

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**207981Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

Lonsurf (trifluridine and tipiracil)

**Date:** September 18, 2015

**To:** File for NDA 207981

**From:** John K. Leighton, PhD, DABT

Director, Division of Hematology Oncology Toxicology  
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Lonsurf conducted by Drs. Khasar and Fox, and secondary memorandum and labeling provided by Dr. Helms. Following finalization of the primary and secondary reviews, additional discussion occurred about the proper description of the established pharmacological class (EPC) for this combination product. As the anticancer activity of the drug is through the action of the trifluridine portion of the drug, the original recommendation was to use the EPC for this portion of the drug to describe the EPC for LONSURF. Upon further review and discussion, and consistent with practices with other combination products in which one component of the product is present primarily to affect the pharmacokinetics of the other component, the Division revised the recommendation for the EPC for LONSURF to be "LONSURF is a combination of trifluridine, a nucleoside metabolic inhibitor, and tipiracil, a thymidine phosphorylase inhibitor, indicated for..."

I concur with Dr. Helms' conclusion that Lonsurf may be approved for the proposed indication and that no additional nonclinical studies are needed.

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/s/  
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JOHN K LEIGHTON  
09/18/2015

## MEMORANDUM

**Date:** August 25 , 2015  
**From:** Whitney S. Helms, PhD.  
Supervisory Pharmacologist  
Division of Hematology Oncology Toxicology for Division of Oncology Products 2  
**To:** File for NDA #207981  
FTD:TPI (LONSURF)  
**Re:** Approvability of Pharmacology and Toxicology

Non-clinical studies examining the pharmacology and toxicology of FTD:TPI provided to support NDA 207981 for the treatment of patients with metastatic colorectal cancer who have been previously treated with, (b) (4) fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy, an anti-VEGF biological therapy, and an anti-EGFR therapy were reviewed in detail by G. Sachia Khasar, PhD, and Emily M. Fox, PhD. The submission included studies of orally administered FTD:TPI in mice, rats, and monkeys that investigated the drug's pharmacology, pharmacokinetics, safety pharmacology, general toxicology, genetic toxicity (in vivo and in vitro), and reproductive toxicity.

FTD:TPI is a combination product comprised of trifluradine (FTD) and tipiracil in a 1:0.5 ratio. FTD is a thymidine analog that was previously approved for the treatment of epithelial keratitis cause by herpes simplex virus. Pharmacology studies showed that FTD interferes with DNA synthesis in cultured mammalian cells and the drug was incorporated into the DNA of human cancer cells in vitro. FTD was able to inhibit in vivo tumor growth following its administration either alone or as a part of FTD:TPI. Conversely, TPI was not able to inhibit tumor growth on its own in pharmacology studies submitted to support NDA 207981. TPI did, however, enhance and prolong the exposure of animals treated with FTD:TPI to FTD in multiple in vivo studies. This prolongation of FTD exposure is consistent with studies showing that TPI inhibits thymidine phosphorylase as this enzyme has phosphorylytic activity against FTD resulting in its degradation. Because the anti-tumor activity of FTD:TPI is driven by the activity of the FTD portion of the drug, the established pharmacologic class of this drug is consistent with that of a nucleoside metabolic inhibitor.

The major targets of FTD:TPI in toxicology studies conducted in rats and monkeys included bone marrow, and gastrointestinal tract. Myelosuppression was the major toxicity observed clinically and GI-toxicity was a commonly reported adverse event, thus, the toxicology studies were predictive of the major clinical toxicities reported to date. In addition, the metabolism of the two components is sufficiently similar between species that no additional studies are warranted to investigate metabolite-mediated toxicity.

In rats additional toxicity not observed in clinical studies of FTD:TPI included changes in the teeth including whitish and fractured incisors along with disarrangement of the odontoblasts and osteodentin. Though these changes are unlikely to be relevant in the intended patient population, they may be important in a pediatric setting and are, therefore, included in the

Pediatric Use section of the label. Both FTD and TPI were present in the milk of lactating rats following administration of FTD:TPI.

FTD:TPI was both mutagenic and clastogenic in assays for genotoxicity. FTD was responsible for the genotoxicity of FTD:TPI as TPI alone was negative in in vitro genotoxicity assays. Carcinogenicity studies were not conducted to support the use of FTD:TPI in patients with metastatic colorectal cancer and are not warranted to support the use of the drug in these patients.

While studies to assess FTD:TPI-mediated effects on fertility were not required to support the approval of the drug for the treatment of patients with advanced cancer, the Applicant submitted studies examining the effects of FTD:TPI on both male and female fertility. Though neither this study nor the additional embryo-fetal development study included a toxicokinetic assessment, both studies employed the same doses used in the 13-week repeat dose study in rats allowing for exposure comparisons between the animal data and clinical exposure at the recommended dose. Administration of FTD:TPI did not show an effect on either male or female fertility at doses resulting in exposures at or above those seen clinically at the recommended dose of 35 mg/m<sup>2</sup> twice daily. In females, however, FTD:TPI administration at a dose approximately equal to the clinical exposure at the recommended dose did result in an increase in early post-implantation loss.

In the embryo-fetal development study, administration of FTD:TPI to pregnant rats during the period of organogenesis resulted in decreased fetal body weights at doses  $\geq$  74 mg/kg (approximately 0.33 times the clinical exposure at 35 mg/m<sup>2</sup> twice daily). Increased post-implantation loss occurred at the 221 mg/kg dose of FTD:TPI (FTD dose of 150 mg/kg and approximately 0.92 times the human exposure at the 35 mg/m<sup>2</sup> twice daily dose). At the same dose level, external (kinked tail, ectrodactyly, cleft palate, and anasarca), visceral (great vessel anomalies), and skeletal abnormalities occurred. A warning for embryofetal risk and a recommendation for females to use contraception during treatment with FTD:TPI are included in the label.

**Recommendations:** I concur with the conclusion of Drs. Khasar and Fox that the pharmacology and toxicology data are sufficient to support the approval of NDA 207981 for LONSURF for the treatment of patients with. There are no outstanding nonclinical issues that would prevent the approval of LONSURF for the proposed indication.

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/s/  
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WHITNEY S HELMS  
08/25/2015

**DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH**

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 207981  
Supporting document/s: 003  
Applicant's letter date: December 19, 2014  
CDER stamp date: December 19, 2014  
Product: Lonsurf (trifluridine:tipiracil)  
Indication: Metastatic colorectal cancer  
Applicant: Taiho Oncology Inc.  
202 Carnegie Center Suite 100  
Princeton, NJ 08540  
Review Division: Division of Hematology Oncology Toxicology  
(Division of Oncology Products 2)  
Reviewers: G. Sachia Khasar, PhD  
Emily M Fox, PhD  
Supervisor/Team Leader: Whitney Helms, PhD  
Division Director: John Leighton, PhD  
(Patricia Keegan, MD)  
Project Manager: Gina Davis

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# 1 Executive Summary

## 1.1 Introduction

Taiho Oncology Inc. has submitted NDA 207981 to support the approval of Lonsurf (TAS-102) for the treatment of patients with metastatic colorectal cancer who have been previously treated with, (b) (4) fluoropyrimidine- oxaliplatin-, and irinotecan-based chemotherapy, an anti-VEGF biological therapy, and an anti-EGFR therapy. Lonsurf is a fixed combination of the nucleoside analog trifluridine (FTD) and the thymidine phosphorylase inhibitor, tipiracil hydrochloride (TPI) presented at a 1:0.5 ratio of FTD:TPI. The recommended dose of Lonsurf is 35 mg/m<sup>2</sup> (based on trifluridine component) taken twice daily on Days 1-5 and 8-12 of a 28-day cycle within one hour of morning and evening meals.

## 1.2 Brief Discussion of Nonclinical Findings

The Applicant conducted in vitro and in vivo studies investigating the pharmacological activity of both FTD and TPI, the two components of trifluridine:tipiracil (FTD:TPI). FTD itself was previously approved for the treatment of epithelial keratitis caused by herpes simplex virus and was shown to interfere with DNA synthesis in cultured mammalian cells. Consistent with its activity as a thymidine analog, FTD was incorporated into the DNA of human cancer cells following 4 and 24 hours of incubation with concentrations of FTD that have been achieved clinically at the recommended dose of 35 mg/m<sup>2</sup> FTD:TPI given twice daily. Incubation with FTD also resulted in transient depletion of the intracellular pool of thymidine, consistent with its ability to non-covalently bind and inhibit thymidylate synthase. FTD inhibited the in vitro proliferation of various human cancer cell lines with IC<sub>50</sub> values ranging from 0.214 μM to 24.4 μM. FTD:TPI exhibited in vivo anti-tumor activity in various human colorectal cancer xenograft models in nude mice, including *KRAS* wild-type (COL-1) and cetuximab-resistant *KRAS* mutant (HCT-116) xenografts. Further, FTD:TPI exhibited in vivo anti-tumor activity against MX-1 human breast cancer xenografts relatively insensitive to the oral fluoropyrimidine anticancer drug TS-1.

TPI inhibits the activity of thymidine phosphorylase. In contrast to FTD, the Applicant showed that treatment of mice implanted with various tumor models with TPI alone resulted in no effect on tumor growth compared to control treated mice, though FTD:TPI had anti-tumor activity. In pharmacokinetic analyses, the administration of TPI along with FTD resulted in in vivo FTD exposures of greater than or equal to 100-fold higher than those following administration of FTD alone. Significant increases in FTD exposure following FTD:TPI administration compared to FTD alone also occurred in the 13-week repeat-dose toxicology study in the monkey. Thus, the data support the conclusion that the major role of TPI in FTD:TPI is to enhance the exposure of FTD.

FTD and TPI preferentially distributed to plasma rather than blood cells in rat, monkey, and human blood. Following single oral administration of ([<sup>14</sup>C]FTD)FTD:TPI or ([<sup>14</sup>C]TPI)FTD:TPI to lactating rats, radioactivity was excreted into milk. Thus, women should be advised to avoid breastfeeding during treatment with FTD:TPI and, based on

its short half-life of 2 hours, for at least one day following the final dose. TPI was not substantially metabolized in vitro in human hepatocytes, although the minor metabolite 6-hydroxymethyluracil (6-HMU) was detected in human plasma and urine at trace levels. 6-HMU was also detected in rat plasma, urine, and feces following single oral administration of [ $^{14}\text{C}$ -TPI]FTD:TPI or  $^{14}\text{C}$ -TPI, providing nonclinical exposure for this metabolite. In human hepatocytes, FTD was metabolized in vitro to 5-(trifluoromethyl)uracil (FTY), uracil-5-carboxylic acid (5-CU), and 5-carboxy-2'-deoxyuridine (5-CdUrd), with FTY being the major metabolite. Consistent with the in vitro findings, trifluridine was metabolized to FTY in human plasma along with 5-CU and 5-CdUrd at low or trace levels. Following a single oral administration of FTD:TPI in which either FTD or TPI was radiolabelled, the majority of FTD-associated radioactivity was excreted in the urine, whereas TPI-associated radioactivity was excreted primarily in the feces. Consistent with clinical findings, the major FTD metabolite detected in rat and monkey plasma and urine was FTY. Adequate exposure to FTY occurred in animals to account for potential metabolite-mediated toxicity. Although 5-CU and 5-CdUrd were not detected in rat or monkey plasma or urine in the nonclinical PK studies conducted by the Applicant, published studies have demonstrated that 5-CU and 5-CdUrd have been detected in urine following single intravenous administration of  $^{14}\text{C}$ -FTD to monkeys. Given these published data, the low amounts of 5-CU and 5-CdUrd detected in human plasma, and the advanced cancer indication, further metabolite evaluation is not warranted at this time.

The rat and monkey were the major species used to test the safety of FTD:TPI in toxicology studies. Animal exposure to FTD following 13 weeks of administration of FTD:TPI (approximately 2:1 ratio) at the high dose level was approximately equal to or greater than (0.92-fold in the rat at 221 mg/kg FTD:TPI, 2.3-fold in the monkey at 29.42 mg/kg) the clinical exposure measured by AUC of 23697 ng·h/mL at the recommended dose of 35 mg/m<sup>2</sup> twice daily. Major target organs of toxicity in both species included the hematopoietic system and gastrointestinal (GI) tract. Following single oral administration of [ $^{14}\text{C}$ -FTD]FTD:TPI to rats, tissue distribution was high in the GI tract, also consistent with clinical findings of GI toxicity. In in vivo safety pharmacology studies in male Sprague-Dawley rats, single oral doses of FTD:TPI up to 640 mg/kg had no significant effect on general physical condition, respiratory rate, tidal volume, or minute volume. Similarly, FTD:TPI had no significant effect on CNS up to 24 hours post administration, though its distribution to the brains of rats was ~7% of that in plasma, suggesting that FTD:TPI is able to cross the blood-brain barrier, at least at low levels. FTD:TPI did not significantly inhibit in vitro hERG-mediated potassium current in stably transfected HEK293 cells at concentrations up to 300  $\mu\text{M}$ , which is much higher than the clinical  $C_{\text{max}}$  of FTD achieved at the recommended human dose of FTD:TPI (~16  $\mu\text{M}$ , ~4857 ng/mL). Consistent with the in vitro findings, single and repeated administration of FTD:TPI had no significant effect on QT/QTc prolongation in in vivo animal studies and no clear effects of FTD:TPI on QTc prolongation have been reported in clinical trials. FTD:TPI and FTD were positive in genetic toxicology tests (mutations and chromosomal aberrations), while TPI was negative. The major toxicity of myelosuppression (including decreased WBC and RBC); mild bone marrow

hypocellularity, as well as the GI tract toxicities reported clinically were predictable from nonclinical toxicology studies in rats and monkeys.

In pharmacokinetic studies in pregnant rats both FTD and TPI were able to cross the placental barrier. FTD:TPI had no effect on fertility in male or female rats; however, administration of the drug either early in development or during the period of organogenesis resulted in decreased numbers of viable fetuses. Toxicokinetic data was not collected in the rat embryofetal development (EFD) study, but, the same doses were used in the rat EFD study and the 13-week repeat-dose toxicology study, allowing for clinical exposure comparison. Based on the toxicokinetic data from the long-term study in rats, increased embryo-fetal lethality occurred at maternal exposures similar to clinical exposures at the clinically recommended dose. Other observations included decreased fetal weights at doses greater than or equal to 74 mg/kg, as well as delayed ossification, and visceral and skeletal abnormalities at the 221 mg/kg dose level. A warning for the risk of effects on embryofetal development is warranted in the label for FTD:TPI. In addition, based on the embryofetal risk, a clinical half-life of the drug of approximately 2 hours, and positive findings for genotoxicity, patients are advised to use contraception during treatment with FTD:TPI and, in males, for 3 months following the final dose of the drug.

### **1.3 Recommendations**

#### **1.3.1 Approvability**

The Applicant submitted sufficient pharmacology and toxicology studies to support the use of TAS-102 (Lonsurf) in the proposed patient population. There are no outstanding pharmacology/toxicology issues that would prevent the approval of Lonsurf, therefore, the pharmacology/toxicology team recommends the approval of this application.

#### **1.3.2 Additional Non Clinical Recommendations**

None

#### **1.3.3 Labeling**

More detailed labeling recommendations will be addressed in a separate review, if warranted.

## **2 Drug Information**

### **2.1 Drug**

CAS Registry Number: 183204-72-0

Generic Name: Tipiracil

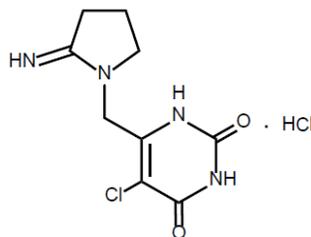
Code Name: TPI; TAS-1-462

Chemical Name: 2,4(1H,3H)-Pyrimidinedione, 5-chloro-6-[(2-imino-1-pyrrolidinyl)methyl]-, hydrochloride (1:1)

Molecular Formula/Molecular Weight:  $C_9H_{11}ClN_4O_2 \cdot HCl/279.12$

Structure

**Figure 1: Tipiracil hydrochloride**



CAS Registry Number: 70-00-8

Generic Name: Trifluridine

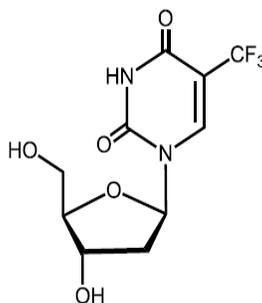
Code Name: FTD;  $F_3TdR$ ;  $F_3dThd$

Chemical Name: 2'-deoxy-5-(trifluoromethyl)uridine thymidine,  $\alpha,\alpha,\alpha$ -trifluoro-

Molecular Formula/Molecular Weight:  $C_{10}H_{11}F_3N_2O_5/296.20$

Structure

**Figure 2: Trifluridine**



Pharmacologic Class: FTD is an antineoplastic thymidine-based nucleoside analog; TPI is a thymidine phosphorylase inhibitor

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 57,674 (owned by the Applicant); DMF 28368; DMF (b) (4) (Letters of authorization provided)

## 2.3 Drug Formulation

Trifluridine/Tipiracil Hydrochloride (FTD/TPI) Film-Coated Tablets (FCT) (15/7.065 mg) contain 15 mg of trifluridine and 7.065 mg tipiracil hydrochloride. They are white, round, biconvex, immediate-release film-coated tablets.

Trifluridine/Tipiracil Hydrochloride (FTD/TPI) Film-Coated Tablets (FCT) (20/9.420 mg) contain 20 mg of trifluridine and 9.420 mg of tipiracil hydrochloride. They are pale red, round, biconvex, immediate-release film-coated tablet.

