

Phase 1 Study of the Safety, Tolerability, and Pharmacokinetics of TH-302, a Hypoxia-Activated Prodrug, in Patients with Advanced Solid Malignancies

Glen J. Weiss¹, Jeffrey R. Infante³, E. Gabriela Chiorean⁴, Mitesh J. Borad^{1,2}, Johanna C. Bendell³, Julian R. Molina⁵, Raoul Tibes¹, Ramesh K. Ramanathan¹, Karen Lewandowski¹, Suzanne F. Jones³, Mario E. Lacouture⁶, Virginia K. Langmuir⁷, Hank Lee⁷, Stew Kroll⁷, and Howard A. Burris, III³

Abstract

Purpose: The objectives of this phase 1, first-in-human study were to determine the dose-limiting toxicities (DLT), maximum tolerated dose (MTD), safety, pharmacokinetics, and preliminary activity of the hypoxia-activated prodrug TH-302 in patients with advanced solid tumors.

Experimental Design: TH-302 was administered intravenously over 30 to 60 minutes in two regimens: three times weekly dosing followed by 1 week off (arm A) and every 3-week dosing (arm B).

Results: Fifty-seven patients enrolled (arm A: $N = 37$ and arm B: $N = 20$). The TH-302 dose was escalated from 7.5 to 670 mg/m² in arm A and from 670 to 940 mg/m² in arm B. The most common adverse events were nausea, skin rash, fatigue, and vomiting. Hematologic toxicity was mild and limited. Grade 3 skin and mucosal toxicities were dose limiting at 670 mg/m² in arm A; the MTD was 575 mg/m². In arm B, grade 3 fatigue and grade 3 vaginitis/proctitis were dose limiting at 940 mg/m²; the MTD was 670 mg/m². Plasma concentrations of TH-302 and the active metabolite Br-IPM (brominated version of isophosphoramidate mustard) increased proportionally with dose. Two partial responses were noted in patients with metastatic small cell lung cancer (SCLC) and melanoma in arm A at 480 and 670 mg/m². Stable disease was observed in arms A and B in 18 and 9 patients, respectively.

Conclusions: The MTD of TH-302 was 575 mg/m² weekly and 670 mg/m² every 3 weeks. Skin and mucosal toxicities were DLTs. On the basis of responses in metastatic melanoma and SCLC, further investigations in these indications were initiated. *Clin Cancer Res*; 17(9); 2997–3004. ©2011 AACR.

Introduction

Within most solid tumors, there are significant areas of hypoxia, which contain cancer cells that are resistant to traditional chemotherapy and radiation treatment (1–4). Thus, therapeutics that can specifically target these resistant hypoxic zones may provide enhanced antitumor activity and clinical benefit. Other hypoxia targeting agents (tirapazamine, PR-104, and AQ4N) have been investigated in

clinical studies but none have gained regulatory approval (5–7). Thus, there remains a clinical need for highly active compounds with acceptable safety profiles that will target hypoxic tumor cells that are inadequately treated by standard therapies.

TH-302 is a prodrug consisting of a 2-nitroimidazole hypoxia trigger covalently linked to a brominated version of isophosphoramidate mustard (Br-IPM; ref. 8). It is not a substrate for common efflux pumps or key cytochrome P450 enzymes. As a second-generation hypoxia-activated prodrug (HAP), TH-302 was also designed to overcome some of the potential limitations of earlier HAPs. It was selected (a) for its ability to diffuse into hypoxic regions without activation by DT diaphorase; (b) to be preferentially activated in significant or severe hypoxia unlikely to be present in nonpathologic tissues in the body; (c) with a potential, on activation, to have limited diffusion to surrounding tissue. This latter characteristic, the so-called "bystander effect," may enable an HAP which has its activation limited to the subregion of the tumor with severe hypoxia, to have single-agent activity by treating both the hypoxic and normoxic components of the tumor. The potential limits of the diffusion would indicate that the HAP may be synergistically combined with another therapeutic with activity in the normoxic regions of the tumor.

