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ORIGINAL ARTICLE

Pharmacokinetics of TH-302: a hypoxically activated prodrug of bromo-isophosphoramide mustard in mice, rats, dogs and monkeys

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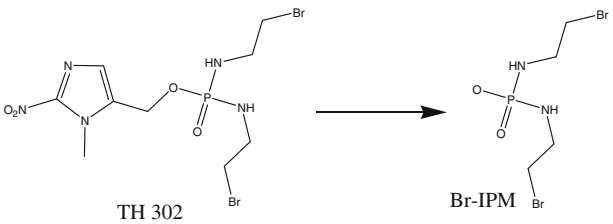
Abstract
Purpose To characterize the pharmacokinetics of the prodrug, TH-302, and its active metabolite, bromo-IPM (Br-IPM), in nonclinical species.
Methods TH-302 was administered in single oral, intraperitoneal and intravenous bolus doses to mice, rats, dogs and monkeys as well as in acute and chronic safety studies in rats and dogs as a 30-min intravenous infusion given once a week for 3 weeks. Assessments were made using liquid chromatography–tandem mass spectrometry.
Results TH-302 was extensively distributed with high systemic clearance exceeding hepatic plasma flow in all species studied, resulting in half-lives ranging between 8 min (mice) and over 4 h (rats). In rats, TH-302 exhibited linear kinetics following intravenous administration and good oral bioavailability. In acute and chronic safety studies, there was no accumulation of TH-302 following once weekly dosing for 3 weeks in the rat and dog. Br-IPM plasma concentrations were a small fraction of the TH-302 plasma concentrations with significantly smaller percentages present in dogs than in rats. Allometric scaling predicted that the systemic clearance and steady-state volume of distribution in humans would be 38.8 l/h/m^2 and 34.3 l/m^2, respectively, resulting in a terminal elimination half-life of about 36 min. These values were similar to those observed in patients with solid tumors (27.1 l/h/m^2, 23.5 l/m^2 and 47 min).
Conclusions TH-302 exhibited good safety, efficacy and pharmacokinetic properties in nonclinical species, translating into favorable properties in humans.

Keywords Pharmacokinetics · TH-302 · Prodrug · Bromo-isophosphoramide mustard

Introduction

Most solid tumors have significant areas of hypoxia that contain cells that are resistant to traditional chemotherapy and radiation treatment [1–3]. Thus, therapeutics that can specifically target these resistant hypoxic zones should provide additional anti-tumor activity and clinical benefit. Some agents that were designed to be activated by hypoxic regions of tumors have been studied previously or are currently under study, including tirapazamine [4–7], PR-104 [8] and banoxantrone (AQ4N) [9].

TH-302, a new prodrug that is activated in hypoxic regions of tumors to an alkylating mustard toxin, has shown excellent preclinical activity [10, 11]. TH-302 is a nitroimidazole-linked prodrug of a brominated version of isophosphoramide mustard (Br-IPM) with the following structure:



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When exposed to hypoxic conditions, TH-302 is reduced by intracellular reductases to release Br-IPM. Br-IPM can then act as a DNA cross-linking agent and may also diffuse to adjacent cells in normoxic regions and thus act as a cytotoxic agent outside of the hypoxic activation zone. In vitro cytotoxicity and clonogenic assays indicate that TH-302 has little activity under normoxic conditions but is highly cytotoxic under hypoxic conditions [10, 11]. TH-302 has been studied in mouse orthotopic and ectopic models of cancer and has demonstrated anti-tumor activity as monotherapy and increased activity including cures in combination with standard chemotherapeutics [10, 11]. In vivo excision assays have demonstrated that the activity of this agent is dependent on the level of tissue oxygen levels [10, 11].

As part of the preclinical characterization of TH-302, pharmacokinetic studies were conducted in CD-1 and nude mice, Sprague–Dawley rats, beagle dogs and Cynomolgus monkeys.

Materials and methods

Materials

TH-302 (1-methyl-2-nitro-1H-imidazole-5-yl) *N*,*N*′-bis (2-bromoethyl) diamidophosphate, m. w. = 449) and d8-TH-302 were synthesized in the Threshold Medicinal Chemistry Department and were at least 96 and 97% pure, respectively, based on HPLC purity analysis. All other reagents and solvents were of reagent grade (or a suitable alternative) and were obtained principally from Sigma Chemical Company (St. Louis, MO, USA), Fisher Scientific Company (Fair Lawn, NJ, USA), VWR International (Radnor, PA, USA) and other approved vendors.

Studies in preclinical species (mice, rats, dogs and monkeys)

All procedures were approved by an Institutional Animal Care and Use Committee. The animals were housed under standard conditions and had free access to water and standard laboratory diet specific for that species.

Pharmacokinetic studies in mice

Single-dose administration

Adult male CD-1 mice or female nude mice (Charles River Laboratories, Hollister, CA, USA) were fasted overnight prior to drug administration. TH-302 was administered either as 50 mg/kg dose intraperitoneally (IP) or via oral gavage (PO) in normal saline to CD-1 mice and intravenously (IV) or IP to female nude mice. Blood was collected from three mice per time point at pre-dose, 2, 5, 15, 30 and 60 min post-dose following IP administration and at pre-dose, 15, 30, 60, 90 and 120 min following oral gavage from the CD-1 mice. For the nude mice, blood was collected at 5, 15, 30, 60 and 120 min post-dose following both IV and IP administration, with an additional blood sample at 2 min post-dose following the IV dose. Blood was collected via cardiac puncture into sodium fluoride/potassium oxalate-containing tubes and gently mixed, and the sample was immediately centrifuged. The resulting plasma was frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

Pharmacokinetic studies in rats

Single-dose administration

Adult male Sprague–Dawley rats (200–230 g, Shanghai Laboratory Animal Center, Shanghai, China) were surgically prepared with indwelling carotid cannulae 1 day before drug administration. Rats were fasted overnight before dosing and were fed 4 h after dosing. The animals had free access to water and were conscious and unrestrained throughout the study. Each rat ($n = 3$ per dose level) was given a single IV bolus dose (1, 5, 20 and 50 mg/kg) of TH-302 over 1 min via tail vein. TH-302 was administered as a solution in a vehicle composed of 50% PEG-400 and 50% normal saline. Blood samples (400 µl) were collected at pre-dose, 2, 5, 15, 30 min and at 1, 2, 4, 6 and 8 h post-dose into sodium fluoride/potassium oxalate-containing tubes. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