**Table 1: Composition of FTD/TPI FCT (15 mg and 20 mg)**

Component	Quality standard	Function	Quantity (mg)/tablet	
			FTD/TPI FCT 15 mg	FTD/TPI FCT 20 mg
(b) (4)				
Trifluridine (FTD)	In-house	Active ingredient	15.000	20.000
Tipiracil hydrochloride (TPI)	In-house	Active ingredient	(b) (4)	
Lactose monohydrate	NF	(b) (4)		
Pregelatinized starch	NF	(b) (4)		
Stearic acid	NF			
(b) (4)				
Hypromellose	USP			
Polyethylene glycol	NF	(b) (4)		
Titanium dioxide	USP			
Ferric oxide	NF			
(b) (4)				
Magnesium stearate	NF	(b) (4)		
<b>Total</b>			<b>122.7</b>	<b>165.6</b>

q. s. = quantity sufficient

(b) (4)

<sup>b)</sup> Trace quantities appear in the final product

(b) (4)

NF = National Formulary; USP = United States Pharmacopeia

(Excerpted from Applicant's submission)

## 2.4 Comments on Novel Excipients

None

## 2.5 Comments on Impurities/Degradants of Concern

(b) (4)

At the proposed limit of (b) (4), the daily exposure of (b) (4) from administration of TAS-102 at the clinically recommended dose will not exceed (b) (4). Based on the available toxicity data, this dose is acceptable for the proposed patient population.

## 2.6 Proposed Clinical Population and Dosing Regimen

Lonsurf is indicated for the treatment of patients with metastatic colorectal cancer who have been previously treated with, (b) (4) fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF biological therapy, and an anti-EGFR therapy.

The recommended dose of Lonsurf in adults is 35 mg/m<sup>2</sup>/dose administered orally twice daily for 5 days a week with 2 days rest for 2 weeks, followed by a 14-day rest (1 treatment cycle). This treatment cycle is repeated every 4 weeks.

## 2.7 Regulatory Background

December 28 1998: Taiho Oncology filed IND 57674 for development of TAS-102

June 23, 2009: IND 57674 was inactivated

June 24, 2011: IND 57674 was re-activated

October 25, 2013 OSE granted initial request for Lonsurf

March 24, 2014: TAS-102 approved in Japan for the treatment of patients with unresectable advanced or recurrent colorectal cancer (mCRC).

July 31 2014: Pre-NDA meeting held

September 12, 2014: Fast Track granted  
 December 19, 2014: Application received  
 February 15, 2015: Application filed.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

##### Pharmacology

Study #	Study title
M01-2006-0025	Comparison of Uptake into Cells and DNA of FTD and Nucleoside Analogues
20061-004	Evaluation of the effects of $\alpha,\alpha,\alpha$ -trifluorothymidine (FTD) in suppressing the proliferation of various human cancer cell lines
11TA05	Survival-prolonging effects of TAS-102 to mice xenograft model intraperitoneally implanted KM20C, human colon adenocarcinoma cells
11TA01	Antitumor effects of TAS-102 toward human colon cancer cell lines with mouse xenograft implanted COL-1 and HCT-116
11TA02	Efficacy study of TAS-102 on MX-1 human breast cancer cell line using TS-1 as comparator
03-12-012	Analysis of transcription factor binding motifs in differentially expressed genes associated with TAS-102 administration
20111-003	Evaluation of the sustainability of inhibitory effect of FTD on TS by measurement of intracellular dTTP pool
03-13-004	Cytotoxicity test by concomitant use of FTD and dThd analog-type antiviral drug

##### Safety Pharmacology

Study #	Study title
B040836	Safety Pharmacology Study of TAS-102 Effects on the Central Nervous System in Rats
B040837	Safety Pharmacology Study of TAS-102 Effects on the Respiratory System in Conscious Rats
B040835	Safety Pharmacology Study of TAS-102 Effects on the Cardiovascular System in Conscious Monkeys
B061099	Safety Pharmacology Study of FTD Effects on the Cardiovascular System in Conscious Monkeys
B050268	Safety Pharmacology Studies of FTD Effects on hERG Current
B050270	Safety Pharmacology Studies of TPI Effects on hERG Current

##### Pharmacokinetics

Study #	Study title
C-O149	Contribution Study of TPI on Pharmacokinetics of FTD in Monkey
12DA41	Pharmacokinetic Evaluation of the Optimum Dosage Ratio of FTD to TPI in Monkey
AE-6932-G	Placental and embryo fetal transfer of TAS-102 using [ $^{14}\text{C}$ ]FTD or [ $^{14}\text{C}$ ]TPI in pregnant rats
AE-6933-G	Excretion into milk of TAS-102 using [ $^{14}\text{C}$ ]FTD or [ $^{14}\text{C}$ ]TPI in nursing rats
AE-2350-3G	Pharmacokinetic Studies on TAS-102 (III) Absorption, Distribution, Metabolism and Excretion in Rats using $^{14}\text{C}$ -FTD

Study #	Study title
AE-2350-2G	Pharmacokinetic Studies on TAS-102 (II) Absorption, Distribution, Metabolism and Excretion in Rats using <sup>14</sup> C-TPI
AE-4140	Pharmacokinetic Study of TAS-102 Structural Analysis of Metabolite in Rats After Oral Administration of <sup>14</sup> C-TPI
AE-6930-G	Pharmacokinetic Evaluation of TAS-102 using [ <sup>14</sup> C]FTD or [ <sup>14</sup> C]TPI in cynomolgus monkeys
12DA 8	Metabolite Analysis for FTD and TPI Using Cryopreserved Human Hepatocytes
12DB03	Metabolic study of <sup>14</sup> C-FTD using human liver microsomes
11DA34	Study of Blood Cell Distribution of FTD and TPI Using Blood of Human, Monkey, and Rat

#### General Toxicology/Toxicokinetics

Study #	Study title
07CA07	A 13-Week Oral Repeated Dose Toxicity Study of TAS-102 in Rats with a 9-Week Recovery Period
B-6227	A 13-Week Oral Repeated Dose Toxicity Study of TAS-102 in Cynomolgus Monkeys with a 9-week Recovery Period

#### Genetic toxicology

Study #	Study title
00CA 1	Reverse mutation test of TAS-102 in bacteria
06CA06	A Reverse Mutation Test of FTD in Bacteria
B050590	Bacterial Reverse Mutation Study of TPI
00CA 2	Chromosomal aberration test on TAS-102 in CHL cells
01CA 5	Micronucleus test of TAS-102
06CA 2	Micronucleus test of FTD in Mice
B050591	Micronucleus test of TPI in Mice
06CA05	A Chromosome Aberration test of FTD in CHL/IU cells
B050592	A Chromosome Aberration test of TPI in CHL/IU cells

#### Reproductive toxicology

R-908	Study of fertility and early embryonic development to implantation in rats treated orally with TAS-102- Administration to males
R-904	Study of Fertility and Early Embryonic Development to Implantation in Rats Treated Orally With TAS-102-Administration to Females
04CA 8	Reproductive and Developmental Toxicity Study of TAS-102 Study for Effects on Embryo-Fetal Development in Rats by Oral Administration

#### Special Toxicology

B110945	In vitro 3T3 NRU Phototoxicity test of FTD
B110946	In vitro 3T3 NRU Phototoxicity test of TPI

### 3.2 Studies Not Reviewed

#### Pharmacology

Study #	Study title
11TA03	Comparative Study of Antitumor Effect between Oral and Sustained Administration of FTD in Animal Model with MX-1 Human Breast Cancer Cell Line Implanted Subcutaneously
11TA04	Comparative Study of Antitumor Effect between Single and Divided Administration of TAS-102 in MX-1 Human Breast Cancer Cell Line
20061-003	A Study to Determine the Dose Levels of TAS-102 Useful Against Human Gastric Carcinoma SC-2

#### Safety Pharmacology

Study #	Study title
B050587	Safety Pharmacology Study of TPI Effects on the Respiratory System in Conscious Rats
B050588	Safety Pharmacology Study of TPI Effects on the Central Nervous System in Rats
B050589	Safety Pharmacology Study of TPI Effects on the Cardiovascular System in Conscious Monkeys
B061097	Safety Pharmacology Study of FTD Effects on the Central Nervous System in Rats
B061098	Safety Pharmacology Study of FTD Effects on the Respiratory System in Conscious Rats

#### Pharmacokinetics

Study #	Study title
06DB01	Analytical Method Validation for the Determination of FTD and FTY in Mouse Plasma by LC/MS/MS
60347-V1	Validation of High Performance Liquid Chromatographics Methods for the Determination of TAS-102 in Dog and Monkey Plasma
97A05	Analytical Method Validation for the Determination of TPI in Dog and Rat Plasma
A-1031	TPI, FTD, and FTY: Validation of Analytical Method for Determining Concentration in Rat Plasma
C-A310	Validation of Analytical Method for the Determination of TPI in Rat Plasma by LC/MS/MS
C-A311	Stability Study of TPI in Rat Plasma by LC/MS/MS
C-A312	Validation of Analytical Method for the Determination of TPI in Monkey Plasma by LC/MS/MS
C-A313	Stability Study of TPI in Monkey Plasma by LC/MS/MS
C-A316	Validation of Analytical Method for the Determination of FTD and FTY in Rat Plasma by LC/MS/MS
C-A317	Stability Study of FTD and FTY in Rat Plasma by LC/MS/MS
C-A318	Validation of Analytical Method for the Determination of FTD and FTY in Monkey Plasma by LC/MS/MS
C-A319	Stability Study of FTD and FTY in Monkey Plasma by LC/MS/MS
C-AT120008	Partial Validation of Analytical Method for the Determination of TPI, FTD, and FTY in Monkey Plasma by LC/MS/MS
07DA 1	Pharmacokinetic Study of TAS-102 After Single Oral Administration in Mice

Study #	Study title
12DA 3	Investigation of the Absorption Site of FTD and TPI in Rat Digestive Tract
AE-6931-G	Quantitative whole-body autoradiography of TAS-102 using [ <sup>14</sup> C]FTD or [ <sup>14</sup> C]TPI in pigmented rats
P041295	Caco-2 Cell Permeability Study of FTD and TPI
11DA38	Plasma Metabolite Profiling in "Clinical Pharmacology Study to Investigate the Food Effect on Pharmacokinetics of TAS-102"
99C42	Preliminary In Vitro Metabolic Study of TPI
P05-10408	Search for TPI Metabolites in Human Plasma and Urine after Dosing of TAS-102 for TAS-102 Phase I Clinical Study for Solid Tumor Patients
09DB 2	Uptake Study of TPI Using Stable Cell Line Expressing hOCT2
11DA 9	Cytochrome P-450 1A2 and 3A4 Induction by FTD and TPI Using Cryopreserved Human Hepatocytes.
11DB 4	Uptake Study of TPI Using Cells Stably Expressing hOAT3
12DA05	Interaction Study on Plasma Protein Binding of FTD
12DA 7	Uptake Study of FTD and TPI using Membrane Vesicles Expressing MDR1
13DB 6	Uptake Studies of 14C-FTD using OATP1B1/1B3-Expressing Cells and of 14C-TPI using OAT1-Expressing Cells
13DB 8	Uptake Study of [ <sup>14</sup> C]FTD and [ <sup>14</sup> C]TPI using Human BCRP-expressing Membrane Vesicles
XT115147	In Vitro Evaluation of FTD and TPI as Inhibitors of Cytochrome P450 Enzymes in Human Liver Microsomes
XT133075	In Vitro Evaluation of FTY, FTD, and TPI as Inducers of Cytochrome P450 Expression in Cultured Human Hepatocytes
XT135055	In Vitro Evaluation of 5-(Trifluoromethyl)uracil (FTY) as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes

#### Reproductive Toxicology

00CC37	Embryo-fetal development
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#### Special Toxicology Study

13CC20	Combination Toxicity Study of TAS-102 and Azidothymidine in Rats
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### 3.3 Previous Reviews Referenced

IND 57,674

## 4 Pharmacology

### 4.1 Primary Pharmacology

#### M01-2006-0025: Comparison of Uptake into Cells and DNA of FTD and Nucleoside Analogues

**Methods:** The Applicant compared the amount of uptake of  $\alpha,\alpha,\alpha$ -trifluorothymidine (FTD; lot # 046019) into DNA and cells compared to that of thymidine (a natural substance) and other nucleoside analogues [1-beta-D-arabinofuranosylcytosine (Ara-C), gemcitabine (dFdC), and 5-fluoro-2'-deoxyuridine (FdUrd)]. NUGC-3 human gastric cells and MCF-7 human breast cancer cells were incubated with a mixture of unlabeled

and tritium-labeled FTD, thymidine, Ara-C, dFdC, and FdUrd at their IC<sub>50</sub> values (see exposure levels in Table 2 and Table 3). After 4 and 24 hrs, harvested cells were counted and drug uptake into tumor cells was quantified using high performance liquid chromatography (HPLC). DNA was extracted, absorbance of the DNA solution was measured at 260 nm, and DNA concentration and total uptake of the tritium labeled drug into DNA was calculated.

**Results:** Following 4 and 24 hrs of incubation, the amount of FTD uptake into the DNA of NUGC-3 and MCF-7 cells was smaller than that of thymidine, but greater than that of the other nucleoside analogues. FTD uptake into the cells of both cancer cell lines was also observed. These data show that FTD is taken up by cells and incorporated into the DNA.

**Table 2: FTD Uptake into the DNA and cells of NUGC-3 Cells**

Drug	Exposure level (μM)	Duration of exposure (hr)	Uptake into cells	Uptake of triphosphate into cells	Uptake into DNA
			pmol / 10 <sup>6</sup> cells Mean ± SD	pmol / 10 <sup>6</sup> cells Mean ± SD	pmol / μg DNA Mean ± SD
Thd	2.64	4	16.1 ± 18.1	4.27 ± 4.96	12.9 ± 7.40
		24	25.8 ± 13.0	19.8 ± 8.70	7.66 ± 4.60
FTD	2.64	4	52.7 ± 3.02	4.89 ± 0.263	0.504 ± 0.0797
		24	70.9 ± -	15.6 ± -	0.779 ± 0.556
Ara-C	0.217	4	8.32 ± 1.44	0.899 ± 0.444	0.0364 ± 0.0000823
		24	2.85 ± 0.473	0.694 ± 0.0727	0.0918 ± 0.0210
dFdC	0.00446	4	10.2 ± 1.41	1.41 ± 0.0519	0.0114 ± 0.00123
		24	2.36 ± 0.869	2.04 ± 0.184	0.0258 ± 0.00457
FdUrd	0.102	4	12.4 ± 0.728	N.D. ± -	0.000710 ± 0.0000436
		24	60.8 ± 3.46	N.D. ± -	0.0244 ± 0.000556

(Excerpted from Applicant's Submission)

**Table 3: FTD Uptake into the DNA and cells of MCF-7 Cells**

Drug	Exposure level (μM)	Duration of exposure (hr)	Uptake into cells	Uptake of triphosphate into cells	Uptake into DNA
			pmol / 10 <sup>6</sup> cells Mean ± SD	pmol / 10 <sup>6</sup> cells Mean ± SD	pmol / μg DNA Mean ± SD
Thd	0.545	4	26.8 ± 2.23	14.1 ± 1.01	6.30 ± 0.937
		24	35.0 ± 15.1	12.1 ± 4.66	4.27 ± 1.27
FTD	0.545	4	26.0 ± 6.01	5.42 ± 1.75	0.520 ± 0.234
		24	34.0 ± 4.98	3.91 ± 0.0380	0.984 ± 0.0328
Ara-C	0.694	4	32.1 ± 3.52	13.4 ± 0.457	0.0922 ± 0.0887
		24	71.5 ± 24.7	7.88 ± 3.10	0.452 ± 0.105
dFdC	0.00104	4	0.681 ± 0.184	0.230 ± 0.0606	0.00294 ± 0.000765
		24	2.52 ± 0.0375	0.582 ± 0.00350	0.0141 ± 0.00378
FdUrd	1.05	4	105 ± 3.41	N.D. ± -	0.0233 ± 0.00241
		24	571 ± 118	N.D. ± -	0.111 ± 0.0150

(Excerpted from Applicant's Submission)

### 20061-004: Evaluation of the effects of α,α,α-trifluorothymidine (FTD) in suppressing the proliferation of various human cancer cell lines

**Methods/Results:** The Applicant evaluated the effects of FTD and 5-fluorouracil (5-FU; a metabolic antagonist) on the proliferation of human cancer cell lines. Human cancer cell lines were plated in 96-well plates (n=3) and treated with FTD (lot #046019) or 5-FU dissolved in pure water at concentrations up to 1000 μM for 3 days. WST-8 was then added to each well, and absorbance at 450 nM was measured to determine cell viability. FTD and 5-FU inhibited the proliferation of various human cancer cell lines with IC<sub>50</sub> values of 0.214 μM to 24.4 μM and 3.18 μM to 17.7 μM, respectively. Thus, FTD

exhibited in vitro anti-proliferative activity against various human cancer cell lines. FTD was more effective than 5-FU at suppressing the proliferation of human gastric, prostatic, head/neck, and leukemia cells. FTD inhibited proliferation of human colorectal cancer cells with an IC<sub>50</sub> of 10.7 µM compared to 4.96 µM with 5-FU.