Authors' Affiliations: ¹Virginia G. Piper Cancer Center at Scottsdale Healthcare (VGPCC)/TGen; ²Mayo Clinic Cancer Center-Arizona, Scottsdale, Arizona; ³Sarah Cannon Research Institute, Nashville, Tennessee; ⁴Indiana University Simon Cancer Center, Indianapolis, Indiana; ⁵Mayo Clinic Cancer Center, Rochester, Minnesota; ⁶Memorial Sloan Cancer Center, New York; and ⁷Threshold Pharmaceuticals, Redwood City, California

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Corresponding Author: Glen J. Weiss, Virginia G. Piper Cancer Center at Scottsdale Healthcare/TGen, 10510 N 92nd St, Suite 200, Scottsdale, AZ 85238. Phone: 4803231350; Fax: 4803231359; E-mail: gweiss@tgen.org

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Translational Relevance

TH-302 is a second-generation hypoxia-activated prodrug (HAP) designed to overcome some of the potential limitations of earlier HAPs. TH-302 has shown broad activity as monotherapy and in combination with a large range of chemotherapies in numerous ectopic, orthotopic, and metastatic xenograft murine models. These data corroborate that the active moiety of TH-302 may diffuse to areas outside the hypoxic region, which would provide promise as a single-agent therapeutic. The current first-in-human study was designed to establish the safety, tolerability, dose limiting toxicities, and preliminary activity of single-agent TH-302 in patients with solid tumors. The study showed efficacy in a wide range of cancer types, providing the basis for future rationally designed monotherapy and combination studies.

TH-302 is activated by a process that involves a 1-electron reduction mediated by ubiquitous cellular reductases, such as the NADPH cytochrome P450, to generate a radical anion prodrug (RP). In the presence of oxygen (normoxia), the RP reacts rapidly with oxygen to generate the original prodrug and superoxide. Under normal oxygen conditions, TH-302 is relatively inert, remaining intact as a prodrug. However, when exposed to severe hypoxic conditions ($<0.5\% \text{ O}_2$), the RP can either fragment directly or undergo further reduction at the nitroimidazole site of the prodrug, leading to fragmentation and the release of Br-IPM (Fig. 1; ref. 8). Br-IPM can then act as a DNA cross-linking agent. The extremely low oxygen tension trigger was chosen to selectively activate the prodrug in the pathologic hypoxic conditions present in solid tumors but not in most normal tissues as shown by pimonidazole staining, which has a similar chemical structure and activation (9, 10).

Preclinically, TH-302 has shown broad activity as monotherapy and in combination with a large range of chemotherapies in numerous ectopic, orthotopic, and metastatic xenograft murine models (8, 11–13). These data corroborate that the active moiety of TH-302 may diffuse to areas outside the hypoxic region, which would provide promise as a single-agent therapeutic. *In vivo* data have also shown that in combination with chemotherapies that are active in normoxic regions, the therapeutic effect is enhanced including complete responses in a range of models (8, 11).

The TH-302 DLTs in both rat and dog multiple dose toxicology studies were reversible leukopenia, neutropenia, and lymphopenia. Histopathology revealed effects on the bone marrow with the absence of major effects in other organs. The no-observed adverse effect level (NOAEL) was 12.5 mg/kg in rats and 8 mg/kg in dogs. On the basis of these data, an allometric scaled starting dose of 7.5 mg/m², one-tenth the NOAEL, was selected. The current first-in-

human study was designed to establish the safety, tolerability, DLTs, and preliminary activity of single-agent TH-302 in patients with solid tumors.

Patients and Methods

Patient selection

Adult patients aged 18 years and older with histologically or cytologically confirmed advanced solid malignancy previously treated with one or more chemotherapy regimens, or for whom no effective therapy was available, were eligible. Patients were required to have at least 1 measurable target lesion and ECOG performance status of 0 or 1. Adequate hematologic (absolute neutrophil count $\geq 1,500$ cells/ μL , platelet count $\geq 100,000$ / μL , and hemoglobin ≥ 9.0 g/dL), renal (serum creatinine \leq upper limit of normal ULN), and hepatic functions (bilirubin ≤ 1.5 ULN; aspartate aminotransferase and alanine aminotransferase ≤ 2.5 ULN or ≤ 5.0 ULN if liver metastases were present) were required. Patients with systemic hypoxemia, defined as requiring supplemental oxygen or with less than 90% oxygen saturation after a 2-minute walk, and patients with prior high-dose chemotherapy were not eligible.