In an acute nonclinical safety study, male and female Sprague–Dawley rats (207–391 g, Charles River Laboratories, Kingston, NY, USA) were administered a single 30-min intravenous infusion of 50, 100 and 200 mg/kg TH-302 as a solution containing 5% sucrose in saline for injection. Blood samples (500 µl, 3 rats per time point) were collected from the jugular vein pre-dose and at 30 (end of infusion) and 40 min and at 1, 1.5, and 2.5 h post-dose into tubes containing sodium fluoride/potassium oxalate. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

Repeat-dose administration

In a chronic nonclinical safety study, TH-302 was administered by intravenous infusion over 30 min, once weekly for 3 weeks. Male and female Sprague–Dawley rats (200–375 g, Charles River Laboratories, Kingston, NY,

USA) were treated with doses of 12.5, 25 and 50 mg/kg TH-302 as a solution containing 5% sucrose in saline for injection. Blood samples (500 µl, 3 rats per time point) were collected on Days 1 and 15, pre-dose and at 30 (end of infusion) and 40 min and at 1, 1.5 and 2.5 h post-dose into tubes containing sodium fluoride/potassium oxalate. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

Pharmacokinetic studies in dogs

Single-dose administration

Adult male beagle dogs (12–15 kg, Jiaan Laboratories Animal Breeding, Inc., Shanghai, China) were fasted overnight before dosing and were fed 4 h after dosing. The animals had free access to water and were conscious and unrestrained throughout the study. Each dog ($n = 3$) was given a single intravenous bolus dose (20 mg/kg) of TH-302 over 1 min as a solution in a vehicle composed of 50% PEG-400 and 50% normal saline. Blood samples (1 ml) were collected via the femoral vein into tubes containing sodium fluoride/potassium oxalate at pre-dose, 2, 5, 15 and 30 min and at 1, 2, 4, 6, 8 and 24 h after dosing. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

In an acute nonclinical safety study, male and female beagle dogs (6–12 kg, Marshall Bioresources, North Rose, NY) were administered a single 30-min intravenous infusion of 4, 8, 16 and 32 mg/kg TH-302 as a solution containing 5% sucrose in saline for injection. Blood samples (2 ml) were collected pre-dose and at 30 (end of infusion) and 40 min and at 1, 1.5 and 2.5 h post-dose into tubes containing sodium fluoride/potassium oxalate. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

Repeat-dose administration

In a chronic nonclinical safety study, TH-302 was administered by intravenous infusion over 30 min, once weekly for 3 weeks. Male and female beagle dogs (6–12 kg, Marshall Bioresources, North Rose, NY) were treated with doses of 4, 8 and 16 mg/kg TH-302 as a solution containing 5% sucrose in saline for injection. Blood samples (2 ml) were collected on Days 1 and 15, pre-dose and at 30 (end of infusion) and 40 min and at 1, 1.5, and 2.5 h post-dose into tubes containing sodium fluoride/potassium oxalate. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −70°C until they were analyzed.

Pharmacokinetic studies in monkeys

Adult male Cynomolgus monkeys (2.5–5 kg, China Local Breeder, Beijing, China) were fasted overnight before dosing and were fed 4 h after dosing. The animals had free access to water and were conscious and unrestrained throughout the study. Each monkey ($n = 3$) was given a single intravenous bolus dose (20 mg/kg) of TH-302 over 1 min as a solution in a vehicle composed of 50% PEG-400 and 50% normal saline via cephalic vein. Blood samples (1 ml) were collected via the cephalic or saphenous vein into tubes containing sodium fluoride/potassium oxalate at pre-dose, 2, 5, 15, 30 and 45 min and at 1, 2, 4, 6, 8 and 24 h after dosing. Following centrifugation, the plasma was separated and frozen on dry ice, and the samples were stored at −80°C until they were analyzed.

Plasma sample analysis

Mouse plasma sample analysis for TH-302

Mouse plasma samples (0.1 ml) were fortified with propranolol (4 µg/ml) as an internal standard and treated with acetonitrile containing 0.1% formic acid for direct deproteinization. After vortex-mixing for 1 min and centrifuging for 10 min at 2,000 rpm, 450 µl of supernatant was transferred to a glass tube and 10 µl injected onto the LC/MS/MS. The HPLC system consisted of a liquid chromatograph (Shimadzu Inc. USA) equipped with an isocratic pump, an autosampler and a degasser. The column was a Thermo Beta-Basic-18 DASH HTS, 5 µm (20 mm × 2.1 mm) maintained at 25°C, and the flow rate of the mobile phase was 0.5 ml/min. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The initial mobile phase composition was 95% A/5% B. After sample injection, the mobile phase composition was changed to 5% A/95% B over 1.6 min and held there for an additional 1.0 min. The mobile phase was then returned to initial conditions and the column re-equilibrated for 1 min. The HPLC was interfaced to an API3000 (triple-quadrupole) instrument, Applied Biosystems, Inc. (Canada), with an ESI interface. Ultrahigh-purity nitrogen was used as the nebulizing gas at a flow rate of 8 l/min. Data acquisition utilized selected reaction monitoring (SRM). Mass transitions for the ions representing the (M + H) species for TH-302 and propranolol (as the internal standard) of $450.1 > 140.2$ and $266.1 > 116.3$, respectively, were monitored. The data acquisition and control system were created using Analyst 1.4 software from Applied Biosystems, Inc. (Canada). Plasma standard curves ranged from 20 to 15,000 ng/ml. The curves were fitted with a linear regression equation.

Concentrations of TH-302 <20 ng/ml were reported as BQL (below the quantification limit of the method).