**Table 4: Anti-proliferative Activity of FTD Against Human Cancer Cell Lines**

Cell line	Origin	IC <sub>50</sub> (µM)	
		FTD	5-FU
NUGC-3	Gastric carcinoma	3.46	8.50
HCT-15	Colorectal carcinoma	10.7	4.96
A549	Lung carcinoma	3.50	3.18
MDA-MB-435	Breast carcinoma	14.4	4.39
SK-OV-3	Ovarian carcinoma	24.4	14.0
HeLa	Uterine carcinoma	8.15	9.12
J82	Bladder carcinoma	8.66	9.15
DU145	Prostatic carcinoma	1.48	7.70
CFPAC-1	Pancreatic carcinoma	2.05	3.25
KB	Head/neck carcinoma	5.84	17.7
CCRF-CEM	Leukemia	0.214	8.40

(Excerpted from Applicant's Submission)

### 11TA05: Survival-prolonging effects of TAS-102 to mice xenograft model intraperitoneally implanted KM20C, human colon adenocarcinoma cells

#### Methods

Drugs, lots: TAS-102: FTD lot #065022 + TPI lot #7A0104  
Irinotecan hydrochloride hydrate (CPT-11)  
Tegafur-gimeracil-oteracil potassium compound (S-1)

Dose, route of administration (ROA), frequency of dosing: TAS-102: 150 mg/kg/day, oral, twice daily on Days 1-28  
CPT-11: 100 mg/kg/day, intravenous (IV), Days 1, 8, 15, 22; S-1: 8.3 mg/kg/day, oral, once daily on Days 1-28

Dose Volume: 10 mL/kg

Formulation/Vehicle: TAS-102 and S-1: 0.5% hydroxypropyl methylcellulose (HPMC); CPT-11: physiological saline solution

Species/Strain: 5 wk old male nude mice

Number/Sex/Group: 10 mice/group

Study design: 2 x 10<sup>7</sup> cells/mL of KM20C cells suspended in phosphate-buffered saline (PBS) were implanted intraperitoneally in nude mice (Day 0). Dosing was initiated on Day 1. The survival time (days), the difference in survival curves (lifespan effect), the increase in life span (ILS), and the body weight change between Days 0 and 29 were determined.

**Results:** The ability of TAS-102 to prolong survival was investigated in nude mice bearing human KM20C colon adenocarcinoma xenografts and compared to that of CPT-11 and S-1, which are used clinically to treat patients with progressive, recurrent colorectal cancer. Twice daily oral treatment with TAS-102 at a dose of 150 mg/kg/day resulted in a median survival time of 70 days and an ILS of 86.7% compared to vehicle-treated mice. Analysis of the lifespan effect using a Log-Rank test demonstrated that

treatment with TAS-102 resulted in significantly longer survival and a greater increase in lifespan compared to control, CPT-11, and S-1 ( $p < 0.01$ ). Treatment with TAS-102 for 29 days resulted in 11% mean body weight loss compared to Day 0; ~5% body weight loss was observed in vehicle-treated mice on Day 29 compared to Day 0. Thus, treatment with TAS-102 prolonged survival in nude mice implanted with human colon adenocarcinoma cells with minimal body weight loss.

**Table 5: Effect of TAS-102 on human KM20C colon cancer xenografts in nude mice**

Group	Dose (mg/kg/day)	Treatment	No. of animals	Survival time (day)										Survival Median	ILS <sup>a)</sup> (%)	BWC <sup>b)</sup>	
				Individuals												(g, Mean ± SD)	(%, Mean ± SD)
Control (0.5%HPMC)	–	Day 1~28, p.o.(b.i.d.)	10	30	32	32	33	37	38	40	43	43	44	38	–	-1.20 ± 2.08	-5.25 ± 9.19
TAS-102	150	Day 1~28, p.o.(b.i.d.)	10	56	57	61	66	68	72	74	79	79	87	70 <sup>**§§##</sup>	86.7	-2.45 ± 1.99	-10.85 ± 8.61
CPT-11	100	Day 1,8,15,22, i.v.(q.d.)	10	46	48	49	58	59	61	61	64	64	65	60 <sup>**</sup>	60.0	3.89 ± 1.06	17.18 ± 4.70
S-1	8.3	Day 1~28, p.o.(q.d.)	10	39	40	41	43	44	44	44	48	49	49	44 <sup>**</sup>	17.3	-0.82 ± 1.54	-3.63 ± 6.93

a): Median increase of life span : ILS (%) = [(median survival time of treated group) / (median survival time of Control group) – 1] × 100

b): Body weight change. BWC on Day 29 were calculated according to the following formula:

$$\text{BWC (g)} = (\text{BW on Day 29}) - (\text{BW on Day 0}), \quad \text{BWC (\%)} = [(\text{BW on Day 29}) - (\text{BW on Day 0})] / (\text{BW on Day 0}) \times 100$$

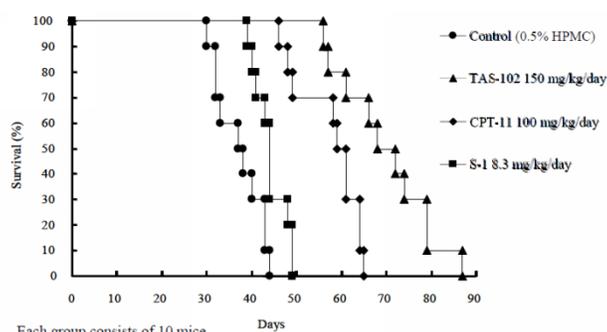
\*\* :  $p < 0.01$  by Log-Rank test as compared to the Control group.

§§ :  $p < 0.01$  by Log-Rank test as compared to the CPT-11 group.

## :  $p < 0.01$  by Log-Rank test as compared to the S-1 group.

(Excerpted from Applicant's Submission)

**Figure 3: Effect of TAS-102 on the survival of nude mice implanted with human KM20C colon cancer xenografts**



Each group consists of 10 mice.

(Excerpted from Applicant's Submission)

### 11TA01: Antitumor effects of TAS-102 toward human colon cancer cell lines with mouse xenograft implanted COL-1 and HCT-116

#### Methods

Drugs, lots: TAS-102: FTD lot #065022 + TPI lot #7A0104  
Cetuximab, lot #110350

Dose, route of administration (ROA), frequency of dosing: TAS-102: 150 mg/kg/day, oral, twice daily on Days 1-14  
Cetuximab: 40 mg/kg/day, IP, once daily on Days 1, 4, 8, and 11

Dose Volume: 10 mL/kg

Formulation/Vehicle: TAS-102: 0.5% HPMC; cetuximab: physiological saline

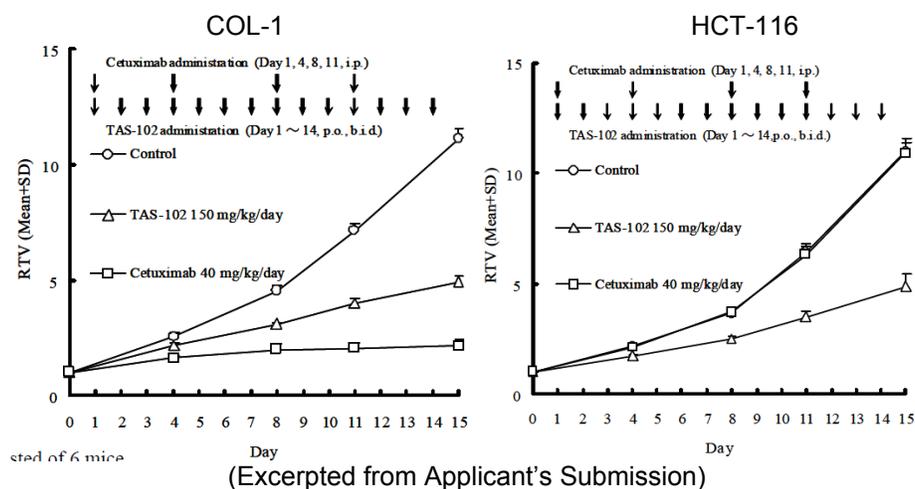
Species/Strain: 5 wk old male nude mice

Number/Sex/Group: 6 mice/group

Study design: ~2 mm COL-1 and HCT-116 tumor fragments previously passaged through mice were implanted subcutaneously in the right flank of nude mice. Dosing was initiated 10-13 days after implantation (designated Day 1) when mean tumor volumes were ~120 mm<sup>3</sup> (COL-1) and 145 mm<sup>3</sup> (HCT-116). Tumors were measured on Days 0, 4, 8, 11, and 15 using calipers. Body weight was measured every day from Day 0 to 15 (control and TAS-102) or on Days 0, 1, 4, 8, 11, and 15 (cetuximab). Relative tumor volume (RTV) = (TV on Day 15) / (TV on Day 0). Tumor growth inhibition rate (TGI, %) = [(mean RTV of control) - (mean RTV of treated group)] / (mean RTV of control) x 100.

**Results:** The anti-tumor activity of TAS-102 was investigated in nude mice bearing the human colon cancer xenografts COL-1 (*KRAS* wild-type) and HCT-116 (*KRAS* mutant). IP administration of cetuximab inhibited COL-1 (80.8% TGI) but not HCT-116 xenograft growth (0.6% TGI), indicating that cetuximab was ineffective against *KRAS* mutant tumors in this model. Twice daily treatment with TAS-102 at a dose of 150 mg/kg/day resulted in statistically significant inhibition of COL-1 and HCT-116 xenograft growth (55.8% and 55.5% TGI, respectively) without inducing overt toxicity. These data suggest that TAS-102 exhibits similar anti-tumor activity against *KRAS* wild-type and mutant (cetuximab resistant) tumors.

**Figure 4: Effect of TAS-102 on human colon cancer xenografts in nude mice**



### 11TA02: Efficacy study of TAS-102 on MX-1 human breast cancer cell line using TS-1 as comparator

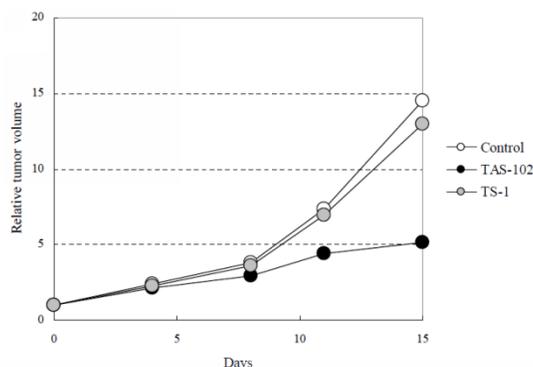
#### Methods

Drugs, lots: TAS-102: FTD lot #065022 + TPI lot #7A0104  
 TS-1, tegafur (FT) lot #9G83  
 Dose, route of administration (ROA), TAS-102: 150 mg/kg/day, oral, twice daily on Days 1-14  
 TS-1: 8.3 mg/kg/day, oral, once daily on Days 1-14

frequency of dosing:  
 Dose Volume: 10 mL/kg  
 Formulation/Vehicle: 0.5% HPMC  
 Species/Strain: 6 wk old male nude mice  
 Number/Sex/Group: 8 mice/group  
 Study design: A ~2 mm MX-1 tumor fragment was inoculated subcutaneously in the right flank of nude mice. Dosing was initiated when mean tumor volumes were 100-300 mm<sup>3</sup>. Tumors and body weight were measured on Days 0 (grouping day), 4, 8, 11, and 15. RTV = (TV on Day 15) / (TV on Day 0). %IR = [1 - (mean RTV of treated group) / (mean RTV of control group)] x 100.

**Results:** The anti-tumor activity of TAS-102 was investigated in nude mice bearing MX-1 human breast cancer xenografts. Twice daily treatment with TAS-102 at a dose of 150 mg/kg/day resulted in statistically significant MX-1 xenograft inhibition compared to control and the oral fluoropyrimidine anticancer drug TS-1; tumor growth inhibition was 64.3% and 10.5% for TAS-102 and TS-1, respectively. Treatment with TAS-102 and TS-1 induced a mean body weight loss of 5.7% and 2.4%, respectively, on Day 15 compared to Day 0. These data suggest that TAS-102 exhibits anti-tumor activity against MX-1 xenografts relatively insensitive to the oral fluoropyrimidine anticancer drug TS-1.

**Figure 5: Effect of TAS-102 on human MX-1 breast cancer xenografts in nude mice**



(Excerpted from Applicant's Submission)

**03-09-008: Efficacy study of administration of TPI alone in animal models with various human tumors implanted subcutaneously**

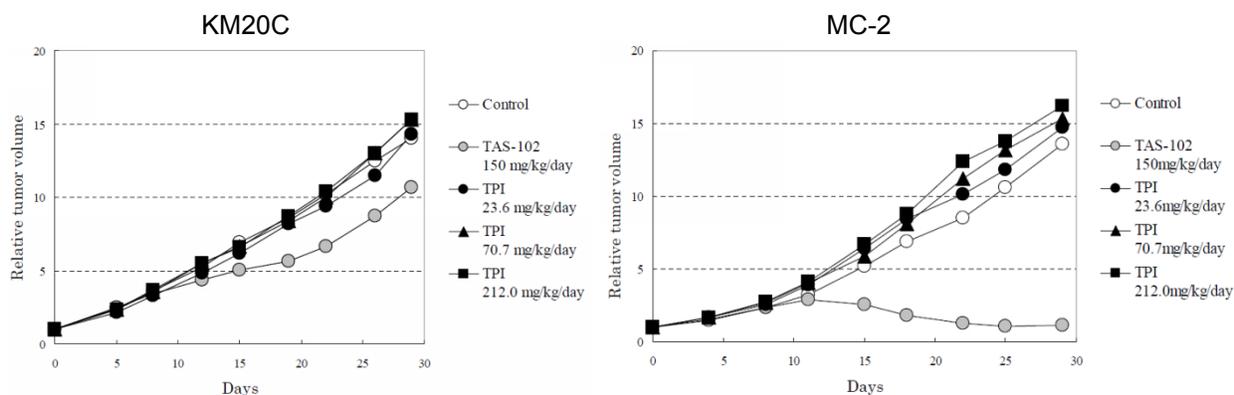
**Methods**

Drugs, lots: TAS-102: FTD lot #056020  
 TPI lot #7A0104  
 Dose, route of administration (ROA), frequency of dosing: TAS-102: 150 mg/kg/day, oral, twice daily on Days 1-14  
 TPI: 23.6, 70.7, and 212.2 mg/kg/day, oral, twice daily on Days 1-14  
 Dose Volume: 10 mL/kg

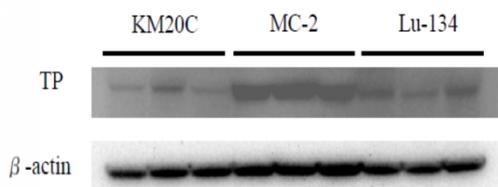
Formulation/Vehicle: HPMC  
 Species/Strain: 5 wk old male nude mice  
 Number/Sex/Group: 7 (KM20C), 6 (MC-2), or 5 (Lu-134 ) mice/group  
 Study design: ~2 mm KM20C, MC-2, and Lu-134 tumor fragments previously passaged subcutaneously through mice were implanted into the right chest of nude mice. Mice were grouped according to approximately equal tumor volume on Day 0, and then dosing was initiated. Tumors and body weights were measured twice a week. Mice that were not included in the grouping were followed until their tumor volume reached ~400-700 mm<sup>3</sup>. Tumors were then removed and homogenized, and tumor lysates were analyzed by western blotting for TP and  $\beta$ -actin.  $RTV = (TV \text{ on Day } 15) / (TV \text{ on Day } 0)$ .  $\%IR = [1 - (\text{mean } RTV \text{ of treated group}) / (\text{mean } RTV \text{ of control group})] \times 100$ .

**Results:** The Applicant investigated the contribution of thymidine phosphorylase inhibitor (TPI) to the anti-tumor activity of TAS-102 in nude mice bearing human KM20C colon cancer, human MC-2 breast cancer, and human Lu-134 lung cancer xenografts. Twice daily treatment with TAS-102 at a dose of 150 mg/kg/day resulted in statistically significant decreases in KM20C, MC-2, and Lu-134 xenograft growth by Day 15 with IR values of 27.1%, 50.5% (shown in Figure 6), and 61.3%, respectively. TAS-102 treatment induced  $\leq 10\%$  body weight loss. In contrast, twice daily treatment with TPI at clinically relevant doses (23.6 to 212 mg/kg/day) did not demonstrate appreciable anti-tumor activity in TP-expressing xenografts (see Figure 7), with Day 15 IR values of  $\leq 10.6\%$  (23.6 mg/kg/day TPI),  $\leq -13.7\%$  (70.7 mg/kg/day TPI), and  $\leq 18.4\%$  (23.6 mg/kg/day TPI) in KM20C, MC-2, and Lu-134 xenografts, respectively. In fact, treatment with TPI appeared to increase the tumor growth rate of MC-2 xenografts, which expressed the highest level of TP. These data suggest that TPI does not contribute to the anti-tumor activity of TAS-102.

**Figure 6: Effect of TAS-102 on human cancer xenografts in nude mice**



(Excerpted from Applicant's Submission)

**Figure 7: Thymidine phosphorylase expression in human cancer xenografts**

(Excerpted from Applicant's Submission)

**03-12-012: Analysis of transcription factor binding motifs in differentially expressed genes associated with TAS-102 administration**

**Methods:** The Applicant investigated the effect of TAS-102 administration on gene expression and transcription factor binding motifs present in shared promoter regions of differentially expressed genes. Mice subcutaneously implanted with KM20C human colon cancer cells were orally administered TAS-102 twice daily at 150 mg/kg/day for 7 days. Tumor samples were taken from TAS-102 and control-treated mice 1 hr after the second administration on Days 1, 3, and 7. DNA microarrays ( (b) (4), 4 x 44K) were used to analyze gene expression levels. Pscan, a web-based database for transcription factor binding motifs (Zambelli, Pesole, et al. 2009), was used to analyze genes in which expression increased  $\geq 2$ -fold or decreased  $\geq 50\%$ .

