prophylactic G-CSF	No. of Patients	Median Cycles (range)	DLTs
240	No	3	8 (8 - 14)	0
240	Yes	3	3 (2 - 6)	0
300	Yes	47	5 (1 - 14)	3
340	Yes	4	1.5 (1 - 8)	2

Adverse Events (AEs)

- No study drug-related deaths have been reported.
- The most frequent non-laboratory adverse events were nausea and fatigue, which were generally either grade 1 or grade 2 (**Table 3**).
- Skin and mucosal adverse events were dose limiting in the single agent TH-302 Phase 1 study. Skin rash (30%) and hyperpigmentation (25%) and stomatitis (37%) were reported.
- Severe non-laboratory AEs (Grade 3/4/5) regardless of relationship to study drug occurring in more than one patient were fatigue (3), tachycardia (2), deep vein thrombosis (2), small intestine obstruction (2), hydronephrosis (2) and urticaria (2).
- Five patients discontinued from the study for an AE (perirectal abscess, airway obstruction by tumor, acute renal failure due to ureteral obstruction by tumor, erythematous rash, hand-foot syndrome).

Table 3: Adverse events regardless of relationship to study drug occurring in 25% of more Patients (all cycles)

Adverse event	Number of Patients with an AE (%) by Dose in mg/m^2					
	240 (N=3)	240 + G-CSF (N=3)	300 + G-CSF (N=47)	340 + G-CSF (N=4)	Total (N=57)	Grade 3/4
Fatigue	2	2	31	2	37 (65%)	3 (5%)
Nausea	3	1	30	3	37 (65%)	0 (0%)
Alopecia	2	1	22	2	27 (47%)	NA
Stomatitis	1	1	19	0	21 (37%)	0 (0%)
Back pain	1	2	14	1	18 (32%)	1 (2%)
Constipation	0	2	14	2	18 (32%)	1 (2%)
Diarrhoea	0	1	16	0	17 (30%)	1 (2%)
Rash	2	1	14	0	17 (30%)	0 (0%)
Anorexia	0	1	13	2	16 (28%)	0 (0%)

*CTCAE v3

Hematologic Toxicity

- Without G-CSF, significant neutropenia was observed at the lowest dose of TH-302 of (240 mg/m^2)
- With G-CSF, grade 4 neutropenia was observed in 2 of 4 patients at 340 mg/m^2 and a 3rd patient had grade 4 thrombocytopenia. All 3 patients had risk factors for increased hematologic toxicity
- At the MTD the frequency of grade 3/4 neutropenia was 24%
- Five events (9%) of febrile neutropenia were reported
- Hematologic toxicity is provided by dose group (**Table 4**).

Table 4: Hematologic Toxicity* (All cycles)

Hematologic	Number of Patients with Grade 3/4 (%) by Dose in mg/m^2					
Grade 3/4	240 (N=3)	240 + G-CSF (N=3)	300 + G-CSF (N=46)	340 + G-CSF (N=4)	Total (N=56)	Grade 4
Neutropenia	100%	33%	24%	75%	32%	21%
Thrombocytopenia	33%	0%	26%	50%	27%	11%
Anemia	0%	33%	22%	50%	23%	0%

*CTCAE v3

Other Laboratory Data

- There has been no evidence of renal or liver toxicity related to TH-302 and no other consistent laboratory abnormalities.

Pharmacokinetics

- The pharmacokinetics of doxorubicin and doxorubicinol are not altered by TH-302 based on comparisons with historical data
- The mean doxorubicin maximum concentration, AUC and terminal half-life were 2.4 ug/mL, 2.6 ug-h/mL and 17.1 h.
- The mean doxorubicin maximum concentration, AUC and terminal half-life were 0.04 ug/mL, 1.3 ug-h/mL and 25.8 h.
- TH-302 and Br-IPM data are summarized in Figure 1 and Figure 2

Figure 1: TH-302 plasma concentrations

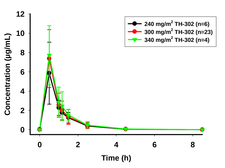
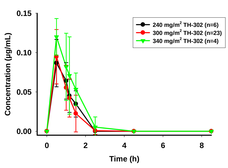


Figure 2: Br-IPM plasma concentrations



TH-302 Activity

- 54 patients had at least one evaluable post-treatment tumor assessment (**Figure 3**)
- 18 patients (33%) had a partial response including 12 patients with a confirmed partial response and 4 patients with ongoing response pending confirmation.
- 28 patients (52%) had a best response of stable disease
  - 8 discontinued with progressive disease; all had received at least 5 cycles
  - 12 discontinued for reason other than progressive disease after a median of 4 cycles
  - 8 are ongoing on-study after a median of 7.5 cycles
- Best response is provided for each sarcoma subgroup classification (**Table 5**). The stable disease or better rate was at least 75% in each of the subgroups.