Single-dose rat, dog and monkey plasma sample analysis for TH-302

Rat, dog and monkey plasma samples were processed by liquid–liquid extraction. To plasma samples (0.1 ml) were added 20 µl methanol, 50 µl d8-TH-302 as internal standard (1 µg/ml) and 500 µl methyl *tert*-butyl ether (MTBE). After vortex-mixing for 1 min and centrifuging for 5 min at 17,000×*g*, 450 µl of supernatant was transferred to a glass tube and then evaporated to dryness under a vacuum. The residue was reconstituted with 100 µl methanol and 10 µl injected onto the LC/MS/MS. The HPLC system consisted of an Agilent 1100 series (Agilent Technologies Inc. USA) liquid chromatograph equipped with an isocratic pump, an autosampler and a degasser. A Luna, 5 µm, C18 (100 mm × 2.0 mm) column was used for the rat and dog samples, and a Agilent Eclipse XDB-C18, 5 µm (4.60 × 150 mm), was used for the monkey samples maintained at 25°C, and the flow rate of the mobile phase (water/4 mM ammonium acetate/acetonitrile, 8/2/90, v/v) was 0.25 ml/min. The HPLC was interfaced to an API3000 (triple-quadrupole) instrument, Applied Biosystems, Inc. (Canada), with an ESI interface. Ultrahigh-purity nitrogen was used as the nebulizing gas at a flow rate of 8 l/min. Data acquisition utilized selected reaction monitoring (SRM). Mass transitions for the ions representing the (M + H) species for TH-302 and d8-TH-302 (as the internal standard) of 450.1 > 140.2 and 458.3 > 140.2, respectively, were monitored. The data acquisition and control system were created using Analyst 1.4 software from ABI Inc. Plasma standard curves ranged from 2.5 to 5,000 ng/ml for rat and dog samples and 10 to 5,000 ng/ml for the monkey samples. Concentrations of TH-302 <2.5 ng/ml for rat and dog samples and <10 ng/ml for monkey samples were reported as BQL. The precision and accuracy of the assay in rat plasma samples ranged from 8.4–15.0 to 97.4–103.9%, respectively, over a concentration range of 1–5,000 ng/ml. In dog plasma samples, the precision and accuracy ranged from 2.6–7.8 to 94.5–108.8%, respectively, between 8 and 5,000 ng/ml, while in monkey plasma samples, the respective values are 6.1–7.5 and 99.2–129.5%, respectively, over a concentration range of 30–5,000 ng/ml.

Repeat-dose rat and dog plasma sample analysis for TH-302 and Br-IPM

TH-302 and Br-IPM in plasma were analyzed by a LC/MS/MS method. The method involves the treatment of dog plasma with acetonitrile/formic acid (100:5, v/v) to precipitate proteins. After centrifugation, the supernatant was analyzed by reverse-phase high-performance liquid chromatography using a Waters Atlantis dC18 column maintained at 35°C. The HPLC system consisted of a Shimadzu LC-10ADVP (Shimadzu Corp., Japan) liquid chromatograph equipped with an isocratic pump, an autosampler and a degasser. The mobile phase was nebulized using heated nitrogen in a positive ESI source/interface, and the ionized compounds were detected using a tandem quadrupole mass spectrometer (API4000, Applied Biosystems, Inc., Canada). The quantitation of TH-302 and Br-IPM is accomplished using standard curves with d8-TH-302 and d8-Br-IPM as internal standards in the range from 10–10,000 and 50–5,000 ng/ml, respectively. Concentrations of TH-302 and Br-IPM less than 10 and 50 ng/ml were reported as BQL. The precision and accuracy of the assay for TH-302 between 30 and 7,500 ng/ml in rat and dog plasma samples were 3.3–9.5 and 93.9–101.6 and 4.5–8.2 and 95.0–101.1%, respectively. The respective values for Br-IPM in rat and dog plasma samples were 4.2–8.9 and 98.4–105.3 and 3.0–4.4 and 101.9–103.3% between 150 and 3,750 ng/ml.

Determination of pharmacokinetic parameters

Plasma concentrations versus time data for TH-302 and Br-IPM were analyzed by standard noncompartmental methods [12] using WinNonlin Pro 5.1 (Pharsight Corporation, Mountain View, CA). Concentrations reported as BQL were set equal to zero for calculation of the pharmacokinetic parameters and summary statistics. The maximum plasma concentration (C_{max}) and time to maximum concentration (T_{max}) were determined directly from the concentration–time data. Data in the terminal log-linear phase were analyzed by linear regression to estimate the terminal rate constant (k) and half-life ($t_{1/2} = 0.693/k$). Total AUC was computed as the sum of AUC_{last} through the last measurable concentration (C_{last}) using the linear trapezoidal rule and the terminal area [calculated by dividing C_{last} by k], i.e., $AUC = AUC_{last} + C_{last}/k$. Total plasma clearance (Cl) after intravenous dosing was calculated as dose/AUC, and the volume of distribution at steady state (V_{ss}) was calculated as Cl × (AUMC/AUC), where AUMC is the total area under the first moment of the plasma concentration versus time curve.

Allometric scaling

Pharmacokinetic data from mice, rats, dogs and monkeys after a single intravenous bolus administration were used to predict the pharmacokinetics of TH-302 in humans using allometric scaling methods [13, 14]. The pharmacokinetic parameters (Cl and V_{ss}) of TH-302 were scaled across

species as a function of body weight (W) by utilizing the following allometric equation:

$$Y = a \cdot W^b \quad (1)$$

where a and b are the allometric coefficient and exponent, respectively. This equation may also be written in the following form:

$$\log Y = \log a + b \cdot \log W \quad (2)$$

where log a is the y intercept and the exponent, b denotes the proportionality between Y and W. The above equation was fit to the logarithmically transformed data using unweighted linear least-squares regression analysis. Statistical significance of the correlation was tested using the Student's t test. A P value <0.05 was considered to be statistically significant.

Using the predicted pharmacokinetic parameters from the allometric scaling, predicted human plasma concentration versus time profiles were generated, assuming a body surface area of 1.73 m^2.

Results

Single-dose pharmacokinetics/toxicokinetics

Mice

Female nude mice were given a single dose of TH-302 (50 mg/kg IV or IP). Pharmacokinetic parameters were calculated based on mean concentrations of TH-302 in plasma ($n = 3$ mice per time point) collected up to 2 h post-dose. Following the IV and IP doses, the plasma $t_{1/2}$ of TH-302 was 9.73 and 8.27 min, respectively. TH-302 had a high plasma clearance (4.37 l/h/kg) and a volume of distribution at steady state approximating total body water (0.54 l/kg). These pharmacokinetic parameters are summarized in Table 1 and Fig. 1.