**Results:** The Pscan-based analysis created six lists of differentially expressed genes (shown in Table 6). Pscan demonstrated that binding motifs of the TATA box-binding protein (TBP) were predominant in the promoter regions of genes that were downregulated on Day 3 of TAS-102 administration. No statistically meaningful transcription factor binding motifs were observed among the genes upregulated by TAS-102. TBP binding sequences are rich in A-T and tend to incorporate FTD. Since FTD is a thymidine analog and is highly electronegative, FTD incorporation into DNA is thought to affect DNA functions such as transcription factor binding. Taken together, these data suggest that the TAS-102-induced decrease in TBP-dependent genes may be due to the incorporation of FTD into A-T-rich promoter regions, thus influencing DNA and transcription factor interactions.

**Table 6: The number of differentially expressed genes following in vivo TAS-102 administration**

	Number of differentially expressed genes		
	Day 1	Day 3	Day 7
Down (decreased expression by 50% or more)	11	75	133
Up (increased expression by 2-fold or more)	10	31	128

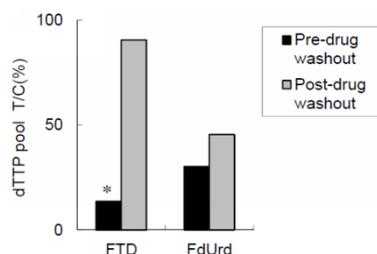
(Excerpted from Applicant's Submission)

### 20111-003: Evaluation of the sustainability of inhibitory effect of FTD on TS by measurement of intracellular dTTP pool

**Methods:** The Applicant evaluated the persistence of TS inhibitory action of FTD using HPLC. Since TS inhibition decreases the dTTP pool, HPLC was used to evaluate changes in the thymidine-5'-triphosphate (dTTP) pool in human HeLa cervical cancer cells following 4 hours of exposure to 30  $\mu\text{M}$  FTD and 4 hours of washout. Exposure to 100  $\mu\text{M}$  of the TS inhibitor 5-fluoro-2'-deoxyuridine (FdUrd) was used as a positive control, and distilled water was used as a negative control. The ratio of dTTP in treated cells/control cells was expressed as the T/C %.

**Results:** 4 hrs of incubation with FTD and FdUrd resulted in T/Cs of 13.6% and 30.3%, respectively, indicating that FTD inhibited TS activity as measured by a decrease in the dTTP pool. Following an additional 4 hrs of washout, T/Cs were 90.7% and 45.5% for FTD and FdUrd, respectively. Thus, FTD-induced reduction of the dTTP pool recovered more quickly following washout compared to that induced by FdUrd, suggesting that FdUrd-induced TS inhibition was more persistent. According to the Applicant this is likely because FdUMP, but not FTP, inhibits TS through formation of a strong covalent bond.

**Figure 8: Effect of FTD on the persistence of TS inhibition in HeLa cells**



\*: a portion of the samples fell outside the dTTP pool concentration calibration curve  
(Excerpted from Applicant's Submission)

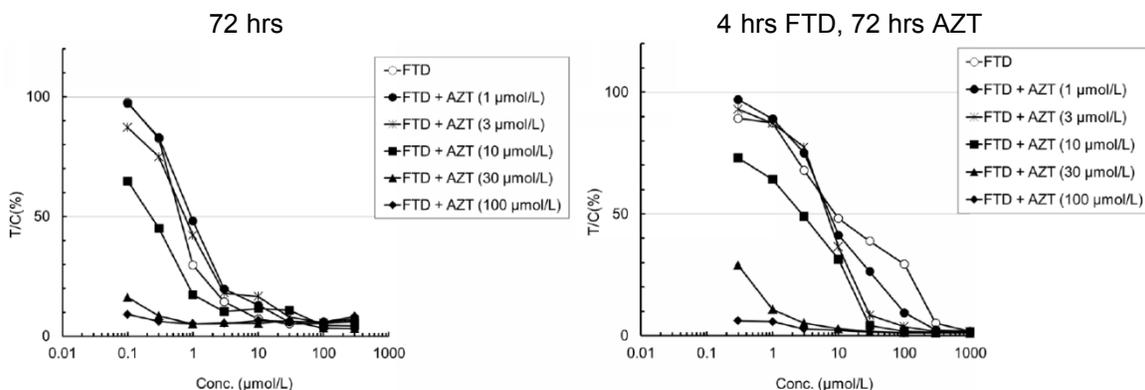
### 03-13-004: Cytotoxicity test by concomitant use of FTD and dThd analog-type antiviral drug

**Methods:** The Applicant evaluated the in vitro interactions between FTD and thymidine (dThd) analog-type antiviral drugs. Human colon HCT116 cells and human gastric NUGC-3 cells plated in 96-well plates were treated with FTD (lot #065022) for either 4 hrs (0.3-1000  $\mu\text{M}$ ) or 72 hrs (0.1-300  $\mu\text{M}$ ) concomitantly with either 1-100  $\mu\text{M}$  3'-azido-3'-deoxythymidine (AZT, zidovudine), 2', 1-100  $\mu\text{M}$  3'-didehydro-3'-deoxythymidine (d4T, stavudine), 3-300  $\mu\text{M}$  2'-deoxy-L-thymidine (LdT, telbivudine) or the positive control dThd (0.03-3  $\mu\text{M}$ ) for 72 hrs. FTD, AZT, d4T, and LdT are dThd analogs. The cells were then fixed and stained with 0.05% crystal violet solution for 30 minutes, and a microplate spectrophotometer was used to measure absorbance at 550 nm in order to measure cell viability.

**Results:** As expected, treatment with dThd reduced FTD-induced inhibition of HCT116 and NUGC-3 cells at 4 and 72 hrs. Treatment with d4T or LdT had no effect on FTD-

induced inhibition of HCT116 or NUGC-3 proliferation at either time point assessed (data not shown). Treatment with AZT at concentrations >10  $\mu\text{M}$  enhanced FTD-induced inhibition of NUGC-3 and HCT116 cells following 4 or 72 hrs of FTD incubation.

**Figure 9: Effects of AZT and FTD treatment on NUGC-3 Cell Growth**

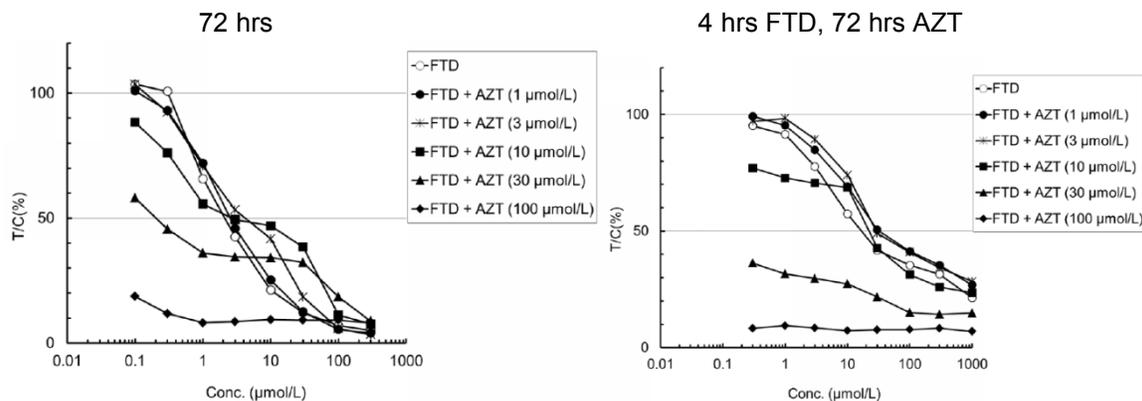


$\Delta\text{ABS}$  = mean absorbance (ABS) values in wells of drug treatment groups – mean ABS values of blanks

$\%T/C = (\Delta\text{ABS at each drug concentration} / \Delta\text{ABS of the control}) \times 100$

(Excerpted from Applicant's Submission)

**Figure 10: Effects of AZT and FTD treatment on HCT116 Cell Growth**



(Excerpted from Applicant's Submission)

According to the Applicant, the clinical  $C_{\text{max}}$  of AZT is 0.549  $\mu\text{g/mL}$  ( $\sim 2 \mu\text{M}$ ). Thus, it is unlikely that AZT will reach high enough concentrations in humans to enhance the anti-tumor effects of TAS-102.

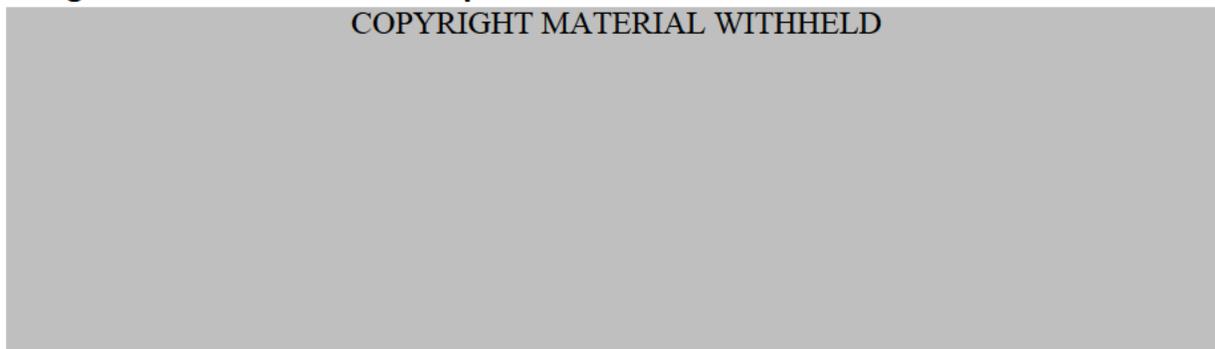
**Summary of literature reference provided by the Applicant (EDR 4.3):**

Fukushima M, Suzuki N, Emura T, Yano S, Kazuno H, Tada Y, Yamada Y, Asao T, 2000, Structure and Activity of Specific Inhibitors of Thymidine Phosphorylase to Potentiate the Function of Antitumor 2'-Deoxyribonucleosides, *Biochemical Pharmacology*, 59: 1227-1236.

Fukushima et al. evaluated a new class of 5-halogenated pyrimidine analogs substituted at the 6-position as potential competitive inhibitors of thymidine phosphorylase (TPase). Although substitution of various halogens at the 5-position of 6-[(2-iminopyrrolidin-1-yl)methyl] uracil did not affect TPase inhibitory activity, substitution of the chlorine with a

methyl group at the 5-position reduced its TPase inhibitory activity, suggesting that 5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4(1*H*,3*H*)-pyrimidinedione hydrochloride (TPI) was a potent inhibitor of TPase. TPI was shown to be a competitive inhibitor of recombinant human TPase with an apparent  $K_i$  value of  $1.7 \times 10^{-8}$  M (see Figure 11).

**Figure 11: Lineweaver-Burk plot of TPI inhibition of recombinant human TPase**



(Figure excerpted from Fukushima et al.)

TPI did not inhibit the activity of uridine phosphorylase, thymidine kinase, orotate phosphoribosyltransferase, (b) (4), suggesting it was selective for TPase. In vitro incubation with  $1 \times 10^{-6}$  M TPI inhibited the phosphorolytic activity on the thymidine analog 5-trifluoromethyl-29-deoxyuridine (F<sub>3</sub>dThd, FTD) using enzyme preparations from human, monkey, mouse, and rat liver, human and monkey intestine, and human colon tumors, but not rodent intestine or dog liver and intestine, suggesting a similar distribution of TPase in humans and monkeys. Oral administration of F<sub>3</sub>dThd and TPI at a molar ratio of 1:1 resulted in F<sub>3</sub>dThd  $C_{max}$  and AUC values ~70-fold and 100-fold higher, respectively, than those following administration of F<sub>3</sub>dThd alone.

## 4.2 Secondary Pharmacology

None submitted.

## 4.3 Safety Pharmacology

**Study title: Safety Pharmacology Study of TAS-102 Effects on the Central Nervous System in Rats**

Study no.:	B040836
Study report location:	EDR 4.2.1.3
Conducting laboratory and location:	<span style="background-color: grey; color: grey;">(b) (4)</span>
Date of study initiation:	September 6, 2004
GLP compliance:	Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)
QA statement:	Yes
Drug, lot #, and % purity:	TAS-102: FTD and TPI at molar ratio of

1:05. FTD, 202016, 100.1%. TPI,  
030317, 99.5%.

## Methods

Doses: 0, 40 (FTD 27.2 + TPI 12.8), 160 (FTD 108.8 + TPI 51.2), and 640 (FTD 435 + TPI 205) mg/kg TAS-102  
 Frequency of dosing: Single dose  
 Route of administration: Oral  
 Dose volume: 10 mL/kg  
 Formulation/Vehicle: 0.5% HPMC in distilled water for injection  
 Species/Strain: Male Sprague-Dawley Rats  
 Number/Sex/Group: 6 males/group  
 Age: 6 weeks old  
 Weight: 200.7 – 227.4 g  
 Behaviors evaluated: Awareness, mood, motor activity, CNS excitation, motor incoordination, muscle tone, reflex, eyes, secretion/excretion, general, and death.

**Results:** The Applicant evaluated the effects of TAS-102 on general physical condition and behavior after single oral administration of TAS-102 to male rats. Irwin's multiple observation method was utilized before and 1, 2, 4, 6, 8, and 24 hrs after TAS-102 administration. Oral administration of TAS-102 up to 640 mg/kg had no effect on general condition or CNS behavior up to 24 hrs post-dose.

**Figure 12: Effects of single oral administration of TAS-102 on general physical condition and behavior in male rats**

Test substance	Dose (mg/kg p.o.)	Signs	Before	Time after administration (hour)					
				1	2	4	6	8	24
Vehicle <sup>1)</sup>	-	No observable effect	6/6 <sup>2)</sup>	6/6	6/6	6/6	6/6	6/6	6/6
TAS-102	40	No observable effect	6/6	6/6	6/6	6/6	6/6	6/6	6/6
	160	No observable effect	6/6	6/6	6/6	6/6	6/6	6/6	6/6
	640	No observable effect	6/6	6/6	6/6	6/6	6/6	6/6	6/6

1) 0.5 w/v% hydroxy propyl methylcellulose solution

2) Number of animals showing the sign / Number of animals tested

(Excerpted from Applicant's Submission)

**Study title: Safety Pharmacology Study of TAS-102 Effects on the Respiratory System in Conscious Rats**

Study no.: B040837  
 Study report location: EDR 4.2.1.3  
 Conducting laboratory and location:

(b) (4)

Date of study initiation: October 5, 2004  
 GLP compliance: Yes (Japan Ministry of Health and

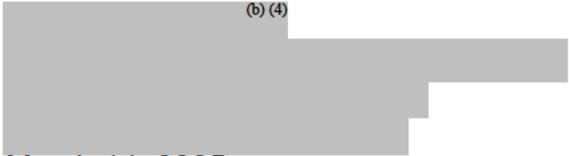
Welfare Ordinance No. 21, 1997)  
 QA statement: Yes  
 Drug, lot #, and % purity: TAS-102: FTD and TPI at molar ratio of 1:05. FTD, 202016, 100.1%. TPI, 030317, 99.5%.

#### Methods

Doses: 0, 40 (FTD 27.2 + TPI 12.8), 160 (FTD 108.8 + TPI 51.2), and 640 (FTD 435 + TPI 205) mg/kg TAS-102  
 Frequency of dosing: Single dose  
 Route of administration: Oral  
 Dose volume: 10 mL/kg  
 Formulation/Vehicle: 0.5% HPMC in distilled water for injection  
 Species/Strain: Male Sprague-Dawley Rats  
 Number/Sex/Group: 8 males/group  
 Age: 6 weeks old  
 Weight: 202– 245 g  
 Study design: Respiratory rate (f), tidal volume (TV), and minute volume (MV) were determined before and 1, 2, 4, 6, 8, and 24 hrs after administration using whole body plethysmography. MV was calculated by multiplying TV by f.

**Results:** The Applicant evaluated the effects of single oral administration of TAS-102 on the respiratory system of male rats. Single oral administration of TAS-102 up to 640 mg/kg had no statistically significant effects on respiratory rate, tidal volume, or minute volume in male rats up to 24 hrs post-dose.

#### Study title: Safety Pharmacology Studies of FTD Effects on hERG Current

Study no.: B050268  
 Study report location: EDR 4.2.1.3  
 Conducting laboratory and location:  (b) (4)  
 Date of study initiation: March 14, 2005  
 GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)  
 QA statement: Yes  
 Drug, lot #, and % purity: FTD, 202016, 100.1%

#### Methods

Concentrations: 3, 30, and 300  $\mu$ M  
 Formulation/Vehicle: 1% physiological saline  
 Cell Line: HEK293 cells stably expressing the human

ether-a-go-go related gene (hERG)  
 Positive Control: 0.1  $\mu$ M E-4031  
 Study design: The cell membrane was ruptured and then patch clamped to -80 mV. The whole cell clamp method was used to measure hERG-mediated current by stepping to +20 mV for 1.5 sec, -40 mV for 1.5 sec, and then to a holding potential of -80 mV; this voltage protocol was continuously applied once every 15 sec. Concentrations of the vehicle, FTD, and E-4031 (n=5 cells/treatment) were applied for 10 min. The experimental temperature was 23.1-25.5°C. Tail peak current was analyzed by Clampfit 6.0.5 software.