Treatment plan

All patients received TH-302 as an intravenous infusion over 30 to 60 minutes, with 60-minute infusions administered in patients receiving 1,000 mg or above of TH-302. On the basis of preclinical data suggesting superior efficacy with more frequent dosing, a weekly dosing regimen was initially explored (arm A) with TH-302 administered on days 1, 8, and 15 of a 28-day cycle. The starting dose was 7.5 mg/m². A modified accelerated titration design was used with a cohort size of 1 to 3 patients to allow multiple sites to enroll in a cohort and 100% dose escalation between cohorts until a dose-limiting toxicity (DLT) or study drug-related grade 2 toxicity (excluding grade 2 nausea, vomiting, diarrhea, alopecia, and fatigue) occurred (14). Then, the cohort size was to be increased to 3 to 6 patients and dose escalations decreased to 40%. After the maximum tolerated dose (MTD) was established in arm A, a once-every-3-week regimen was then explored (arm B) to investigate differences in DLT and activity with less frequent dosing. The starting dose in arm B was 670 mg/m² administered on day 1 of a 21-day cycle. The cohorts contained 3 to 6 patients and dose escalations were 40%. The MTD for both arms was defined as the highest dose level at which 0 or 1 of 6 patients experienced a DLT.

DLTs were assessed during cycle 1 and were defined as grade 3 or 4 nonhematologic toxicity (except nausea, vomiting, and diarrhea unless it could not be medically managed and grade 3 transaminasemia lasting ≤ 7 days), as well as febrile neutropenia, grade 4 neutropenia lasting greater than 5 days, grade 4 thrombocytopenia or a requirement for a platelet transfusion, grade 4 anemia unexplained by the underlying disease, or the inability to start cycle 2 within 2 weeks of the scheduled date due to unresolved toxicity. Grade 2 nonhematologic toxicity could