Male CD-1 mice were given a single IP or single oral dose of 50 mg/kg TH-302 (Table 1; Fig. 1). Pharmacokinetic parameters were calculated based on mean concentrations of TH-302 in plasma [$n = 3$ (IP) or 4 (PO) per time point] collected up to 1 h post-dose. The plasma half-lives of TH-302 following IP and PO administration were 7.91 and 25.7 min, respectively. Following oral administration, TH-302 was rapidly absorbed with mean peak plasma concentration reached at the first sampled time point of 15 min.

Rats

In a dose-escalating study, male Sprague–Dawley rats ($n = 3$/dose group) were given single IV bolus doses of 1, 5, 20 and 50 mg/kg TH-302. TH-302 exhibited linear

Table 1 Pharmacokinetics of TH-302 in mice (composite data), rats, dogs and monkeys (mean ± SD)

Parameter	Unit	CD-1 mouse[a]		Nude mouse[a]		Rat ($n = 3$)			Dog ($n = 3$)		Monkey ($n = 3$)
		IP	PO	IV	IP	IV	IV[b]	IV	IV	PO[a]	IV
Route											
Dose	mg/kg	50	50	50	50	1	5	20	50	100	20
T_{max}	h	0.083	0.250	0.033	0.083	0.033 ± 0.00	0.033 ± 0.00	0.033 ± 0.00	0.033 ± 0.00	0.250	0.033 ± 0.00
C_{max}	μg/ml	38.5	0.93	74.4	32.6	0.802 ± 0.165	3.82 ± 0.0265	13.5 ± 1.52	35.5 ± 3.01	15.8	20.7 ± 6.84
AUC	μg-h/ml	10.6	0.707	11.4	10.6	0.221 ± 0.054	0.826 ± 0.133	3.30 ± 0.36	8.47 ± 0.02	13.1	3.19 ± 1.43
Cl	l/h/kg	–	–	4.37	–	4.69 ± 1.05	6.14 ± 0.99	6.09 ± 0.68	5.91 ± 0.25	–	7.66 ± 4.62
V_{ss}	l/kg	–	–	0.542	–	2.05 ± 0.69	5.09 ± 3.66	2.29 ± 0.61	1.67 ± 0.04	–	2.92 ± 2.83
$t_{1/2}$	h	0.132	0.428	0.138	0.162	2.03 ± 0.24	3.75 ± 3.58	4.37 ± 0.42	1.32 ± 0.80	1.59	0.415 ± 0.356
Oral F	%	–	–	–	–	–	–	–	–	77.3	–

AUC, total area under the concentration–time curve from 0 to ∞; *IV*, intravenous; *IP*, intraperitoneal; *PO*, oral

[a] Composite data, $n = 3$ per time point

[b] $n = 2$

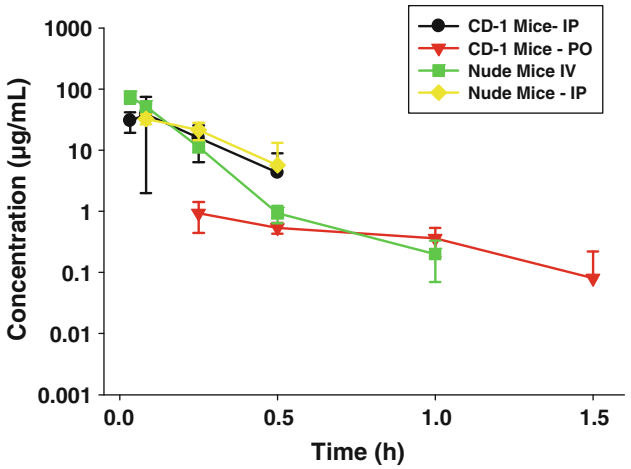

Fig. 1 *Plot* of plasma concentration versus time data for TH-302 in CD-1 mice following a single intravenous (IV), oral (PO) and intraperitoneal (IP) dose of 50 mg/kg TH-302 in CD-1 and nude mice. The values are a composite from different sets of animals and represent mean (±SD) concentrations from 3 mice per time point

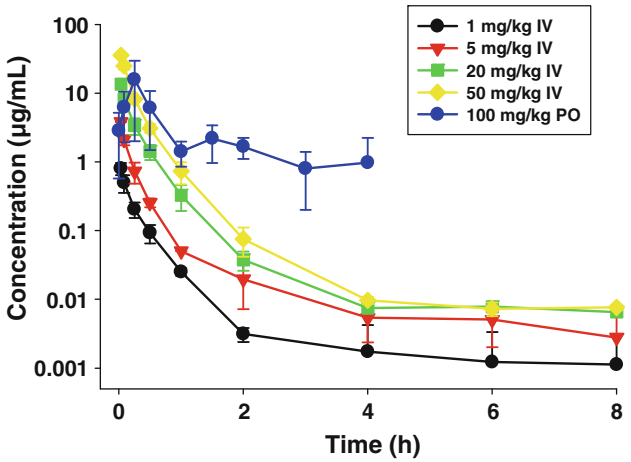

Fig. 2 *Plot* of plasma concentration versus time data for TH-302 in Sprague–Dawley rats following a single intravenous (IV) and oral (PO) dose of TH-302. The values following the IV doses represent mean (±SD) concentrations from three rats at each dose level. The values following the PO dose are a composite from different sets of animals and represent mean (±SD) concentrations from three rats per time point

pharmacokinetics over this dose range (Table 1; Fig. 2). The mean terminal plasma $t_{1/2}$ of TH-302 ranged between 1.32 and 4.37 h following the IV dose. Mean plasma clearance was high (4.69–6.14 l/h/kg), and mean steady-state volume of distribution was large (1.67–5.09 l/kg).

Male Sprague–Dawley rats ($n = 3$ per time point) were given a single oral dose of 100 mg/kg TH-302 (Table 1; Fig. 2). TH-302 was rapidly and well absorbed with mean peak plasma concentration reached at the first sampled time point of 15 min and an absolute oral bioavailability of 77.3%. The terminal plasma $t_{1/2}$ of TH-302 was 1.59 h.

In the acute safety study, following a single 30-min intravenous infusion of 50, 100 and 200 mg/kg TH-302, C_{max} and *AUC* increased linearly and in a dose-proportional manner with increasing dose in both male and female rats (Table 2). Mean C_{max} and *AUC* were qualitatively similar for males and females. The mean apparent terminal half-life of TH-302 was independent of dose, time and gender, ranging between 0.268 and 0.390 h (16.1–23.4 min).