**Results:** The Applicant used the whole cell clamp method to assess the ability of FTD to inhibit repolarization current through the hERG potassium channel. The positive control E-4031 significantly inhibited hERG-mediated potassium current by 91.1% ( $p < 0.05$ ), whereas FTD inhibited hERG-mediated potassium current by 1.8%, 1.8%, and 2.5% (corrected for vehicle inhibition) at 3, 30, and 300  $\mu$ M, respectively. FTD-mediated current inhibition was not dose-dependent and did not reach statistical significance compared to vehicle. Since the percent inhibition with FTD was  $< 50\%$ , the Applicant did not calculate an  $IC_{50}$  for hERG inhibition. These results indicate that FTD has low potential for causing QTc prolongation.

**Table 7: Effect of FTD on hERG-mediated potassium current**

Test substance	Concentration ( $\mu$ mol/L)	Number of preparations	% before <sup>1)</sup> (mean $\pm$ S.D.)	% inhibition <sup>2)</sup>
Vehicle <sup>3)</sup>	---	5	98.5 $\pm$ 2.3	-
FTD	3	5	96.7 $\pm$ 2.9	1.8
	30	5	96.7 $\pm$ 6.4	1.8
	300	5	96.0 $\pm$ 3.3	2.5
E-4031	0.1	5	8.9 $\pm$ 5.1 #	~ <sup>4)</sup>

1) : Relative percent of the value just before application to the one after application of vehicle, the test substance, or E-4031.

2) :  $100 - (\text{mean \%before of the test substance group}) / (\text{mean \%before of the vehicle control group}) \times 100$ .

3) : Physiological saline (external solution containing 1.0 vol% physiological saline).

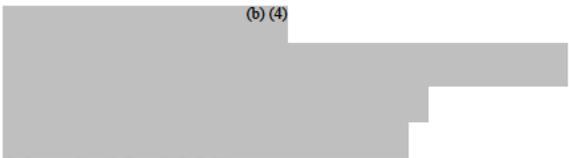
4) : The value was not corrected because water for injection was used as the vehicle to dissolve E-4031, which was different from the vehicle control group (1.0 vol% physiological saline).

No values were significantly different between the vehicle control group and the test substance groups.

# : Significantly different from the control value by Student's *t*-test,  $p < 0.05$ .

(Excerpted from Applicant's Submission)

**Study title: Safety Pharmacology Studies of TPI Effects on hERG Current**

Study no.: B050270  
Study report location: EDR 4.2.1.3  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: March 16, 2005  
GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)  
QA statement: Yes  
Drug, lot #, and % purity: TPI, 030317, 99.5%

**Methods**

Concentrations: 1, 10, and 100  $\mu$ M  
Formulation/Vehicle: 1% physiological saline  
Cell Line: HEK293 cells stably expressing hERG  
Positive Control: 0.1  $\mu$ M E-4031  
Study design: The whole cell clamp method was used to measure hERG-mediated current as described above in Study B050268. The experimental temperature was 22.8-25.7°C. Tail peak current was analyzed by Clampfit 6.0.5 software.

**Results:** The Applicant used the whole cell clamp method to assess the ability of TPI to inhibit repolarization current through the hERG potassium channel. The positive control E-4031 significantly inhibited hERG-mediated potassium current by 93.7% ( $p < 0.05$ ), whereas TPI inhibited hERG-mediated potassium current by -0.8%, 1.8%, and 0.4% (corrected for vehicle inhibition) at 1, 10, and 100  $\mu$ M, respectively. TPI-mediated current inhibition was not dose-dependent and did not reach statistical significance compared to vehicle. Since the percent inhibition with TPI was  $< 50\%$ , the Applicant did not calculate an  $IC_{50}$  for hERG inhibition. These results indicate that TPI has low potential to cause QTc prolongation.

**Table 8: Effect of TPI on hERG-mediated potassium current**

Test substance	Concentration (µmol/L)	Number of preparations	% before <sup>1)</sup> (mean ± S.D.)	% inhibition <sup>2)</sup>
Vehicle <sup>3)</sup>	---	5	97.9 ± 2.8	-
TPI	1	5	98.7 ± 3.2	-0.8
	10	5	96.1 ± 5.3	1.8
	100	5	97.5 ± 10.3	0.4
E-4031	0.1	5	6.3 ± 1.9 #	- <sup>4)</sup>

1) : Relative percent of the value just before application to the one after application of vehicle, the test substance, or E-4031.

2) :  $100 - (\text{mean \%before of the test substance group}) / (\text{mean \%before of the vehicle control group}) \times 100$ .

3) : Physiological saline (external solution containing 1.0 vol% physiological saline).

4) : The value was not corrected because water for injection was used as the vehicle to dissolve E-4031, which was different from the vehicle control group (1.0 vol% physiological saline).

No values were significantly different between the vehicle control group and the test substance groups.

# : Significantly different from the control value by Student's *t*-test,  $p < 0.05$ .

(Excerpted from Applicant's Submission)

### Study title: Safety Pharmacology Study of TAS-102 Effects on the Cardiovascular System in Conscious Monkeys

Study no.: B040835  
 Study report location: EDR 4.2.1.3  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: September 14, 2004  
 GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)  
 QA statement: Yes  
 Drug, lot #, and % purity: TAS-102: FTD and TPI at molar ratio of 1:05. FTD, 202016, 100.1%. TPI, 030317, 99.5%.

#### Methods

Doses: 0, 10 (FTD 6.8 + TPI 3.2), 40 (FTD 27.2 + TPI 12.8), and 160 (FTD 108.8 + TPI 51.2) mg/kg TAS-102  
 Frequency of dosing: Single dose  
 Route of administration: Oral  
 Dose volume: 5 mL/kg  
 Formulation/Vehicle: 0.5% HPMC in distilled water for injection  
 Species/Strain: Male cynomolgus monkeys implanted with a telemetry device  
 Number/Sex/Group: 4 males; each monkey was administered with

vehicle followed by 10, 40, and then 160 mg/kg TAS-102 with each administration separated by an interval of 7-8 days

Age: 4 years old

Weight: 3.1-3.4 kg

Study design: Heart rate, blood pressure (systolic, diastolic, and mean), and electrocardiographic parameters (PR interval, QRS duration, and QT and QTc intervals) were determined using a telemetry system before and 1, 2, 4, 6, 8, and 24 hrs after administration.

**Results:** The Applicant evaluated the effects of single oral administration of TAS-102 on the cardiovascular system of male cynomolgus monkeys implanted with a telemetry device. There was a statistically significant 9-12% decrease in diastolic and mean blood pressure 4 hrs after administration of TAS-102 at the 40 mg/kg and 160 mg/kg dose levels compared to vehicle; however, diastolic and mean blood pressure values were slightly increased compared to baseline. There was a statistically significant 6% prolongation of the mean QTc interval 1 hr after administration of TAS-102 at the 40 mg/kg dose level compared to vehicle, but not at the high dose level. Single oral administration of TAS-102 up to 160 mg/kg did not have any significant effects on any other cardiovascular parameters.

**Study title: Safety Pharmacology Study of FTD Effects on the Cardiovascular System in Conscious Monkeys**

Study no.: B061099

Study report location: EDR 4.2.1.3

Conducting laboratory and location:

(b) (4)

Date of study initiation: August 8, 2006

GLP compliance: Yes (Japan Ministry of Health and Welfare Ordinance No. 21, 1997)

QA statement: Yes

Drug, lot #, and % purity: FTD, 202016, 100.1%

**Methods**

Doses: 0, 6.8, 27.2, and 108.8 mg/kg

Frequency of dosing: Single dose

Route of administration: Oral

Dose volume: 5 mL/kg

Formulation/Vehicle: 0.5% HPMC in distilled water for injection

Species/Strain: Male cynomolgus monkeys implanted with a telemetry device

Number/Sex/Group: 4 males; each monkey was administered with

vehicle followed by 6.8, 27.2, and then 108.8 mg/kg FTD with each administration separated by an interval of 6-7 days.

Age: 4 years old

Weight: 3-3.9 kg

Study design: Heart rate, blood pressure (systolic, diastolic, and mean), and electrocardiographic parameters (PR interval, QRS duration, and QT and QTc intervals) were determined using a telemetry system before and 1, 2, 4, 6, 8, and 24 hrs after administration.

**Results:** The Applicant evaluated the effects of single oral administration of FTD on the cardiovascular system of male cynomolgus monkeys implanted with a telemetry device. There was a statistically significant 27% decrease in mean heart rate 1 hr after administration of FTD at the 27.2 mg/kg dose level compared to vehicle, which correlated with a 17% increase in the mean QT interval. These parameters were not significantly changed compared to baseline, however, and administration of FTD at the high dose level did not significantly affect mean heart rate or result in QT or QTc prolongation. Thus, single oral administration of FTD up to 108.8 mg/kg did not significantly affect cardiovascular parameters.

## 5 Pharmacokinetics/ADME/Toxicokinetics

### C-O149: Contribution Study of TPI on Pharmacokinetics of FTD in Monkey

#### Methods

Drug, lot #, purity: FTD, 065022, 100%  
 TPI, 7A0104, 99.7%

Dose, ROA, frequency: First administration: 30 mg/kg FTD IV, once  
 Second administration: 10 mg/kg, oral, once (1 week after first administration)  
 Third administration: 10 mg/kg FTD and 4.71 mg/kg TPI, oral, once (1 week after second administration)

Dose volume: IV: 1 mL/kg  
 Oral: 5 mL/kg

Formulation/Vehicle: IV: physiological saline  
 Oral: 0.5% HPMC

Species/Strain: ~3 year old male cynomolgus monkeys

Number/Group: 4 monkeys, each monkey received each administration

Sample collection: IV: 5, 10, 15, 30, 45, 60, 90, 120, and 360 min post-dose  
 Oral: 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 hrs post-dose

**Results:** The Applicant examined the bioavailability of FTD and the effects of TPI on the PK parameters of FTD in cynomolgus monkeys. There were no mortalities or clinical signs observed following a single IV administration of FTD at the 30 mg/kg dose level or two oral administrations of FTD given a week apart at the 10 mg/kg dose level either

alone or in combination with 4.71 mg/kg TPI. The calculated oral bioavailability of FTD when given alone versus in combination with TPI was 3% versus 336.5%. FTD was rapidly metabolized to its metabolite trifluoromethyl-pyrimidinedione (FTY) following oral administration, but co-administration of TPI with FTD increased FTD exposure ~104-fold and enhanced the oral bioavailability of FTD ~112-fold.

**Table 9: Pharmacokinetic parameters following administration of FTD and TPI to cynomolgus monkeys**

Dose (mg/kg)	First administration (I.V.)		Second administration (P.O.)		Third administration (P.O.)		
	FTD 30, TPI 0		FTD 10, TPI 0		FTD10, TPI 4.71		
PK parameter	FTD	FTY	FTD	FTY	FTD	FTY	TPI
C <sub>0</sub> (ng/mL)	122000	-	-	-	-	-	-
C <sub>max</sub> (ng/mL)	-	54500	298	9850	16000	4120	712
t <sub>max</sub> (min)	-	26	-	-	-	-	-
t <sub>max</sub> (h)	-	-	0.9	1.1	1.5	1.8	2.3
t <sub>1/2</sub> (min)	13.4	41.0	18.9	62.7	67.5	108	110
AUC <sub>0-4</sub> (ng min/mL)	1610000	5870000	13700	1080000	1740000	617000	133000
AUC <sub>0-∞</sub> (ng min/mL)	1620000	5870000	14100	1080000	1740000	617000	133000
AUC <sub>inf</sub> (ng min/mL)	1610000	5880000	16800	1080000	1740000	650000	137000
Vd (mL/kg)	374	-	-	-	-	-	-
Vd/F (mL/kg)	-	-	20700	-	585	-	5560
CL (mL/min/kg)	19.1	-	-	-	-	-	-
CL/F (mL/min/kg)	-	-	740	-	5.87	-	35.6
MRT (min)	12.7	71.4	46.9	108	115	168	198
Bioavailability (%)	-	-	3.0	-	336.5	-	-
AUC <sub>ratio</sub>	-	-	-	-	116	-	-

(Excerpted from Applicant's Submission)

## 12DA41: Pharmacokinetic Evaluation of the Optimum Dosage Ratio of FTD to TPI in Monkey

### Methods

Drug, lot #, purity: FTD and TPI (lots and purities not indicated)  
Dose, ROA, frequency:

Group	Dose level (mg/kg)		Molar ratio (FTD : TPI)	Individual number
	FTD	TPI		
1	10	0	1 : 0	1001, 1002, 1003, 1004
2	10	1.88	1 : 0.2	1001, 1002, 1003, 1004
3	10	4.71	1 : 0.5	1001, 1002, 1003, 1004
4	10	9.42	1 : 1	1001, 1002, 1003, 1004

ROA, dose volume: Oral, 5 mL/kg  
Frequency: Single  
Formulation/Vehicle: 0.5% HPMC  
Species/Strain: Male cynomolgus monkeys  
Number/Group: 4/group. 6 days of rest between administration of next dose level.  
Sample collection: 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 hrs post-dose

**Results:** The Applicant examined the PK of FTD and FTY after single oral administration of FTD alone or in combination with TPI to cynomolgus monkeys. Administration of FTD and TPI at molar ratios (FTD:TPI) of 1:0, 1:0.2, 1:0.5, and 1:1

was evaluated. The  $C_{max}$  and  $AUC_{all}$  of FTD were significantly increased following co-administration of TPI compared to administration of FTD alone. There were no significant differences in FTD  $C_{max}$  in animals administered different doses of TPI, but the FTD  $AUC_{all}$  values were significantly higher in Groups 3 (1:0.5) and 4 (1:1) compared to Group 2 (1:0.2). The FTD exposure at the molar ratio of 1:0.5 was approximately 84% of the exposure at the 1:1 ratio.

**Table 10:  $C_{max}$  and  $AUC_{all}$  after single oral administration of FTD and TPI to cynomolgus monkeys**

A) FTD								
Individual number	Dosage ratio (FTD : TPI)							
	1 : 0		1 : 0.2		1 : 0.5		1 : 1	
	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)
1001	269	9590	12200	1030000	13800	1430000	21200	1830000
1002	437	15200	14400	949000	19100	1470000	22200	1840000
1003	158	8840	11600	955000	13200	1170000	17100	1600000
1004	244	9830	14200	1120000	15100	1530000	13600	1500000
Mean	277	10900	13100	1010000	15300	1400000	18500	1690000
SD	117	2900	1400	80000	2700	160000	4000	170000
CV (%)	42.2	26.6	10.7	7.92	17.6	11.4	21.6	10.1

B) FTY								
Individual number	Dosage ratio (FTD : TPI)							
	1 : 0		1 : 0.2		1 : 0.5		1 : 1	
	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)
1001	10600	810000	5290	594000	3970	451000	5850	550000
1002	13500	1030000	6120	700000	6760	707000	5230	541000
1003	9640	873000	4300	594000	4480	608000	3900	460000
1004	12200	1070000	5440	555000	3720	438000	2130	322000
Mean	11500	946000	5290	611000	4730	551000	4280	468000
SD	1700	124000	750	62000	1390	130000	1650	106000
CV (%)	14.8	13.1	14.2	10.1	29.4	23.6	38.6	22.6

C) TPI								
Individual number	Dosage ratio (FTD : TPI)							
	1 : 0		1 : 0.2		1 : 0.5		1 : 1	
	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)	$C_{max}$ (ng/mL)	$AUC_{all}$ (ng•min/mL)
1001	—	—	260	44300	566	78600	1170	193000
1002	—	—	167	36800	606	143000	765	168000
1003	—	—	215	34700	514	74700	955	154000
1004	—	—	291	43000	641	99800	1220	195000
Mean	—	—	233	39700	582	99000	1030	178000
SD	—	—	54	4700	55	31300	210	20000
CV (%)	—	—	23.2	11.8	9.38	31.6	20.4	11.2

SD, and CV mean "standard deviation", and "coefficient of variation", respectively.

— : Not determine

(Excerpted from Applicant's Submission)

**Table 11: Ratio of  $C_{max}$  and  $AUC_{all}$  of FTD in cynomolgus monkeys**

Individual number	Dosage ratio (FTD : TPI)				Individual number	Dosage ratio (FTD : TPI)			
	1 : 0	1 : 0.2	1 : 0.5	1 : 1		1 : 0	1 : 0.2	1 : 0.5	1 : 1
	$C_{max}$	$C_{max}$	$C_{max}$	$C_{max}$		$AUC_{all}$	$AUC_{all}$	$AUC_{all}$	$AUC_{all}$
1001	1.27	57.5	65.1	100	1001	0.52	56.3	78.1	100
1002	1.97	64.9	86.0	100	1002	0.83	51.6	79.9	100
1003	0.92	67.8	77.2	100	1003	0.55	59.7	73.1	100
1004	1.79	104	111	100	1004	0.66	74.7	102	100
Mean	1.49	73.7	84.8	100	Mean	0.64	60.6	83.3	100
SD	0.48	20.9	19.5	—	SD	0.14	10.0	12.8	—

Each value represents % of  $C_{max}$  in dosage ratio of 1 : 1 (group 4).