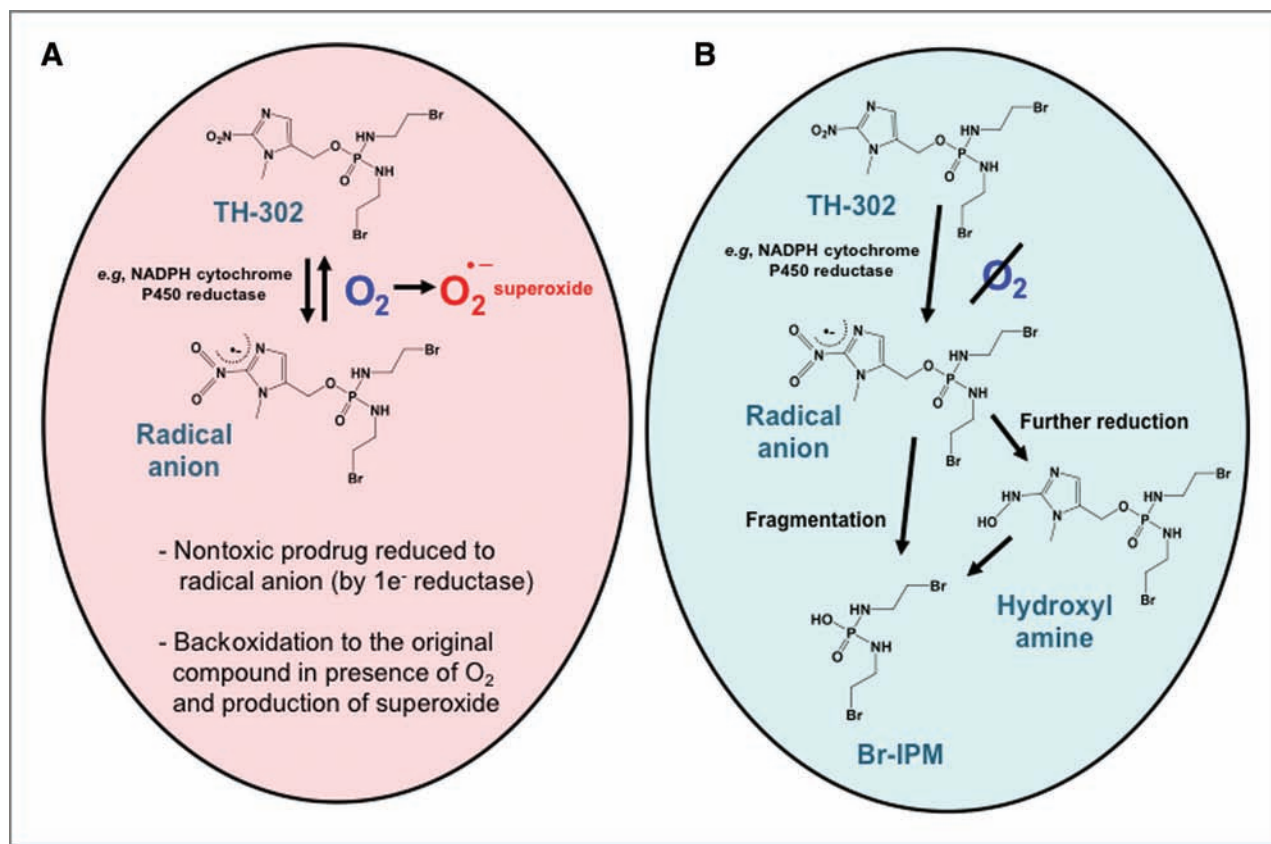


Figure 1. TH-302 is activated by a process that involves a 1-electron ($1e^-$) reduction mediated by ubiquitous cellular reductases, such as the NADPH cytochrome P450, to generate an RP. A, in the presence of oxygen (normoxia), the RP reacts rapidly with oxygen to generate the original prodrug and superoxide. Under normal oxygen conditions, TH-302 is relatively inert, remaining intact as a prodrug. B, when exposed to severe hypoxic conditions ($<0.5\% O_2$), the RP can either fragment directly or undergo further reduction at the nitroimidazole site of the prodrug, leading to fragmentation and the release of Br-IPM.

be assessed as a DLT on the basis of investigator judgment. After the MTD was established, an additional 6 patients were enrolled at the MTD.

Treatment was continued for up to 6 cycles until disease progression, clinical deterioration, or unacceptable toxicity. Subjects with apparent clinical benefit and acceptable toxicity profile were allowed to continue additional cycles after cycle 6.

Evaluations

Laboratory data were collected at screening, before every dose, at week 2 of each cycle in arm B, and at study termination. ECG was done at screening, predose, and at 30, 60, and 150 minutes after the start of the infusion on cycle 1/day 1. Baseline tumor assessments were done within 4 weeks of the first dose of TH-302. Tumor response assessments were repeated after every 2 cycles.

Pharmacokinetic methods and analyses

Plasma samples for measurement of TH-302 and Br-IPM were collected predose and at 13 time points from 15 minutes until 12.5 hours after infusion start on days 1 and 15 of cycle 1 in arm A and on day 1 of cycle 1 in arm B.

Samples were analyzed using a validated LC/MS-MS (liquid chromatography/tandem mass spectrometry) method. Pharmacokinetic parameters were calculated using standard noncompartmental methods using WinNonlin v5.2.

Statistical design including response criteria

No power calculations were done to determine patient sample size. With 12 patients each assessed at the MTD on arms A and B, there is an 80% probability that at least 1 DLT will be observed if the true DLT frequency is 12.5% and at least 3 DLTs will be observed if the true DLT frequency is 33%. All patients who enrolled and received study drug were included in all safety analyses. Tumor assessment was conducted using RECIST (Response Evaluation Criteria in Solid Tumors) version 1.0 (15).

Results

Patient characteristics and drug exposure

Thirty-seven patients were enrolled in arm A from July 2007 to April 2009, and 20 patients were enrolled in arm B from February 2009 to December 2009. All patients received at least 1 dose of TH-302. Patient baseline characteristics are

Table 1. Patient baseline characteristics

	Arm A	Arm B
Sex, n (%)		
Female	13 (35)	9 (45)
Male	24 (65)	11 (55)
Age, y		
Median	64	54
Range	31–79	41–78
ECOG		
0	20	12
1	17	8
Measurable disease at baseline, n (%)	34 (92)	20 (100)
Primary tumor site		
Colorectal	9	2
Prostate	8	0
SCLC	3	1
Head and neck	3	2
NSCLC	2	1
Ovarian	1	3
Pancreas	2	2
Other	9	9
Number of prior therapies		
Median	3	3
Range	1–10	0–7
Prior radiotherapy, n (%)	22 (59)	14 (70)

summarized in Table 1. Patients received a median of 3 prior therapies.

The median time on study was 2 cycles (8 weeks) in both arms and ranged from 1 to 12 cycles. In arm A, 2 patients completed all 6 cycles and 1 patient with non-small cell lung cancer (NSCLC) remained on study for 8 cycles. In arm B, 3 patients completed all 6 cycles and 1 patient with ovarian cancer remains on study for 15 cycles and is continuing at the time of analysis.

DLT and MTD

In arm A, the dose was accelerated in six 100% increments up to 480 mg/m², at which the first study drug-related event of transient grade 2 neutropenia was reported.