Figure 3: Best Response by RECIST

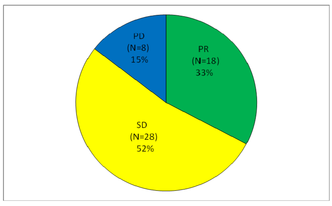


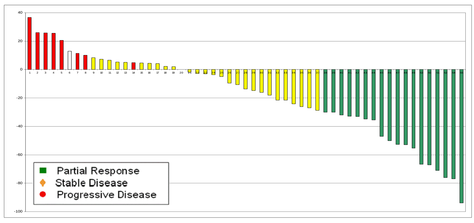
Table 5: Best Response by Sarcoma Classification

		Best Response		
Sarcoma Classification	N	PR	SD	PD
Leiomyosarcoma	19	37%	42%	21%
Malignant fibrous histiocytoma	14	43%	50%	7%
Liposarcoma	12	25%	58%	17%
Other*	9	22%	67%	11%
Total	54	33%	52%	15%

* Other: synovial (3), unclassified (3), angiosarcoma (2), high-grade fibrosarcoma (1).

- A waterfall plot for the change in the sum of the longest diameters for all target lesions is provided in **Figure 4**.

Figure 4: Waterfall Plot for Change in Sum of Longest Diameters of Target Lesions



* Patient 7 and Patient 14 progressed based on the presence of new lesions; Patient 8 progressed based on progression of nontarget lesions

- A Kaplan-Meier plot for progression-free survival (PFS) is provided in **Figure 5**.
- The median PFS was 6.4 months (95% CI: 5.6 to 6.9 months).
- The 6-month progression-free rate was 56%.

Figure 5: Progression-free Survival

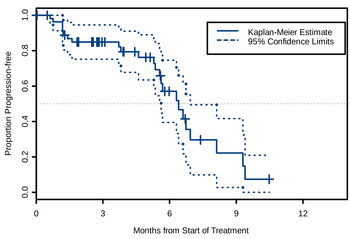
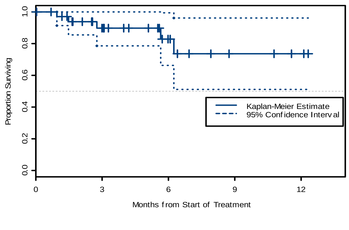


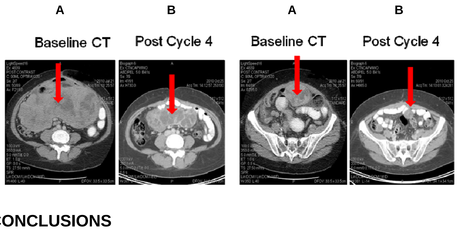
Figure 6: Overall Survival



Case Study

A 65 year old female was original diagnosed with a leiomyosarcoma of the uterus which was excised in August 2009. The patient received adjuvant therapy with gemcitabine and docetaxel for the next three months. Peritoneal metastasis were identified in July 2010 including a large 28 cm mass. The patient initiated TH-302 (300 mg/m^2) and doxorubicin (75 mg/m^2) in August 2010 and has currently completed 4 cycles of the combination.
The end of Cycle 4 assessment demonstrated > 40% reduction in target lesions.
A – Baseline CT
B – Post-Cycle 4 CT demonstrating clear shrinkage of both lesions (RECIST PR)

Figure 7: Case Study



CONCLUSIONS

- A dosing regimen of TH-302 (300 mg/m^2) in combination with doxorubicin (75 mg/m^2) has been established
  - Myelosuppression acceptable
  - Grade 1/2 reversible TH-302 related skin and mucosal toxicities
  - No additive cardiac toxicity and no cumulative toxicity
  - The activity reported in the initial cohort has continued with a response rate of 33% and median PFS of 6.4 months and 6-month PFR of 56% in 57 patients
  - The efficacy appears to be higher than expected for single agent doxorubicin, but requires a controlled trial for confirmation.
- A controlled study designed with SARC with primary efficacy endpoint of overall survival to include 450 subjects with interim futility based on PFS scheduled after approximately half of study is enrolled is being planned

We thank the patients who participated in this trial and the study coordinators, nurses, clinical research assistants and doctors who assisted with the research