Each value represents % of  $AUC_{all}$  in dosage ratio of 1 : 1 (group 4).

(Excerpted from Applicant's Submission)

Since there was no significant difference in the FTD  $AUC_{all}$  following administration of FTD and TPI at ratios of 1:0.5 and 1:1, the Applicant concluded that co-administration of

FTD and TPI at a molar ratio of 1:0.5 resulted in a sufficient amount of TPI to inhibit the degradation of FTD in monkeys. Thus, the optimal molar ratio of FTD:TPI in monkeys was determined to be 1:0.5.

### **AE-6932-G: Placental and embryo fetal transfer of TAS-102 using [<sup>14</sup>C]FTD or [<sup>14</sup>C]TPI in pregnant rats**

#### Methods

Drug, lot #, % purity: [<sup>14</sup>C]FTD, CP-3228, 98.1%  
[<sup>14</sup>C]TPI, CP-3229, ≥95.2%  
Unlabeled FTD, 065022, purity not indicated  
Unlabeled TPI, 7A0104, purity not indicated

Dose: Equivalent to 50 mg/kg TAS-102 (50 mg FTD + 23.6 mg TPI/kg). [<sup>14</sup>C]FTD was combined with unlabeled TPI and FTD to make 50 mg/kg TAS-102, and [<sup>14</sup>C]TPI was combined with unlabeled FTD and TPI.

Radioactive dose: 5 MBq/kg  
Frequency, ROA: Single dose, oral gavage  
Dose Volume: 5 mL/kg  
Formulation/Vehicle: 0.5% HPMC in water  
Species/Strain: Female Sprague Dawley rats fasted 16 hr before dosing  
Number/Sex/Group: 3 rats/group

**Results:** The Applicant investigated placental and embryofetal transfer of TAS-102 after single oral administration of ([<sup>14</sup>C]FTD)TAS-102 or ([<sup>14</sup>C]TPI)TAS-102 at a TAS-102 dose level of 50 mg/kg to fasted pregnant female rats on Day 18 of gestation. Radioactivity concentrations in maternal and fetal tissues were determined at 0.5, 1, 4, 24, and 48 hrs after dosing using a LSC (liquid scintillation counter).

Following a single oral administration of ([<sup>14</sup>C]FTD)TAS-102 on Day 18 of pregnancy, radioactivity concentrations in the maternal plasma and maternal blood peaked at 0.5 hrs post-dose and decreased with time; [<sup>14</sup>C]FTD-related radioactivity was the highest in maternal plasma and maternal blood at 0.5 and 1 hrs post-dose. At 0.5 hr post-dose, [<sup>14</sup>C]FTD-related radioactivity in the fetus, fetal blood, and fetal tissues was 21%, 21%, and ≤28%, respectively, of that in the maternal plasma. Radioactivity concentrations in the placenta, fetus, fetal blood, and fetal tissues peaked at 1 hr post-dose, with the exception of the fetal membrane (24 hrs post-dose) and the amniotic fluid and fetal liver (4 hrs post-dose), and then generally decreased in parallel with maternal plasma with the exception of the fetal membrane. At 4 hrs post-dose, radioactivity concentrations were higher in the fetal liver than in maternal plasma and blood. At 24 and 48 hrs post-dose, radioactivity concentrations were ≥1.5-fold higher in the fetal membrane, fetus, fetal lung, fetal liver, and fetal kidney than in maternal blood and plasma. The percentage distribution of [<sup>14</sup>C]FTD-related radioactivity to the fetus was highest at 1 hr post-dose (0.08% of dosed radioactivity).

**Table 12: Radioactivity concentration in tissues following a single 50 mg/kg oral dose of (<sup>14</sup>C)FTD)TAS-102 to rats on Day 18 of pregnancy**

Tissue	Radioactivity concentration (ng eq. of FTD/g or mL)				
	0.5 hr	1 hr	4 hr	24 hr	48 hr
Maternal plasma	36455 ± 4686	30798 ± 4858	11191 ± 2376	1995 ± 344	804 ± 93
Maternal blood	26588 ± 3728	22272 ± 3051	8138 ± 1465	1889 ± 261	1052 ± 77
Placenta	10457 ± 1475	12594 ± 1479	5080 ± 248	1552 ± 111	888 ± 60
Fetal membrane	3898 ± 866	5366 ± 1168	4819 ± 247	5560 ± 1037	3571 ± 709
Amniotic fluid	1080 ± 195	3396 ± 1101	3451 ± 1220	1582 ± 561	590 ± 72
Fetus	7667 ± 1016	9634 ± 2250	6948 ± 646	2976 ± 148	1080 ± 60
Fetal blood	7562 ± 809	9399 ± 1343	4628 ± 302	1727 ± 127	877 ± 67
Fetal brain	3751 ± 647	6208 ± 847	3206 ± 132	946 ± 155	532 ± 9
Fetal heart	7716 ± 1542	10199 ± 1190	4735 ± 403	1982 ± 222	846 ± 70
Fetal lung	7472 ± 1294	10181 ± 1544	7500 ± 853	3967 ± 204	1914 ± 223
Fetal liver	10325 ± 2227	13841 ± 356	15138 ± 1101	4906 ± 718	1405 ± 131
Fetal kidney	7989 ± 612	11403 ± 1774	9421 ± 1106	4201 ± 112	1590 ± 166
Tissue	Radioactivity content (% of dose)				
	0.5 hr	1 hr	4 hr	24 hr	48 hr
Fetus	0.06 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	0.02 ± 0.01

Data are expressed as the mean values ± S.D. of three animals.

(Excerpted from Applicant's Submission)

**Table 13: Ratio of radioactivity concentration in tissues relative to maternal plasma following a single 50 mg/kg oral dose of (<sup>14</sup>C)FTD)TAS-102 to rats**

Tissue	Ratio to the radioactivity concentration in plasma				
	0.5 hr	1 hr	4 hr	24 hr	48 hr
Maternal plasma	1.00	1.00	1.00	1.00	1.00
Maternal blood	0.73	0.72	0.73	0.95	1.31
Placenta	0.29	0.41	0.45	0.78	1.10
Fetal membrane	0.11	0.17	0.43	2.79	4.44
Amniotic fluid	0.03	0.11	0.31	0.79	0.73
Fetus	0.21	0.31	0.62	1.49	1.34
Fetal blood	0.21	0.31	0.41	0.87	1.09
Fetal brain	0.10	0.20	0.29	0.47	0.66
Fetal heart	0.21	0.33	0.42	0.99	1.05
Fetal lung	0.20	0.33	0.67	1.99	2.38
Fetal liver	0.28	0.45	1.35	2.46	1.75
Fetal kidney	0.22	0.37	0.84	2.11	1.98

(Excerpted from Applicant's Submission)

Following a single oral administration of (<sup>14</sup>C)TPI)TAS-102 on Day 18 of pregnancy, radioactivity concentrations in the maternal plasma and maternal blood peaked at 0.5 hrs post-dose and decreased with time; [<sup>14</sup>C]TPI-related radioactivity was the highest in maternal plasma and maternal blood at 0.5 and 1 hrs post-dose. At 0.5 hr post-dose, [<sup>14</sup>C]TPI-related radioactivity in the fetus, fetal blood, and fetal tissues was 6%, 10%, and ≤7%, respectively, of that in the maternal plasma. Radioactivity concentrations peaked at 4 hrs post-dose in the fetal membrane, amniotic fluid, fetus, and fetal tissues and at 0.5 hrs post-dose in the placenta and fetal blood; radioactivity concentrations appeared to decrease at a slower rate in the placenta, fetal membrane, and fetal tissues/fluids compared to maternal plasma. [<sup>14</sup>C]TPI-related radioactivity was the highest in the fetal membrane at 24 hrs-post dose. [<sup>14</sup>C]TPI-related radioactivity was still detectable in the fetus, placenta, fetal membrane, and fetal fluids/tissues at 48 hrs post-dose, but was undetectable in maternal plasma and blood.

**Table 14: Radioactivity concentration in tissues following a single 50 mg/kg oral dose of (<sup>14</sup>C)TPI)TAS-102 to rats on Day 18 of pregnancy**

Tissue	Radioactivity concentration (ng eq. of TPI/g or mL)				
	0.5 hr	1 hr	4 hr	24 hr	48 hr
Maternal plasma	996 ± 12	747 ± 56	151 ± 29	24 ± 4	N.D.
Maternal blood	838 ± 24	692 ± 27	152 ± 29	18 ± 16	N.D.
Placenta	367 ± 51	316 ± 69	146 ± 20	27 ± 3	24 ± 3
Fetal membrane	85 ± 16	130 ± 12	167 ± 13	53 ± 5	43 ± 9
Amniotic fluid	N.D.	16 ± 14	35 ± 8	31 ± 9	N.D.
Fetus	60 ± 8	76 ± 10	82 ± 11	24 ± 6	24 ± 5
Fetal blood	104 ± 61	87 ± 16	85 ± 14	15 ± 13	20 ± 18
Fetal brain	32 ± 2	37 ± 6	46 ± 5	21 ± 5	20 ± 4
Fetal heart	36 ± 31	72 ± 6	78 ± 21	20 ± 5	23 ± 10
Fetal lung	46 ± 3	57 ± 6	72 ± 11	23 ± 3	21 ± 4
Fetal liver	52 ± 5	93 ± 16	104 ± 7	33 ± 6	26 ± 9
Fetal kidney	66 ± 18	85 ± 11	97 ± 10	32 ± 5	24 ± 2

Tissue	Radioactivity content (% of dose)				
	0.5 hr	1 hr	4 hr	24 hr	48 hr
Fetus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data are expressed as the mean values ± S.D. of three animals.  
N.D.: Not detected

(Excerpted from Applicant's Submission)

**Table 15: Ratio of radioactivity concentration in tissues relative to maternal plasma following a single 50 mg/kg oral dose of (<sup>14</sup>C)TPI)TAS-102 to rats**

Tissue	Ratio to the radioactivity concentration in plasma				
	0.5 hr	1 hr	4 hr	24 hr	48 hr
Maternal plasma	1.00	1.00	1.00	1.00	N.C.
Maternal blood	0.84	0.93	1.01	0.75	N.C.
Placenta	0.37	0.42	0.97	1.13	N.C.
Fetal membrane	0.09	0.17	1.11	2.21	N.C.
Amniotic fluid	N.C.	0.02	0.23	1.29	N.C.
Fetus	0.06	0.10	0.54	1.00	N.C.
Fetal blood	0.10	0.12	0.56	0.63	N.C.
Fetal brain	0.03	0.05	0.30	0.88	N.C.
Fetal heart	0.04	0.10	0.52	0.83	N.C.
Fetal lung	0.05	0.08	0.48	0.96	N.C.
Fetal liver	0.05	0.12	0.69	1.38	N.C.
Fetal kidney	0.07	0.11	0.64	1.33	N.C.

N.C.: Not calculated

(Excerpted from Applicant's Submission)

In conclusion, following single oral administration of (<sup>14</sup>C)FTD)TAS-102 or (<sup>14</sup>C)TPI)TAS-102 on Day 18 of pregnancy, radioactivity transferred to all fetal tissues, indicating placental transfer of TAS-102 to the fetus.

### AE-6933-G: Excretion into milk of TAS-102 using [<sup>14</sup>C]FTD or [<sup>14</sup>C]TPI in nursing rats

#### Methods

Drug, lot #, % purity: [<sup>14</sup>C]FTD, CP-3228, 98.4%  
 [<sup>14</sup>C]TPI, CP-3229, 95.2%  
 Unlabeled FTD, 065022, purity not indicated  
 Unlabeled TPI, 7A0104, purity not indicated  
 Dose: Equivalent to 50 mg/kg TAS-102 (50 mg FTD + 23.6 mg TPI/kg)  
 Frequency, ROA: Single dose, oral gavage  
 Radioactive dose: 5 MBq/kg

Dose Volume: 5 mL/kg  
 Formulation/Vehicle: 0.5% HPMC in water  
 Species/Strain: Female non-fasted Sprague Dawley rats  
 Number/Sex/Group: 3 rats/group

**Results:** The Applicant investigated the transfer of TAS-102 into milk after single oral administration of ( $^{14}\text{C}$ )FTD)TAS-102 or ( $^{14}\text{C}$ )TPI)TAS-102 at a TAS-102 dose level of 50 mg/kg to female nursing rats on postnatal Day (PND) 10. Radioactivity concentrations in milk and plasma were determined using LSC. Following a single oral administration of ( $^{14}\text{C}$ )FTD)TAS-102 to nursing rats, radioactivity was excreted into milk and peaked in milk and plasma at 1 hr post-dose.  $^{14}\text{C}$ )FTD-related radioactivity was higher in plasma than in milk, except for 8 and 12 hrs post-dose when the levels were similar.

**Table 16: Radioactive concentrations in milk and plasma following a single 50 mg/kg oral dose of ( $^{14}\text{C}$ )FTD)TAS-102 to nursing rats**

Time (hr)	Radioactivity concentration (ng eq. of FTD/mL)		
	Milk	Plasma	M/P ratio
1	15637 ± 3698	33440 ± 7149	0.47
2	8213 ± 720	13390 ± 1274	0.61
4	4960 ± 645	6242 ± 1725	0.79
6	6466 ± 1219	7794 ± 1694	0.83
8	6003 ± 1332	6065 ± 1739	0.99
12	3929 ± 735	3832 ± 798	1.03
24	1229 ± 486	2333 ± 331	0.53
72	275 ± 85	877 ± 54	0.31

Data are expressed as the mean values ± S.D. of three animals.

(Excerpted from Applicant's submission)

Following a single oral administration of ( $^{14}\text{C}$ )TPI)TAS-102 to nursing rats, radioactivity peaked in milk and plasma at 4 hr and 1 hr post-dose, respectively. Beginning at 2 hrs post-dose,  $^{14}\text{C}$ )TPI-related radioactivity was higher in milk than in plasma, resulting in a milk/plasma ratio of ~5 at 24 hrs post-dose. Thus, TAS-102 was present in milk following administration of ( $^{14}\text{C}$ )FTD)TAS-102 or ( $^{14}\text{C}$ )TPI)TAS-102.

**Table 17: Radioactive concentrations in milk and plasma following a single 50 mg/kg oral dose of ( $^{14}\text{C}$ )TPI)TAS-102 to nursing rats**

Time (hr)	Radioactivity concentration (ng eq. of TPI/mL)		
	Milk	Plasma	M/P ratio
1	197 ± 41	568 ± 124	0.35
2	514 ± 98	399 ± 49	1.29
4	728 ± 123	183 ± 27	3.98
6	705 ± 166	191 ± 4	3.69
8	705 ± 102	201 ± 70	3.51
12	641 ± 180	167 ± 78	3.84
24	78 ± 17	17 ± 16	4.59
72	N.D.	N.D.	N.C.

Data are expressed as the mean values ± S.D. of three animals.

N.D.: Not detected

N.C.: Not calculated

(Excerpted from Applicant's submission)

### AE-2350-3G: Pharmacokinetic Studies on TAS-102 (III) Absorption, Distribution, Metabolism and Excretion in Rats using <sup>14</sup>C-FTD

#### Methods

Drug, lot, specific activity FTD, 706004, 100%; TPI, 961125, 100%  
 (SA), % purity: <sup>14</sup>C-FTD, CP-2095, 6.995 MBq/mg, ≥ 98%  
 Dose, frequency, ROA, [ <sup>14</sup>C-FTD ]TAS-102: 50 mg/kg (50 mg/kg FTD + 23.6  
 dose volume: mg/kg TPI; 3.7 MBq/kg), once, oral or IV, 5 mL/kg  
<sup>14</sup>C-FTD: 50 mg/kg (3.7 MBq/kg), once, oral  
 Formulation/Vehicle: Oral: 0.5% HPMC; IV: physiological saline for injection  
 Species/Strain: 7 week old male Sprague Dawley rats  
 Number/Group: 4/group

The Applicant examined the absorption, distribution, metabolism, and excretion of TAS-102 following a single oral or IV administration of 50 mg/kg [ <sup>14</sup>C-FTD ]TAS-102 or <sup>14</sup>C-FTD to male rats; radioactivity was measured by LSC. The PK parameters following single administration of 50 mg/kg [ <sup>14</sup>C-FTD ]TAS-102 are shown in Table 18. Mean radioactivity concentrations reached 28460 ng eq.·hr/mL and 25135 ng eq.·hr/mL in non-fasted and fasted rats, respectively. Mean oral bioavailability was calculated to be ~31%. AUC<sub>(0-∞)</sub> was ~1.6-fold lower in fasted rats compared to non-fasted rats.