The TH-302 dose was then escalated 40% to 670 mg/m², where 2 of 5 patients developed DLTs: grade 3 herpes simplex virus (HSV) perianal and rectal ulcers in one patient which resolved after cycle 2 dosing was delayed and reoccurred after cycle 2 was initiated and grade 3 oral mucositis in another patient. The cohort at 480 mg/m² was then expanded to 6 patients with no DLTs. An intermediate dose of 575 mg/m² was then evaluated, and 1 of 6 patients had a DLT (grade 3 urethritis). The arm A MTD was determined to be 575 mg/m². Six additional patients were enrolled at 575 mg/m² and no more DLTs were reported.

In arm B, the dose was started at 670 mg/m² and escalated to 970 mg/m², where 2 patients developed grade 3 DLTs: fatigue with joint pain and vaginitis/proctitis with pain. The cohort at 670 mg/m² was then expanded to 6 patients with no DLTs. The arm B MTD was determined to be 670 mg/m². Of the 14 patients treated at the MTD, there were no DLTs reported (Table 2).

Safety and tolerability

Twenty-six serious adverse events (SAE) occurred in 17 patients on arm A; in 3 patients, these were considered to be related to TH-302. These included the 2 patients with DLTs of HSV rectal and perianal ulcers and oral mucositis with associated dehydration, and a separate event of vomiting. One patient died from an SAE (post-obstructive pneumonia) thought to be related to underlying disease. There were no treatment-related deaths. Six SAEs occurred in 4 patients from arm B including a TH-302-related event in 1 patient. This patient developed grade 3 vomiting and pancytopenia following the second TH-302 dose of 670 mg/m². Three patients in arm A (biliary obstruction, grade 2 genital rash, and a buttock ulcer after local trauma in a diabetic patient; respectively) and 1 patient in Arm B (biliary obstruction) discontinued treatment due to an AE.

Nonlaboratory AEs are summarized in Tables 3 and 4. The most common toxicities were nausea, vomiting, and fatigue; most cases were grade 1 or 2. Nausea and vomiting increased with dose and standard antiemetic prophylaxis for moderately emetogenic therapy (generally serotonin 5-HT₃ receptor antagonist and steroid) was recommended at doses greater than 240 mg/m². Skin and mucosal AE incidence and severity increased with dose. The most common skin AEs were rash and hyperpigmentation. Dose

Table 2. Patient dosing and DLT assessment

TH-302 dose level, mg/m ²	Arm A		Arm B	
	Number of DLTs	Median (range), number of cycles	Number of DLTs	Median (range), number of cycles
7.5–240	0/14	2 (1–8)	NA	
480	0/6	3 (1–4)	NA	
575	1/12	2 (1–5)	NA	
670	2/5	2 (2–3)	0/14	2 (1–13+)
940	NA		2/6	2.5 (1–3)

Table 3. Skin and mucosal adverse events (all cycles) for arms A and B

Arm A AE ^a	Number of patients with an AE by dose (%)				
	7.5–240 (N = 14)	480 (N = 6)	575 (N = 12)	670 (N = 5)	All (N = 37)
Skin	4 (29)	5 (83)	10 (83)	5 (100)	24 (65%)
Grade 3/4	0	0	0	1 (20)	1 (5%)
Mucosal	1 (7)	3 (50)	9 (75)	4 (80)	17 (46%)
Grade 3/4	0	0	1 (8)	2 (40)	3 (8%)

Arm B AE ^a	Number of patients with an AE by dose, %		
	670 (N = 14)	940 (N = 6)	All (N = 20)
Skin	9 (64)	5 (83)	14 (70%)
Grade 3/4	0	1 (17)	1 (5%)
Mucosal	3 (21)	4 (67)	7 (35%)
Grade 3/4	0	1 (17)	1 (5%)

^aIncludes all adverse events considered as skin or mucosal.

reductions or delays due to skin/mucosal toxicity occurred only at doses of 575 mg/m² or higher. Skin and mucosal toxicities were reversible following dose interruption, dose decrease, and/or local intervention (examples are shown in

Supplementary Fig. S1A–D). There has been no evidence of renal or liver toxicity of TH-302 and no other significant laboratory abnormalities.