Dogs

Male beagle dogs ($n = 3$) were given a single IV dose (20 mg/kg) of TH-302. Mean clearance of TH-302 was high (3.28 l/h/kg), and the mean steady-state volume of distribution was large (1.98 l/kg). The mean $t_{1/2}$ value following the IV dose was 0.82 h (Table 1; Fig. 3).

In the acute safety study, following a single 30-min intravenous infusion of 4, 8, 16 and 32 mg/kg TH-302, C_{max} and *AUC* increased linearly, but in a less than dose-proportional manner, with increasing dose in male and female beagle dogs (Table 2). Mean C_{max} and *AUC* were qualitatively similar for males and females. The mean apparent terminal half-life of TH-302 was independent of dose, time and gender, ranging between 0.248 and 0.279 h (14.9–16.7 min).

Monkeys

Male cynomolgus monkeys ($n = 3$) were given a single IV (20 mg/kg) dose of TH-302. Similar to rodents and dogs, TH-302 had high clearance (7.66 l/h/kg) and a large steady-state volume of distribution (2.92 l/kg). Plasma $t_{1/2}$ was 0.415 h following the IV dose (Table 1; Fig. 3).

Repeat-dose toxicokinetics

Rats

The toxicokinetic parameters of TH-302 and Br-IPM, and active moiety, on Days 1 and 15 are summarized in Table 3. As there was no accumulation observed on Day 15 as compared to Day 1, only the steady-state plasma concentration versus time profiles for TH-302 and Br-IPM on day 15 are shown in Fig. 4a and b, respectively. C_{max} and *AUC* of TH-302 increased linearly and in a dose-proportional manner with increasing dose in male and female rats on Days 1 and 15 and were qualitatively and quantitatively similar for males and females. However, Br-IPM plasma concentrations were highly variable and significantly less (4.0–37.1%) than those observed for TH-302. In most cases, C_{max} and AUC_{last} were similar between the genders except at the lowest dose (12.5 mg/kg) where these values were up to 6-fold greater in females as compared to males. There

Table 2 Single-dose toxicokinetic parameters of TH-302 after a 30-min intravenous infusion of TH-302 in rats and dogs

Species	Gender	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/ml)	*AUC* (μg h/ml)	Half-life (h)	Cl (l/h/kg)	V_{ss} (l/kg)
Rat[a]	Females	50	0.517	14.1	7.33	0.319	6.87	2.81
		100	0.500	30.0	16.1	0.286	6.16	2.59
		200	0.683	71.1	40.6	0.390	4.84	2.35
	Males	50	0.517	15.6	7.94	0.333	6.27	2.57
		100	0.550	30.9	17.0	0.268	5.90	2.59
		200	0.550	62.5	37.6	0.323	5.38	2.51
Dog[b]	Females	4	0.517	3.83	2.27	0.269	1.76	0.785
		8	0.525	6.41	3.40	0.251	2.38	0.974
		16	0.517	13.7	7.66	0.279	2.09	0.874
		32	0.517	18.5	9.53	0.256	3.39	1.37
	Males	4	0.617	4.16	2.33	0.268	1.70	0.822
		8	0.525	6.35	3.64	0.260	2.23	0.982
		16	0.525	10.8	6.20	0.258	2.58	1.10
		32	0.525	23.0	11.0	0.248	2.91	1.12

[a] $n = 3$ rats per time point

[b] $n = 2$

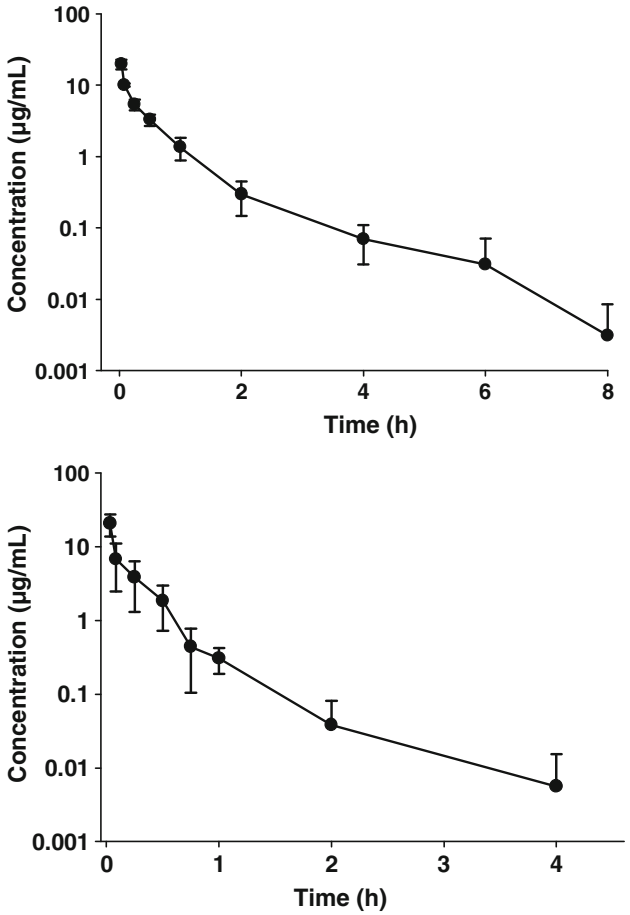

Fig. 3 *Plot* of plasma concentration versus time data for TH-302 in beagle dogs (*upper panel*) and Cynomolgus monkeys (*lower panel*) following a single intravenous dose of TH-302 (20 mg/kg). The values following the IV dose represent mean (±SD) concentrations from 3 animals

was not a dose-dependent increase in Br-IPM C_{max} except in male rats. The apparent terminal half-lives of TH-302 and Br-IPM were independent of dose, time and gender, ranging between 0.252 and 0.400 h (15.1–24.0 min), and 0.0565 and 0.252 h (3.9–15.1 min), respectively (Table 3).