**Table 18: Pharmacokinetic Parameters following single administration of [ <sup>14</sup>C-FTD ]TAS-102 to rats**

Time	Radioactivity concentration (ng eq. of FTD/mL)					
	p. o.				i. v.	
	Non-fasting		Fasting		Non-fasting	
	Blood	Plasma	Blood	Plasma	Blood	Plasma
5 min	—	—	—	—	60599 ± 8595	90421 ± 11860
15	10397 ± 4848	14167 ± 6708	13740 ± 1357	19931 ± 2500	35360 ± 3295	56779 ± 5669
30	20553 ± 1878	28460 ± 3559	16815 ± 2368	23199 ± 2744	19464 ± 4183	29045 ± 7793
1 hr	19161 ± 2670	27446 ± 2895	17795 ± 1355	25135 ± 1780	9109 ± 3001	13115 ± 4803
2	12934 ± 2810	18500 ± 5100	10566 ± 2301	13825 ± 2733	3302 ± 963	4390 ± 1558
4	5125 ± 964	6928 ± 1069	4336 ± 773	5777 ± 1284	1719 ± 368	2159 ± 552
6	4341 ± 1098	5785 ± 1569	2823 ± 196	3651 ± 488	1213 ± 86	1626 ± 150
8	2196 ± 229	2833 ± 272	2008 ± 204	2562 ± 411	1286 ± 176	1725 ± 181
24	1398 ± 150	1603 ± 163	1209 ± 154	1423 ± 231	1077 ± 174	1346 ± 167
72	742 ± 95	786 ± 103	—	—	—	—
T <sub>max</sub>	30 min	30 min	1 hr	1 hr	—	—
t <sub>1/2</sub> (hr)	2.30 (T <sub>max</sub> -8hr) 52.52 (24-72hr)	2.21 (T <sub>max</sub> -8hr) 46.68 (24-72hr)	2.23 (T <sub>max</sub> -8hr) 21.86 (8-24hr)	2.16 (T <sub>max</sub> -8hr) 18.86 (8-24hr)	0.48 (5min-2hr) 43.30 (4-24hr)	0.45 (5min-2hr) 38.31 (4-24hr)
AUC (ng eq.·hr·mL <sup>-1</sup> )	93958 (0-24hr) 145318 (0-72hr)	126296 (0-24hr) 183632 (0-72hr)	80983 (0-24hr)	106569 (0-24hr)	62674 (0-24hr)	88347 (0-24hr)
	201544 (0-∞)	236570 (0-∞)	119111 (0-∞)	145289 (0-∞)	128288 (0-∞)	161485 (0-∞)

Data are expressed as the mean values ± S.D. of four animals.

— : Not determined

(Excerpted from Applicant's submission)

Following oral administration of [ <sup>14</sup>C-FTD ]TAS-102 to rats, the highest level of radioactivity up to 4 hrs post-dose as measured by whole body autoradiograms was in the gastric contents followed by the intestinal contents. <sup>14</sup>C-FTD-related radioactivity reached its maximum levels in the stomach and jejunum at 15 minutes post-dose, in the cecum and colon at 6 hrs post-dose, and in the plasma, blood, and remaining tissues at 1 hr post-dose. Tissue distribution of <sup>14</sup>C-FTD was the highest in the stomach. Radioactivity concentrations were generally higher in plasma than in tissues at 1 hr post-dose except for the kidney, ileum, and urinary bladder. Radioactivity

concentrations were generally higher in plasma than in tissues at 15 min post-dose except for the kidney, stomach, jejunum, and urinary bladder. Radioactivity concentrations in the cerebrum and cerebellum were 7% of that in plasma at 1 hr post-dose, and the percentage of distribution of radioactivity to the cerebrum and cerebellum was  $\leq 0.02\%$  and  $0\%$  of dosed radioactivity, respectively (data not shown). Mean tissue radioactivity concentrations generally decreased with time.

**Table 19: Tissue Distribution following a single oral administration of [ $^{14}\text{C}$ -FTD]TAS-102 to non-fasted rats**

Tissue	Radioactivity concentration ( $\mu\text{g eq. of FTD/g or ml}$ )									
	15 min		1 hr		6 hr		24 hr		72 hr	
Plasma	14167 $\pm$ 6708 ( 1.00 $\pm$ 0.00 )	27446 $\pm$ 2895 ( 1.00 $\pm$ 0.00 )	5785 $\pm$ 1569 ( 1.00 $\pm$ 0.00 )	1603 $\pm$ 163 ( 1.00 $\pm$ 0.00 )	786 $\pm$ 103 ( 1.00 $\pm$ 0.00 )					
Blood	10397 $\pm$ 4848 ( 0.74 $\pm$ 0.01 )	19161 $\pm$ 2670 ( 0.70 $\pm$ 0.03 )	4341 $\pm$ 1098 ( 0.75 $\pm$ 0.03 )	1398 $\pm$ 150 ( 0.87 $\pm$ 0.02 )	742 $\pm$ 95 ( 0.95 $\pm$ 0.02 )					
Cerebrum	748 $\pm$ 442 ( 0.05 $\pm$ 0.01 )	1858 $\pm$ 362 ( 0.07 $\pm$ 0.02 )	633 $\pm$ 169 ( 0.11 $\pm$ 0.01 )	190 $\pm$ 18 ( 0.12 $\pm$ 0.02 )	140 $\pm$ 59 ( 0.18 $\pm$ 0.09 )					
Cerebellum	831 $\pm$ 612 ( 0.05 $\pm$ 0.02 )	2030 $\pm$ 474 ( 0.08 $\pm$ 0.02 )	714 $\pm$ 206 ( 0.13 $\pm$ 0.01 )	256 $\pm$ 21 ( 0.16 $\pm$ 0.02 )	185 $\pm$ 43 ( 0.23 $\pm$ 0.03 )					
Pituitary gland	4268 $\pm$ 1893 ( 0.30 $\pm$ 0.03 )	10020 $\pm$ 2797 ( 0.37 $\pm$ 0.09 )	2123 $\pm$ 47 ( 0.38 $\pm$ 0.08 )	861 $\pm$ 276 ( 0.53 $\pm$ 0.12 )	666 $\pm$ 123 ( 0.87 $\pm$ 0.25 )					
Eyeball	1811 $\pm$ 1145 ( 0.12 $\pm$ 0.02 )	4068 $\pm$ 507 ( 0.15 $\pm$ 0.02 )	1274 $\pm$ 268 ( 0.22 $\pm$ 0.03 )	265 $\pm$ 52 ( 0.16 $\pm$ 0.02 )	182 $\pm$ 24 ( 0.24 $\pm$ 0.03 )					
Harderian gland	4300 $\pm$ 3098 ( 0.29 $\pm$ 0.07 )	6664 $\pm$ 1678 ( 0.25 $\pm$ 0.07 )	2472 $\pm$ 509 ( 0.44 $\pm$ 0.05 )	1391 $\pm$ 131 ( 0.88 $\pm$ 0.14 )	481 $\pm$ 68 ( 0.61 $\pm$ 0.07 )					
Thyroid gland	5256 $\pm$ 1887 ( 0.39 $\pm$ 0.05 )	8716 $\pm$ 1165 ( 0.32 $\pm$ 0.03 )	2646 $\pm$ 1405 ( 0.44 $\pm$ 0.11 )	1277 $\pm$ 466 ( 0.82 $\pm$ 0.37 )	744 $\pm$ 213 ( 0.95 $\pm$ 0.25 )					
Trachea	7103 $\pm$ 7537 ( 0.43 $\pm$ 0.24 )	8317 $\pm$ 3094 ( 0.31 $\pm$ 0.13 )	2458 $\pm$ 1126 ( 0.42 $\pm$ 0.10 )	979 $\pm$ 263 ( 0.62 $\pm$ 0.21 )	762 $\pm$ 118 ( 0.98 $\pm$ 0.19 )					
Mandibular gland	5995 $\pm$ 3861 ( 0.40 $\pm$ 0.08 )	9809 $\pm$ 1565 ( 0.36 $\pm$ 0.06 )	3301 $\pm$ 895 ( 0.57 $\pm$ 0.05 )	736 $\pm$ 57 ( 0.46 $\pm$ 0.02 )	501 $\pm$ 58 ( 0.64 $\pm$ 0.07 )					
Thymus	4684 $\pm$ 3226 ( 0.31 $\pm$ 0.06 )	10256 $\pm$ 1563 ( 0.38 $\pm$ 0.08 )	5426 $\pm$ 852 ( 0.96 $\pm$ 0.13 )	4771 $\pm$ 669 ( 2.97 $\pm$ 0.23 )	2653 $\pm$ 656 ( 3.35 $\pm$ 0.48 )					
Heart	6631 $\pm$ 3748 ( 0.46 $\pm$ 0.06 )	10652 $\pm$ 1773 ( 0.39 $\pm$ 0.06 )	2168 $\pm$ 416 ( 0.38 $\pm$ 0.06 )	450 $\pm$ 55 ( 0.28 $\pm$ 0.02 )	314 $\pm$ 36 ( 0.40 $\pm$ 0.03 )					
Lung	6233 $\pm$ 3386 ( 0.43 $\pm$ 0.03 )	10344 $\pm$ 1974 ( 0.38 $\pm$ 0.06 )	2513 $\pm$ 789 ( 0.43 $\pm$ 0.03 )	818 $\pm$ 85 ( 0.51 $\pm$ 0.02 )	573 $\pm$ 47 ( 0.74 $\pm$ 0.06 )					

Tissue	Radioactivity concentration (ng eq. of FTD/g or mL)					
	15 min	1 hr	6 hr	24 hr	72 hr	
Liver	5191 ± 2941 ( 0.35 ± 0.07 )	6947 ± 1387 ( 0.26 ± 0.06 )	2201 ± 641 ( 0.38 ± 0.03 )	766 ± 122 ( 0.48 ± 0.03 )	407 ± 47 ( 0.52 ± 0.04 )	
Kidney	36208 ± 14883 ( 2.61 ± 0.15 )	59445 ± 8812 ( 2.17 ± 0.23 )	8447 ± 1374 ( 1.50 ± 0.22 )	1080 ± 116 ( 0.67 ± 0.04 )	586 ± 105 ( 0.75 ± 0.11 )	
Adrenal gland	7021 ± 3663 ( 0.49 ± 0.08 )	9016 ± 1500 ( 0.33 ± 0.08 )	2094 ± 152 ( 0.38 ± 0.07 )	691 ± 102 ( 0.44 ± 0.10 )	450 ± 63 ( 0.58 ± 0.10 )	
Spleen	5733 ± 3096 ( 0.40 ± 0.03 )	9841 ± 1457 ( 0.36 ± 0.06 )	3465 ± 706 ( 0.61 ± 0.12 )	1041 ± 143 ( 0.65 ± 0.08 )	1256 ± 284 ( 1.61 ± 0.36 )	
Pancreas	6000 ± 2896 ( 0.43 ± 0.04 )	9591 ± 1279 ( 0.35 ± 0.04 )	2586 ± 671 ( 0.45 ± 0.02 )	486 ± 36 ( 0.30 ± 0.02 )	297 ± 41 ( 0.38 ± 0.04 )	
Fat	890 ± 603 ( 0.06 ± 0.01 )	1377 ± 165 ( 0.05 ± 0.01 )	463 ± 129 ( 0.08 ± 0.02 )	196 ± 33 ( 0.13 ± 0.02 )	163 ± 20 ( 0.21 ± 0.05 )	
Brown fat	3895 ± 2287 ( 0.27 ± 0.05 )	6528 ± 451 ( 0.24 ± 0.01 )	1802 ± 533 ( 0.31 ± 0.05 )	695 ± 58 ( 0.44 ± 0.05 )	450 ± 22 ( 0.58 ± 0.08 )	
Skeletal muscle	4097 ± 1752 ( 0.30 ± 0.07 )	7740 ± 2104 ( 0.29 ± 0.08 )	1448 ± 400 ( 0.25 ± 0.01 )	386 ± 45 ( 0.24 ± 0.03 )	283 ± 23 ( 0.36 ± 0.03 )	
Skin	5063 ± 2828 ( 0.35 ± 0.04 )	9581 ± 1131 ( 0.35 ± 0.05 )	3545 ± 860 ( 0.62 ± 0.10 )	1590 ± 193 ( 0.99 ± 0.05 )	1060 ± 104 ( 1.35 ± 0.08 )	
Bone marrow	6415 ± 3789 ( 0.44 ± 0.06 )	13165 ± 2339 ( 0.48 ± 0.08 )	8678 ± 1580 ( 1.55 ± 0.34 )	3450 ± 717 ( 2.14 ± 0.28 )	809 ± 124 ( 1.04 ± 0.19 )	
Aorta	10698 ± 9233 ( 0.73 ± 0.62 )	12287 ± 2524 ( 0.45 ± 0.11 )	3454 ± 1358 ( 0.59 ± 0.13 )	1546 ± 378 ( 0.96 ± 0.15 )	1130 ± 120 ( 1.46 ± 0.27 )	
Mesenteric lymph node	7780 ± 4191 ( 0.54 ± 0.15 )	10455 ± 1624 ( 0.39 ± 0.10 )	3483 ± 463 ( 0.62 ± 0.08 )	893 ± 145 ( 0.56 ± 0.06 )	539 ± 31 ( 0.69 ± 0.06 )	
Testis	1746 ± 987 ( 0.12 ± 0.03 )	7876 ± 1032 ( 0.29 ± 0.05 )	2249 ± 517 ( 0.40 ± 0.05 )	544 ± 91 ( 0.34 ± 0.04 )	276 ± 34 ( 0.35 ± 0.03 )	
Epididymis	3713 ± 2027 ( 0.25 ± 0.04 )	9020 ± 1271 ( 0.33 ± 0.05 )	2291 ± 687 ( 0.39 ± 0.03 )	751 ± 132 ( 0.47 ± 0.04 )	523 ± 60 ( 0.67 ± 0.10 )	

Tissue	Radioactivity concentration (ng eq. of FTD/g or mL)				
	15 min	1 hr	6 hr	24 hr	72 hr
Prostate gland	5334 ± 3395 ( 0.36 ± 0.16 )	19351 ± 25302 ( 0.73 ± 0.95 )	2262 ± 315 ( 0.40 ± 0.04 )	605 ± 97 ( 0.37 ± 0.03 )	321 ± 11 ( 0.42 ± 0.05 )
Stomach	180135 ± 81623 ( 13.19 ± 3.54 )	23498 ± 9117 ( 0.86 ± 0.34 )	4741 ± 2153 ( 0.80 ± 0.17 )	639 ± 146 ( 0.40 ± 0.07 )	435 ± 57 ( 0.56 ± 0.10 )
Jejunum	94631 ± 62374 ( 6.33 ± 3.91 )	8078 ± 1283 ( 0.30 ± 0.08 )	4118 ± 1410 ( 0.71 ± 0.09 )	1771 ± 618 ( 1.09 ± 0.29 )	567 ± 47 ( 0.73 ± 0.04 )
Ileum	4488 ± 3198 ( 0.30 ± 0.17 )	83487 ± 138044 ( 3.43 ± 5.87 )	3864 ± 976 ( 0.67 ± 0.07 )	1731 ± 344 ( 1.08 ± 0.14 )	597 ± 107 ( 0.77 ± 0.14 )
Cecum	2932 ± 1986 ( 0.20 ± 0.11 )	6090 ± 2820 ( 0.23 ± 0.13 )	17470 ± 4282 ( 3.21 ± 1.20 )	2422 ± 856 ( 1.52 ± 0.59 )	780 ± 179 ( 0.99 ± 0.15 )
Colon	7378 ± 6251 ( 0.50 ± 0.41 )	7339 ± 1521 ( 0.27 ± 0.08 )	11227 ± 4347 ( 1.93 ± 0.55 )	1572 ± 875 ( 0.95 ± 0.47 )	578 ± 84 ( 0.74 ± 0.10 )
Urinary bladder	24320 ± 15639 ( 1.68 ± 0.73 )	135174 ± 103921 ( 4.96 ± 3.67 )	39774 ± 30801 ( 7.61 ± 6.41 )	1237 ± 334 ( 0.77 ± 0.17 )	809 ± 64 ( 1.04 ± 0.13 )

Data are expressed as the mean values ± S.D. of four animals.

Figures in parentheses are expressed as the ratio of concentration in tissue relative to plasma.

(Excerpted from Applicant's submission)

[<sup>14</sup>C-FTD]TAS-102 or <sup>14</sup>C-FTD was spiked into fresh plasma obtained from male animals and healthy human volunteers, and the in vitro protein binding ratio was determined. <sup>14</sup>C-FTD was 37.8-45.4%, 57.1-72.3%, 70-82.5%, 87.8-91.5%, and 96.7-97.3% protein bound in dogs, rats, mice, monkeys, and humans, respectively.

Following a single oral administration of [<sup>14</sup>C-FTD]TAS-102 or <sup>14</sup>C-FTD to non-fasted rats, the majority of radioactivity was excreted in the urine. Similar results were seen in rats fasted for 16 hrs (data not shown).

**Table 20: Excretion of Radioactivity following a single oral administration of [<sup>14</sup>C-FTD]TAS-102 or <sup>14</sup>C-FTD to non-fasted rats**

Time (hr)	Excretion of radioactivity (% of dose)							
	<sup>14</sup> C-FTD]TAS-102				<sup>14</sup> C-FTD			
	50mg TAS-102/kg[(60mg FTD+23.6mg TPI)/kg]				50mg/kg			
	Urine	Feces	Expired air	Total	Urine	Feces	Expired air	Total
0 - 4	42.3 ± 6.0	—	9.8 ± 1.9	—	40.3 ± 2.8	—	13.2 ± 3.2	—
8	53.9 ± 3.6	0.5 ± 0.7	14.4 ± 2.3	68.8 ± 2.2	53.4 ± 4.0	0.2 ± 0.2*	16.5 ± 3.2	70.0 ± 6.3
24	59.8 ± 4.7	19.7 ± 2.8	15.6 ± 2.5	95.0 ± 1.1	60.6 ± 2.0	17.2 ± 3.1	17.3 ± 3.2	95.1 ± 1.9
48	60.1 ± 4.7	20.5 ± 2.8	15.7 ± 2.6	96.2 ± 0.8	61.4 ± 1.7	17.7 ± 2.9	17.3 ± 3.2	96.4 ± 1.3
72	60.2 ± 4.6	20.5 ± 2.8	15.7 ± 2.6	96.5 ± 0.9	61.6 ± 1.5	17.8 ± 2.9	17.4 ± 3.2	96.7 ± 1.1
96	60.4 ± 4.6	20.5 ± 2.8	15.8 ± 2.6	96.7 ± 0.9	61.8 ± 1.5	17.8 ± 2.9	17.4 ± 3.2	97.0 ± 1.0
120	60.5 ± 4.6	20.5 ± 2.8	15.8 ± 2.6	96.8 ± 0.9	61.8 ± 1.5	17.9 ± 2.9	17.4 ± 3.2	97.1 ± 1.1
144	60.5 ± 4.6	20.6 ± 2.8	15.8 ± 2.6	96.9 ± 1.0	62.0 ± 1.4	17.9 ± 2.9	17.4 ± 3.2	97.3 ± 0.9
168	60.6 ± 4.6	20.6 ± 2.8	15.8 ± 2.6	96.9 ± 1.0	62.1 ± 1.4	17.9 ± 2.9	17.4 ± 3.2	97.4 ± 0.8
Carcass (168hr)				0.6 ± 0.2				0.6 ± 0.1

Data are expressed as the mean values ± S.D. of four animals.