Hematologic toxicity was not dose limiting (Table 4). In arm A, grade 4 neutropenia was reported in one patient treated at 575 mg/m² and grade 3 thrombocytopenia was reported in 1 patient at 575 mg/m². Grade 3 anemia and lymphopenia occurred in 1 (3%) and 12 (32%) patients, respectively, and there were greater decreases from baseline at higher TH-302 doses. Change in hemoglobin worsened with increasing dose with a median decrease of 2.4 g/dL overall. In arm B, grade 4 neutropenia and thrombocytopenia were reported in 1 patient at 670 mg/m². Grade 3 or 4 lymphopenia was reported in 3 patients at 670 mg/m².

Table 4. Adverse events regardless of relationship to study drug reported in at 15% or more of subjects

	Arm A (N = 37)		Arm B (N = 20)	
	Any grade	Grade 3/4	Any grade	Grade 3/4
Nonhematologic adverse event				
Nausea	19 (51)	1 (3)	11 (55)	1 (5)
Fatigue	14 (38)	3 (8)	13 (65)	1 (5)
Vomiting	13 (35)	1 (3)	12 (32)	0 (0)
Constipation	7 (19)	1 (3)	5 (25)	1 (5)
Stomatitis	7 (19)	1 (3)	4 (20)	0 (0)
Arthralgia	6 (16)	0 (0)	1 (5)	0 (0)
Back pain	6 (16)	1 (3)	1 (5)	0 (0)
Dehydration	6 (16)	2 (5)	4 (20)	1 (5)
Dyspnea	6 (16)	1 (3)	2 (10)	0 (0)
Pyrexia	5 (13)	0 (0)	4 (20)	1 (5)
Hematologic adverse event				
Neutropenia	4 (11)	1 (3)	4 (20)	1 (5)
Lymphopenia	24 (65)	12 (32)	13 (65)	5 (25)
Thrombocytopenia	11 (30)	1 (3)	4 (20)	1 (5)
Anemia	35 (95)	1 (3)	15 (75)	2 (10)

NOTE: All values in table are expressed as n (%).

Pharmacokinetics

There were no apparent differences between plasma pharmacokinetics on days 1 and 15 for either TH-302 or its active metabolite, Br-IPM. The TH-302 and Br-IPM plasma half-life, time of maximum concentration, clearance, and volume of distribution appeared to be independent of TH-302 dose over a 7.5 to 940 mg/m² dose range (Fig. 2). The median plasma terminal half-life of TH-302 was 0.81 hours and of Br-IPM was 0.70 hours. *T*_{max} was generally observed toward the end of the dosing interval. TH-302 was cleared rapidly from plasma, and its volume of distribution approximated total body water. Area under the curve (AUC) increased in a generally linear dose-proportional manner for both TH-302 and Br-IPM with the AUC of Br-IPM approximately 2% of the AUC of TH-302 (Table 5). Plasma concentrations of Br-IPM were approximately 1% to 2% of the plasma concentrations of TH-302.

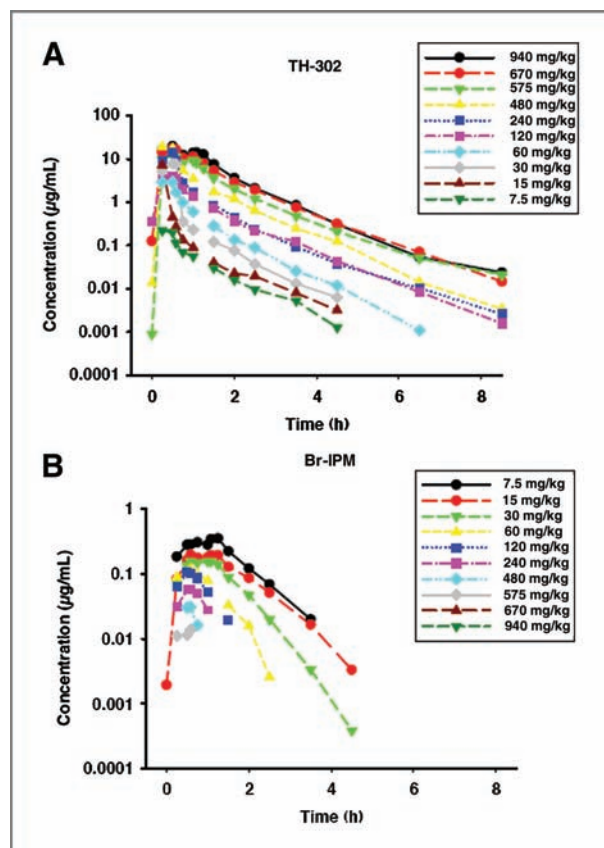


Figure 2. Mean plasma concentrations of TH-302 (A) and Br-IPM (B) vs. time (days 1 and 15 combined). Plasma Br-IPM could not be detected reliably following administration of TH-302 doses below 30 mg/m².