Dogs

The toxicokinetic parameters of TH-302 and Br-IPM on Days 1 and 15 are summarized in Table 4. C_{max} and *AUC* for TH-302 and Br-IPM increased linearly and in a dose-proportional manner with increasing dose in male and female dogs. While mean C_{max} and *AUC* were qualitatively similar for males and females on Days 1 and 15, the mean C_{max} in females was approximately 40% higher than in males. No accumulation of TH-302 was observed on Day 15 as compared to Day 1 except in males at the 8 mg/kg dose group where the mean C_{max} and *AUC* were increased by 59 and 33%, respectively. Time-averaged plasma concentrations (e.g., AUC_{last}, *AUC*) as well as C_{max} of Br-IPM were substantially lower than for TH-302 ranging between 0.00 and 4.36%. Following 4 and 8 mg/kg TH-302, all plasma concentrations of Br-IPM were BQL on Days 1 and 15. At the 16 mg/kg dose group, most plasma concentrations of Br-IPM were also BQL 30 min following the end of the infusion on Days 1 and 15. Mean C_{max} and AUC_{last} were 1.46- to 2.06-fold greater in females as compared to males on Day 1, but less than unity on Day 15. Although no accumulation of Br-IPM was observed at the 8 and 16 mg/kg dose groups in females on Day 15 as compared to Day 1, accumulation of 1.41- to 1.93-fold was seen in males. The large number of BQL values in the terminal elimination

Table 3 Repeat-dose toxicokinetic parameters of TH-302 and Br-IPM after once weekly administration of a 30-min intravenous infusion of TH-302 on Days 1 and 15 in rats

Analyte	Gender	Day	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/ml)	AUC_{last} (μg h/ml)	AUC (μg h/ml)	Half-life (h)	Cl (l/h/kg)	V_{ss} (l/kg)
TH-302	Females	1	12.5	0.550	2.11	1.03	1.05	0.352	11.7	5.91
			25	0.550	4.64	2.35	2.37	0.278	10.5	4.20
			50	0.550	8.08	4.11	4.12	0.335	11.9	5.74
		15	12.5	0.533	1.72	0.962	0.968	0.318	16.7	5.78
			25	0.533	3.44	1.84	1.84	0.306	14.0	5.94
			50	0.533	7.17	4.27	4.29	0.290	15.4	4.77
	Males	1	12.5	0.583	1.39	0.730	0.733	0.279	12.6	6.70
			25	0.617	3.02	1.76	1.77	0.377	13.5	7.62
			50	0.617	5.39	3.16	3.19	0.400	11.4	8.85
		15	12.5	0.617	1.02	0.662	0.664	0.252	18.4	6.68
			25	0.567	4.25	1.95	1.95	0.245	12.7	4.49
			50	0.567	6.04	3.27	3.30	0.296	14.9	6.35
Br-IPM	Females	1	12.5	0.550	0.557	0.139	0.139	0.0565	NC	NC
			25	0.550	0.389	0.170	0.170	0.214	NC	NC
			50	0.530	0.618	0.320	0.320	0.193	NC	NC
		15	12.5	0.550	0.157	0.0860	0.0860	0.196	NC	NC
			25	0.533	0.281	–	NC	NC	NC	NC
			50	0.533	0.616	0.309	0.309	0.241	NC	NC
	Males	1	12.5	0.583	0.516	–	NC	NC	NC	NC
			25	0.583	0.416	0.121	0.121	0.148	NC	NC
			50	0.667	0.325	0.164	0.164	0.133	NC	NC
		15	12.5	0.650	0.0869	0.0452	0.0452	0.148	NC	NC
			25	0.567	0.333	0.0820	0.0820	0.128	NC	NC
			50	0.567	0.444	0.175	0.175	0.252	NC	NC

$n = 3$ rats per time point

phase in most animals precluded the calculation of half-life for Br-IPM. The mean apparent terminal half-life of TH-302 was independent of dose, time and gender, ranging between 0.222 and 0.302 h (13.7–18.1 min) (Table 5).

Allometric scaling

Figure 5 illustrates the interspecies correlations for Cl and V_{ss}. Allometric equations for Cl and V_{ss} were extrapolated to obtain estimates for 70 kg humans. Table 2 lists the parameters of the allometric equations (a and b) and the coefficient of determination (r^2). Correlations between Cl and V_{ss} with body weight were excellent ($r^2 \geq 0.975$). The predicted values of Cl and V_{ss} in humans were 46.9 l/h (27.1 l/h/m^2) and 40.7 l (23.5 l/m^2), respectively, which results in a predicted half-life of 0.601 h (36 min). The predicted TH-302 pharmacokinetic parameters were similar to the observed parameters (38.8 l/h/m^2, 34.3 l/m^2 and 47.0 min, respectively) in patients in the first-in-man study. Figure 6 illustrates the excellent correlation between predicted and observed (mean ± SD) plasma concentration versus time profiles in 3 (6 profiles) and 22 patients (39 profiles) with solid tumors following a 30-min infusion of 7.5 and 480 mg/m^2, respectively.

Conclusions

TH-302 is a bioreductive agent exhibiting up to 550× greater toxicity for hypoxic cells as compared to oxygenated cells in proliferation and clonogenic assays using a number of human cancer cell lines [10, 11]. TH-302 is being developed for the treatment of solid tumors. The studies reported herein were carried out to assess the pharmacokinetics (including absorption, distribution and clearance) of TH-302 in the preclinical species used for efficacy and safety toxicology studies. The studies were carried out in nude and CD-1 mice, Sprague–Dawley rats, beagle dogs and Cynomolgus monkeys as part of the preclinical pharmacokinetic characterization of TH-302.