Antitumor activity

In arm A, 33 patients had at least 1 evaluable posttreatment tumor assessment and 2 patients had partial responses (PR) after cycle 2 that were not confirmed on the next follow-up exam: (a) a patient with refractory small cell lung cancer (SCLC) metastatic to liver and regional lymph nodes

treated at 480 mg/m² had a PR (44% decrease in sum of longest diameters (SLD) of target lesions) at the end of cycle 2 (Supplementary Fig. S2A). After a 3-week dosing delay due to a large empyema requiring surgical intervention, the patient had progressive liver disease, as well as new brain metastases. (2) A patient with progressing malignant melanoma metastatic to lung and liver treated at 670 mg/m² had a PR at the end of cycle 2 but discontinued treatment after a seizure due to previously undiagnosed brain metastases (Supplementary Fig. S2B). Both patients with a PR had elevated lactate dehydrogenase (LDH) at baseline that normalized on treatment (72% and 75% reductions, respectively). Eighteen patients had stable disease (SD) for at least 2 cycles including SD of 12 weeks or more in 10 patients: prostate (*N* = 2), NSCLC (*N* = 2), colorectal (*N* = 2), gastric, hepatocellular, SCLC, and bladder cancer. The median progression-free survival (PFS) was 3.6 months (95% CI: 1.9–3.8). Of the 16 patients in arm B with an evaluable tumor assessment, 9 (56%) patients had SD including SD of 12 weeks or more in 3 patients: ovarian, hepatocellular, and SCLC. The median PFS was 2.1 months (95% CI: 1.4–4.0).

Discussion

TH-302 is a second-generation HAP designed to address and potentially overcome some of the recognized limitations of earlier HAPs. These included shifting the oxygen selectivity to more extreme hypoxia (<0.5%), engineering the prodrug to be insensitive to 2 electron reductases to better enable its diffusion without activation to reach the hypoxic zone, engineering the prodrug to not be metabolized by cytochrome P450s and not be a substrate for clinically relevant drug resistance pumps, and designing the activation to allow the possibility of a bystander effect. In comparison to other HAPs tested in the clinic, TH-302 has a different side effect profile, primarily with DLT from skin and mucosal side effects and minimal single-agent myelosuppression. In addition, responses and durable disease control were observed in a variety of refractory solid tumors.

Table 5. Mean (SD) plasma pharmacokinetics^a

TH-302 dose, mg/m ²	TH-302			Br-IPM		
	Terminal half-life, h	C _{max} , µg/mL	AUC, µg h/mL	Terminal half-life, h	C _{max} , µg/mL	AUC, µg h/mL
120 (<i>N</i> = 3)	0.83 (0.09)	4.2 (0.6)	3.9 (1.3)	0.81 (0.06)	0.06 (0.02)	0.08 (0.03)
240 (<i>N</i> = 2)	0.98 (0.12)	14.7 (11.2)	8.4 (4.6)	0.46 (0.03)	0.11 (0.03)	0.11 (0.03)
480 (<i>N</i> = 6)	0.98 (0.23)	22.5 (9.7)	14.5 (4.1)	0.54 (0.12)	0.15 (0.04)	0.16 (0.05)
575 (<i>N</i> = 12)	0.83 (0.29)	14.7 (9.2)	14.1 (4.6)	0.67 (0.14)	0.19 (0.05)	0.26 (0.08)
670 (<i>N</i> = 17)	0.84 (0.15)	19.7 (7.7)	23.0 (7.7)	0.87 (0.45)	0.22 (0.08)	0.37 (0.17)
940 (<i>N</i> = 5)	0.80 (0.16)	21.2 (5.5)	25.8 (8.1)	0.61 (0.11)	0.37 (0.07)	0.54 (0.19)

^aEstimates are averages of cycle 1, day 1 and cycle 1, day 15 values.