After intravenous administration, TH-302 was well distributed in the body, with a steady-state volume of

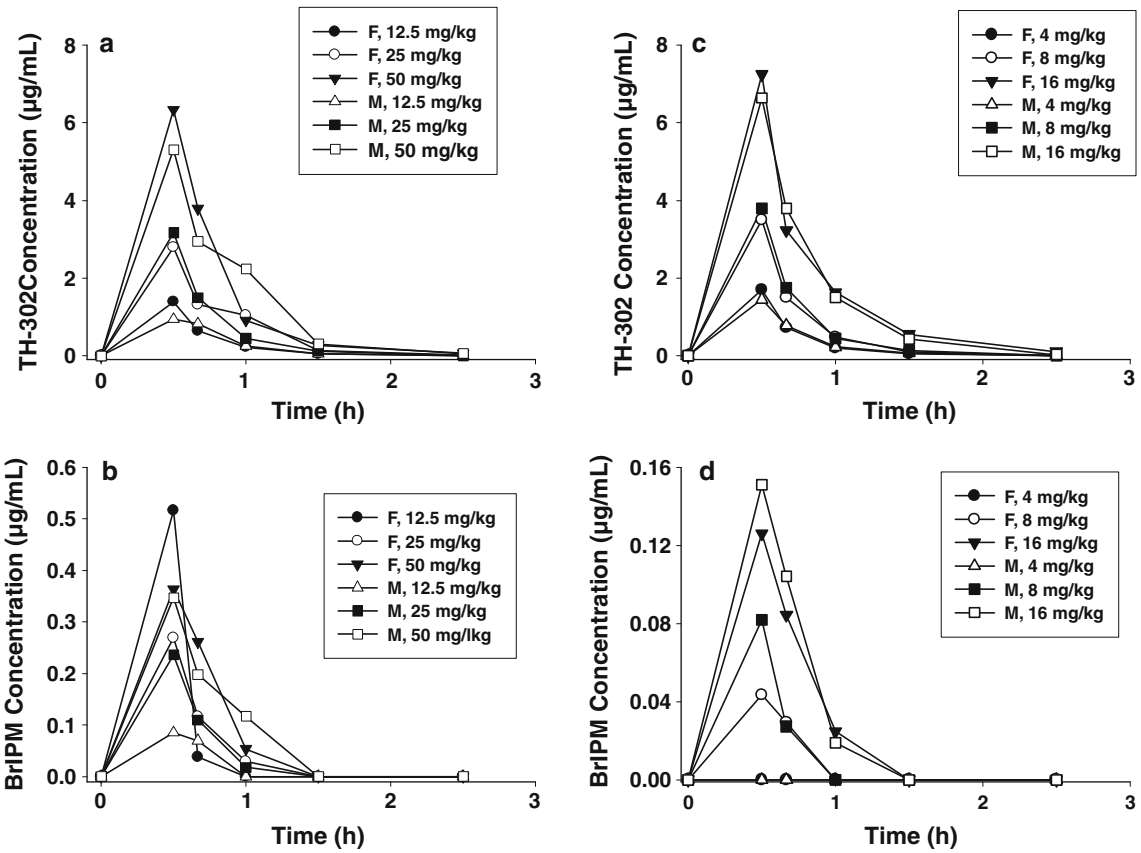

Fig. 4 Steady-state plasma concentration–time profiles of TH-302 and Br-IPM in rats (**a** and **b**) and dogs (**c** and **d**) on Day 15 (**c** and **d**) (in these *figures M* represents male animals and *F* represents female animals)

Table 4 Allometric scaling of pharmacokinetic parameters for TH-302 based on intravenous data from mice, rats, dogs and monkeys

	r^2	*a*	*b*	Predicted value in humans	Observed value in humans ($n = 45$)
Cl (l/h/m^2)	0.984	0.697	0.991	27.1	34.3 ± 12.2
V_{ss} (l/m^2)	0.975	0.252	1.20	23.5	38.8 ± 16.8
Half-life (h)[a]	–	–	–	0.601	0.783 ± 0.234

[a] Half-life computed as 0.693 × Cl/V_{ss}

distribution (V_{ss}) similar (0.542 l/kg in mice) to or greater (1.67–5.09 l/kg in rats, 1.98 l/kg in dogs and 2.92 l/kg in monkeys) than total body water. TH-302 exhibited high clearance (3.28–7.66 l/h/kg) in all animal species studied when compared with their respective hepatic plasma flows (1.07–2.97 l/h/kg) [15]. The high clearance observed indicates that there may be an extra-hepatic clearance pathway involved. TH-302 exhibited a biphasic profile in all species, except in mice where its pharmacokinetics was best described by a single compartment model, possibly due to the lower sensitivity of the bioanalytical assay used. In rat, dog and monkeys, using a more sensitive bioanalytical method, TH-302 plasma concentrations were shown to drop up to two orders of magnitude or greater from the initial concentrations, followed by a prolonged elimination phase. Instead of a half-life observed in mice ranging between 0.132 and 0.438 h, the terminal elimination half-life ranged between 0.415 and 4.37 h in the other species. Although the terminal elimination half-life values varied across the species tested, on average, the short half-life observed in the mice was comparable to the α-phase half-life in the rat, dog and monkey, where the *AUC*s comprising the α- and ß-phases contributed to approximately 87 and 13% of the total area under the plasma concentration versus time curve, respectively, suggesting that the predominant half-life of TH-302 is the distribution (α-phase) half-life, rather than the elimination (*β*-phase) half-life. The rapid decline in plasma concentrations was likely due to a number of factors, including tissue distribution and metabolic clearance.

The repeat-dose pharmacokinetics of TH-302 in rats and dogs, the primary animal species for nonclinical safety

Table 5 Repeat-dose toxicokinetic parameters of TH-302 and Br-IPM after once weekly administration of a 30-min intravenous infusion of TH-302 on Days 1 and 15 in dogs

Analyte	Gender	Day	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/ml)	AUC_{last} (μg h/ml)	AUC (μg h/ml)	Half-life (h)	Cl (l/h/kg)	V_{ss} (l/kg)
TH-302	Females	1	4	0.517	1.81	0.921	0.939	0.229	4.30	1.67
			8	0.523	3.38	1.81	1.83	0.247	4.43	1.83
			16	0.533	6.42	3.51	3.55	0.222	4.48	1.89
		15	4	0.523	1.70	0.868	2.94	0.243	4.71	1.90
			8	0.517	3.50	1.79	2.84	0.248	4.62	1.87
			16	0.613	7.56	4.36	2.52	0.302	3.60	1.85
	Males	1	4	0.547	1.29	0.771	0.787	0.288	5.19	2.45
			8	0.540	2.41	1.48	1.51	0.254	5.38	2.54
			16	0.533	5.91	3.67	3.69	0.288	4.28	2.07
		15	4	0.540	1.45	0.805	2.46	0.299	4.96	2.30
			8	0.530	3.80	1.98	2.82	0.257	4.07	1.68
			16	0.540	6.64	4.01	2.49	0.279	3.90	1.87
Br-IPM	Females	1	4	NC	0.00	0.00	NC	NC	NC	NC
			8	0.525	0.0667	0.0222	NC	NC	NC	NC
			16	0.533	0.155	0.0660	0.135	0.249	NC	NC
		15	4	NC	0.00	0.00	NC	NC	NC	NC
			8	0.517	0.0434	0.0167	NC	NC	NC	NC
			16	0.613	0.141	0.0575	0.108	0.305	NC	NC
	Males	1	4	NC	0.00	0.00	NC	NC	NC	NC
			8	0.539	0.0344	0.0108	NC	NC	NC	NC
			16	0.533	0.106	0.0399	NC	NC	NC	NC
		15	4	–	0.00	0.00	NC	NC	NC	NC
			8	0.530	0.0819	0.0268	NC	NC	NC	NC
			16	0.540	0.151	0.0665	0.182	0.481	NC	NC