The safety and efficacy profile of TH-302 differentiates it from the previous HAPs that have been tested in the clinic. Tirapazamine is a heteroaromatic *N*-oxide with a half-life of less than 1 hour and with no expected bystander effect (16). In the tirapazamine single-agent phase 1 clinical trial, no responses were seen in 28 patients, the DLTs were reversible tinnitus and hearing loss, and muscle cramps were a common side effect (17, 18). AQ4N, an aliphatic *N*-oxide HAP is an inert prodrug which penetrates deeply in tissues where it is selectively activated intracellularly to produce a stable active metabolite, AQ4. AQ4 is an analogue of mitoxanthrone and a potent topoisomerase II inhibitor that binds tightly to DNA and has minimal bystander effect (19). In the single-agent AQ4N phase 1 clinical trial, no responses were seen in 16 patients, the DLTs were grade 5 respiratory failure and grade 3 fatigue, and common side effects included fatigue, diarrhea, nausea, vomiting, anorexia, and blue coloration of body fluids and skin (20). PR-104 is a HAP that forms nitrogen mustard in the presence of hypoxia. In 2 single-agent PR-104 phase 1 studies with a weekly and every 3-week dosing schedules, no objective responses were observed in 27 patients; DLTs were neutropenic fever, infection with normal ANC and myelosuppression, and commonly observed side effects included fatigue, nausea, anemia, and dysgeusia (21, 22).

The key objectives of this study were to establish the safety, tolerability, DLTs, and preliminary activity of single-agent TH-302 as a treatment for patients with advanced cancers. For both dosing schedules, nonhematologic and hematologic toxicities of grade >2 severity were uncommon and TH-302 was generally well tolerated. Prior toxicology studies in rats and dogs had suggested that hematologic toxicity would be dose limiting; however, in humans, skin and mucosal toxicities were dose limiting. The higher ratio of Br-IPM bis-alkylator to TH-302 parent prodrug in plasma samples from rats (5%) and dogs (2%) than in humans (1%) may explain the cross-species differences in extent of myelosuppression if the myelosuppression is related to systemic circulation of Br-IPM.

Skin and mucosal toxicity increased in incidence and severity with dose starting at TH-302 doses of 480 mg/m². Although primarily grade 1 and 2, they affected the tolerability of TH-302 because of pain and discomfort in areas of desquamation or ulceration. Steps implemented to minimize or prevent them included patient education on the importance of diligent hygiene, prophylactic application of barrier creams, and therapeutic application of topical antimicrobial creams and/or mild strength topical corticosteroids.

Mechanisms for the skin and mucosal toxicity are not yet well defined. In normal human skin, the human dermis is well oxygenated; the stratum granulosum of the epidermis can be modestly hypoxic, and portions of both sebaceous glands and hair follicles may be moderately or severely hypoxic (23). The skin toxicities were most evident in the perineal region and were also reported in other areas with skin folds (e.g., inguinal

region, axilla, or under the breast). Erythema and desquamation were observed most commonly. Skin areas with contact pressure were at times associated with rash such as the buttocks, heel, and belt line. Hyperpigmentation was observed in 5% of patients including hyperpigmentation overlying the infusion site vein. The most common mucosal toxicity was stomatitis. Normal mucosa is also noted to have an oxygen gradient with moderate or severe hypoxia in cells lining the lumen (24). Thus, both the skin and mucosal toxicities are consistent with the pharmacology of an HAP and may be explained by the ability of TH-302 to penetrate into tissues and to selectively activate in severely hypoxic regions.

Pharmacokinetic data showed that TH-302 exhibits dose-proportional kinetics over all dose ranges tested. Plasma concentrations of the bio-reduced active metabolite Br-IPM were appreciably lower (approximately 1%) than the TH-302 concentration and, as discussed above, were lower than had been observed in rats and dogs. The lack of systemic Br-IPM is consistent with its prodrug mode of action and the selection of a compound which is not susceptible to activation by hepatic enzymes or DT-diphosphorase. TH-302 undergoes rapid distribution during the first hour following administration and plasma levels achieved at the MTD with the weekly dosing exceed the levels where antineoplastic activity was observed in preclinical models (unpublished data).

In conclusion, the MTD of TH-302 is 575 mg/m² when administered on days 1, 8, and 15 of a 28-day cycle and is 670 mg/m² when administered on day 1 of a 21-day cycle. The tolerability of these dosing schedules together with prior preclinical demonstration of tumor selectivity and hypoxia targeting of TH-302, in a wide range of cancer types, provides the basis for rationally designed monotherapy and combination studies.

Disclosure of Potential Conflicts of Interest

V.K. Langmuir, H. Lee, and S. Kroll are employees of and hold ownership interest in Threshold. M.E. Lacouture receives honoraria from Threshold's Speaker's Bureau. V.K. Langmuir and M.E. Lacouture are consultants to and/or on the advisory board of Threshold.

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