$n = 5$

evaluations, were consistent with the kinetics after single doses. A few differences, however, were noted: Unlike in dogs, there was no gender-related difference in observed plasma concentrations in rats. Quantitatively, after repeat-dose administration of TH-302, again, unlike in rats, Br-IPM C_{max} and AUC values were a significantly smaller fraction of the values for TH-302. Consistent with the short half-lives, there was no or minimal accumulation of TH-302 and Br-IPM in plasma following repeat-dose administration in rats and dogs.

Interspecies scaling is commonly used to predict human pharmacokinetics for dose selection in first-in-man (FIM) studies. Allometry has been used to relate the pharmacokinetics among different species to body weight, assuming biological similarity of anatomy and physiology among mammals. The primary objectives of the TH-302 interspecies scaling were (1) to provide estimates of exposure in terms of AUC in humans and (2) to provide information on predicted plasma concentration–time profiles in humans for the selection of blood sampling times and bioanalytical purposes. Since TH-302 is intended to be administered as a short 30-min intravenous infusion in the FIM study, human plasma concentration versus time profiles were first predicted for intravenous administration using allometric scaling. The parameter estimates from the modeling were subsequently used to generate predicted concentration versus time profiles following a 30-min intravenous infusion, assuming a body surface area of 1.73 m^2. Using the results from the rat and dog toxicology studies as well as the scaling information and assuming the most conservative position, in terms of safety, an intravenous infusion dose of 7.5 mg/m^2 TH-302 was selected as the first dose in the FIM study. Plasma concentration versus time profiles of the 7.5 mg/m^2 and one additional intravenous infusion dose of 480 mg/m^2 TH-302 were predicted to be likely representations of what may be observed in the FIM study (Fig. 6) [16]. The observed plasma concentration versus time profiles show that allometric scaling provided an excellent prediction of the pharmacokinetic profile of TH-302 in humans.

The in vivo data on TH-302 predict an acceptable pharmacokinetic profile in humans and along with the desired

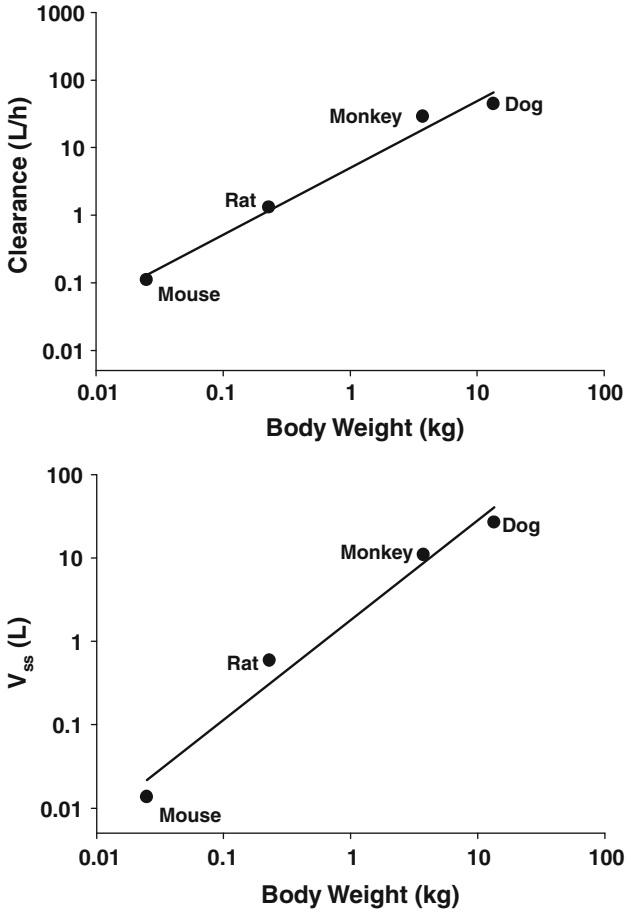

Fig. 5 Allometric relationship of clearance and apparent steady-state volume of distribution (V_{ss}) in animals to body weight

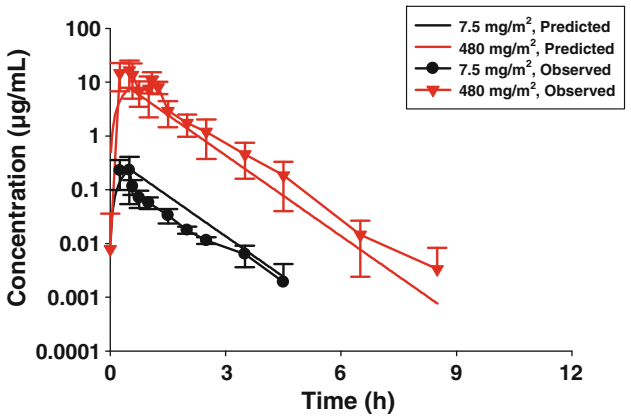

Fig. 6 Observed (mean ± SD) and predicted plasma TH-302 concentration versus time profiles following a single 30-min intravenous infusion of 7.5 and 480 mg/m^2 TH-302 to patients with solid tumors

efficacy in preclinical efficacy models [10, 11] make the compound an attractive candidate for further development. The predictable and dose-dependent pharmacokinetics of TH-302 together with its excellent pre-clinical efficacy were key factors supporting the continued assessment and development of the compound. Consequently, TH-302 was recommended for advancement into clinical trials and is currently being developed as a therapy for solid tumors.

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