\* : Feces sample was not obtained by one of four animals.

— : Not determined

(Excerpted from Applicant's submission)

Following single IV administration of [<sup>14</sup>C-FTD]TAS-102 to non-fasted rats, excretion of radioactivity in the urine, feces, and expired air was 85.6%, 1.9%, and 7.9% of the dose at 168 hrs post-dose, respectively. Excretion of radioactivity in the bile was 0.4% following oral administration of [<sup>14</sup>C-FTD]TAS-102 to bile duct-cannulated rats at 48 hrs post-dose; excretion of radioactivity in the urine and feces was 65.8% and 6.7%, respectively.

Metabolite analysis was conducted using high performance liquid chromatography (HPLC). Following a single oral administration of [<sup>14</sup>C-FTD]TAS-102 to non-fasted rats, the major metabolite detected in plasma was 5-(trifluoromethyl) uracil (FTY), which accounted for 58-82% of the radioactivity in the plasma up to 6 hrs post-dose (see Table 21). Similar results were seen in fasted rats (data not shown).

**Table 21: Metabolites detected in plasma following single oral administration of [<sup>14</sup>C-FTD]TAS-102 to non-fasted rats**

Metabolite	% of radioactivity in plasma							
	15 min	30 min	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
FTD	25.3 ± 2.4	16.1 ± 2.0	9.9 ± 0.8	5.1 ± 1.6	2.4 ± 0.4	1.5 ± 0.7	N.D.	N.D.
FTY	68.3 ± 3.5	78.1 ± 2.2	81.9 ± 1.8	78.8 ± 5.0	65.1 ± 5.3	58.1 ± 2.6	28.4 ± 8.3	6.2 ± 3.2
HFF1	3.0 ± 0.6	2.9 ± 0.7	3.7 ± 0.8	5.3 ± 1.8	5.1 ± 0.7	5.2 ± 0.8	8.4 ± 3.0	2.0 ± 1.6
Others	2.0 ± 1.0	1.3 ± 0.5	1.4 ± 0.4	2.4 ± 0.3	2.9 ± 1.1	6.2 ± 2.5	9.4 ± 4.0	14.7 ± 5.4
Recovery (%)*	98.6 ± 0.4	98.4 ± 0.2	96.9 ± 0.5	91.5 ± 2.0	75.5 ± 4.7	71.0 ± 3.8	46.1 ± 9.1	23.0 ± 5.0
Unextractable fraction (%)	1.4 ± 0.4	1.6 ± 0.2	3.1 ± 0.5	8.5 ± 2.0	24.5 ± 4.7	29.0 ± 3.8	53.9 ± 9.1	77.0 ± 5.0

Plasma was analyzed by HPLC.

Data are expressed as the mean values ± S.D. of four animals.

\* : Recovery of radioactivity from plasma

N.D. : Not detected

(Excerpted from Applicant's submission)

The metabolic profile of [<sup>14</sup>C-FTD]TAS-102 in urine and feces is shown in Table 22. Following single oral administration of [<sup>14</sup>C-FTD]TAS-102, FTY was the main metabolite in urine. FTD and FTY were not detected in feces up to 24 hrs post-dose, but the metabolite HFF1 accounted for 44.3% of radioactivity in the feces.

**Table 22: Metabolites detected in urine and feces following single oral administration of [<sup>14</sup>C-FTD]TAS-102 to non-fasted rats**

Metabolite	% of radioactivity in urine			Metabolite	% of radioactivity in feces
	0-4 hr	4-8 hr	8-24 hr		
FTD	27.1 ± 2.4	15.4 ± 1.4	10.8 ± 1.7	FTD	—
FTY	60.5 ± 1.2	65.3 ± 2.3	60.7 ± 6.8	HFF1	44.3 ± 2.8
HFU1	9.1 ± 2.3	15.2 ± 2.8	20.5 ± 3.3	HFF2	15.0 ± 1.5
Others	3.3 ± 0.5	4.1 ± 0.4	7.9 ± 2.8	HFF3	7.9 ± 1.2
Recovery (%)*	100.0 ± 0.0	100.0 ± 0.0	99.9 ± 0.0	HFF5	3.0 ± 1.2
Unextractable fraction (%)	0.0 <sup>1)</sup> ± 0.0	0.0 <sup>1)</sup> ± 0.0	0.1 ± 0.0	Others	13.0 ± 4.3
				Recovery (%)*	83.2 ± 1.0
				Unextractable fraction (%)	16.8 ± 1.0

Urine was analyzed by HPLC.  
Data are expressed as the mean values ± S.D. of four animals.  
\* : Recovery of radioactivity from urine  
<sup>1)</sup> : < 0.05 %

Feces was analyzed by HPLC.  
Data are expressed as the mean values ± S.D. of four animals.  
— : Not determined  
\* : Recovery of radioactivity from feces

(Excerpted from Applicant's submission)

Excretion of FTD and its metabolite FTY in urine were lower and higher, respectively, following single oral administration of <sup>14</sup>C-FTD compared to [<sup>14</sup>C-FTD]TAS-102, suggesting that TPI inhibits the metabolism of FTD to FTY.

**Table 23: Excretion of FTD and its metabolites in urine following single oral administration of [<sup>14</sup>C-FTD]TAS-102 or <sup>14</sup>C-FTD to non-fasted rats**

<sup>14</sup> C-FTD				[ <sup>14</sup> C-FTD]TAS-102			
Metabolite	% of dose			Metabolite	% of dose		
	0-4 hr	0-8 hr	0-24 hr		0-4 hr	0-8 hr	0-24 hr
FTD	4.8 ± 0.6	5.6 ± 0.5	5.9 ± 0.5	FTD	11.4 ± 0.9	13.2 ± 0.6	13.8 ± 0.7
FTY	30.7 ± 2.2	40.8 ± 4.3	46.0 ± 3.0	FTY	25.6 ± 3.4	33.2 ± 1.8	36.8 ± 2.3
HFU1	3.7 ± 1.2	5.6 ± 1.7	6.8 ± 1.9	HFU1	4.0 ± 1.5	5.7 ± 1.4	6.9 ± 1.6
Others	1.0 ± 0.2	1.6 ± 0.2	2.1 ± 0.2	Others	1.4 ± 0.4	1.9 ± 0.3	2.4 ± 0.5
Unextractable fraction (%)	0.0 <sup>1)</sup> ± 0.0	0.0 <sup>1)</sup> ± 0.0	0.0 <sup>1)</sup> ± 0.0	Unextractable fraction (%)	0.0 <sup>1)</sup> ± 0.0	0.0 <sup>1)</sup> ± 0.0	0.0 <sup>1)</sup> ± 0.0
Excretion (%)	40.3 ± 2.8	53.4 ± 4.0	60.6 ± 2.0	Excretion (%)	42.3 ± 6.0	53.9 ± 3.6	59.8 ± 4.7

Urine was analyzed by HPLC.  
Data are expressed as the mean values ± S.D. of four animals.  
<sup>1)</sup> : < 0.05 %

Urine was analyzed by HPLC.  
Data are expressed as the mean values ± S.D. of four animals.  
<sup>1)</sup> : < 0.05 %

(Excerpted from Applicant's submission)

### AE-2350-2G: Pharmacokinetic Studies on TAS-102 (II) Absorption, Distribution, Metabolism and Excretion in Rats using <sup>14</sup>C-TPI

#### Methods

Drug, lot, specific activity FTD, 706004, 100%; TPI, 961125, 100%  
(SA), % purity: <sup>14</sup>C-TPI, CP-1976, 7.31 MBq/mg, ≥ 98%  
Dose, frequency, ROA, [ <sup>14</sup>C-TPI ] TAS-102: 50 mg/kg (50 mg/kg FTD + 23.6 mg/kg  
dose volume: TPI; 10 MBq/kg), once, oral or IV, 5 mL/kg  
<sup>14</sup>C-TPI: 23.6 mg/kg (10 MBq/kg), once, oral  
Formulation/Vehicle: Oral: 0.5% HPMC; IV: physiological saline for injection  
Species/Strain: 7 week old male Sprague Dawley rats  
Number/Group: 4/group

The Applicant examined absorption, distribution, metabolism, and excretion following a single oral or IV administration of 50 mg/kg [<sup>14</sup>C-TPI]TAS-102 or 23.6 mg/kg <sup>14</sup>C-TPI to male rats; radioactivity was measured by LSC. The PK parameters following single administration of 50 mg/kg [<sup>14</sup>C-TPI]TAS-102 are shown in Table 24. Mean radioactivity

concentrations reached 421 ng eq.·hr/mL and 607 ng eq.·hr/mL in non-fasted and fasted rats, respectively, and oral bioavailability was calculated to be 9%. TPI exposures were relatively comparable in fasted and non-fasted rats, whereas C<sub>max</sub> was ~1.4-fold higher in fasted rats compared to non-fasted rats. T<sub>max</sub> was also achieved earlier in fasted rats, suggesting a higher absorption rate in fasted compared to non-fasted rats.

**Table 24: Pharmacokinetic Parameters following single administration of [<sup>14</sup>C-TPI]TAS-102 to rats**

Time	Radioactivity concentration (ng eq. of TPI/mL)					
	p. o.				i. v.	
	Non-fasting		Fasting		Non-fasting	
	Blood	Plasma	Blood	Plasma	Blood	Plasma
5 min	—	—	—	—	26487 ± 1488	35305 ± 2365
15	102 ± 27	144 ± 33	331 ± 62	487 ± 87	17484 ± 1579	18474 ± 2168
30	298 ± 48	371 ± 59	479 ± 79	607 ± 113	7685 ± 1174	6448 ± 1268
1 hr	372 ± 66	421 ± 79	444 ± 21	488 ± 43	1740 ± 119	1282 ± 116
2	277 ± 66	306 ± 66	233 ± 100	261 ± 123	286 ± 40	261 ± 40
4	171 ± 83	188 ± 98	99 ± 28	103 ± 33	106 ± 18	118 ± 10
6	123 ± 16	128 ± 13	91 ± 35	102 ± 46	87 ± 7	89 ± 12
8	106 ± 24	115 ± 24	70 ± 24	89 ± 39	65 ± 2	69 ± 5
24	20 ± 3	19 ± 2	15 ± 5	13 ± 9	36 ± 4	32 ± 4
72	N. D.	N. D.	—	—	—	—
T <sub>max</sub>	1 hr	1 hr	30 min	30 min	—	—
t <sub>1/2</sub> (hr)	2.70 (T <sub>max</sub> -4hr)	2.61 (T <sub>max</sub> -4hr)	1.48 (T <sub>max</sub> -4hr)	1.35 (T <sub>max</sub> -4hr)	0.29 (5min-2hr)	0.27 (5min-2hr)
	6.78 (6-24hr)	6.39 (6-24hr)	7.03 (6-24hr)	5.94 (6-24hr)	13.95 (4-24hr)	11.59 (4-24hr)
	6.65 (8-24hr)	6.16 (8-24hr)	7.20 (8-24hr)	5.77 (8-24hr)	—	—
AUC (ng eq.·hr·mL <sup>-1</sup> )	2534 (0-24hr)	2769 (0-24hr)	2075 (0-24hr)	2422 (0-24hr)	13985 (0-24hr)	15195 (0-24hr)
	2726 (0-∞)	2938 (0-∞)	2231 (0-∞)	2530 (0-∞)	14687 (0-∞)	15712 (0-∞)

Data are expressed as the mean values ± S.D. of four animals.

— : Not determined

N. D. : Not detected

(Excerpted from Applicant's submission)

Following oral administration of [<sup>14</sup>C-TPI]TAS-102 to rats, the highest level of radioactivity at up to 4 hrs post-dose as measured by whole body autoradiograms was found in the intestinal contents followed by urine in bladder and gastric contents. <sup>14</sup>C-TPI-related radioactivity reached its maximum levels in the stomach, jejunum, urinary bladder, adrenal gland, and pancreas at 15 minutes post-dose, in the brain, eyeball, skeletal muscle, testis, prostate gland, cecum, and colon at 6 hrs post-dose, and in the plasma, blood, and remaining tissues at 1 hour post-dose. Tissue distribution of <sup>14</sup>C-TPI was the highest in the jejunum, ileum, and stomach. Radioactivity concentrations were higher in the liver, kidney, aorta, mesenteric lymph node, stomach, jejunum, ileum, cecum, colon, and urinary bladder than in plasma at 1 hr post-dose. Radioactivity concentrations in the cerebrum and cerebellum were ~3% and 5% of that in plasma at 1 hour post-dose, respectively. Mean tissue radioactivity concentrations generally decreased with time.

**Table 25: Tissue Distribution following a single oral administration of [<sup>14</sup>C-TPI]TAS-102 to non-fasted rats**

Tissue	Radioactivity concentration (ng eq. of TPI/g or mL)						72 hr		
	15 min		1 hr		6 hr			24 hr	
Plasma	144 ± 33 ( 1.00 ± 0.00 )	33	421 ± 79 ( 1.00 ± 0.00 )	79	128 ± 13 ( 1.00 ± 0.00 )	13	19 ± 2 ( 1.00 ± 0.00 )	2	N. D.
Blood	102 ± 27 ( 0.71 ± 0.09 )	27	372 ± 66 ( 0.89 ± 0.03 )	66	123 ± 16 ( 0.96 ± 0.06 )	16	20 ± 3 ( 1.04 ± 0.20 )	3	N. D.
Cerebrum	N. D.		11 ± 2 ( 0.03 ± 0.00 )	2	23 ± 5 ( 0.18 ± 0.04 )	5	12 ± 1 ( 0.64 ± 0.03 )	1	N. D.
Cerebellum	N. D.		22 ± 20 ( 0.05 ± 0.05 )	20	26 ± 5 ( 0.21 ± 0.04 )	5	10 ± 1 ( 0.55 ± 0.09 )	1	N. D.
Pituitary gland	N. D.		143 ± 51 ( 0.35 ± 0.10 )	51	116 ± 29 ( 0.91 ± 0.20 )	29	N. D.		N. D.
Eyeball	13 ± 3 ( 0.09 ± 0.02 )	3	62 ± 16 ( 0.15 ± 0.03 )	16	64 ± 8 ( 0.50 ± 0.03 )	8	19 ± 4 ( 0.98 ± 0.14 )	4	N. D.
Harderian gland	19 ± 4 ( 0.13 ± 0.01 )	4	79 ± 22 ( 0.19 ± 0.06 )	22	71 ± 15 ( 0.55 ± 0.08 )	15	55 ± 6 ( 2.96 ± 0.48 )	6	13 ± 4
Thyroid gland	N. D.		195 ± 35 ( 0.47 ± 0.09 )	35	115 ± 27 ( 0.91 ± 0.24 )	27	N. D.		N. D.
Trachea	79 ± 17 ( 0.58 ± 0.19 )	17	176 ± 128 ( 0.41 ± 0.25 )	128	119 ± 17 ( 0.93 ± 0.10 )	17	N. D.		N. D.
Mandibular gland	40 ± 12 ( 0.27 ± 0.02 )	12	175 ± 25 ( 0.42 ± 0.05 )	25	106 ± 11 ( 0.83 ± 0.07 )	11	31 ± 2 ( 1.64 ± 0.17 )	2	18 ± 3
Thymus	25 ± 6 ( 0.18 ± 0.01 )	6	183 ± 28 ( 0.44 ± 0.07 )	28	109 ± 19 ( 0.85 ± 0.08 )	19	27 ± 4 ( 1.45 ± 0.14 )	4	21 ± 4
Heart	28 ± 6 ( 0.20 ± 0.01 )	6	115 ± 14 ( 0.28 ± 0.05 )	14	90 ± 16 ( 0.70 ± 0.06 )	16	21 ± 1 ( 1.10 ± 0.05 )	1	7 ± 1
Lung	58 ± 15 ( 0.40 ± 0.02 )	15	247 ± 29 ( 0.59 ± 0.05 )	29	128 ± 25 ( 1.00 ± 0.12 )	25	34 ± 2 ( 1.79 ± 0.15 )	2	21 ± 7

Tissue	Radioactivity concentration (ng eq. of TPI/g or mL)						72 hr		
	15 min		1 hr		6 hr			24 hr	
Liver	522 ± 118 ( 3.66 ± 0.43 )	118	1133 ± 326 ( 2.66 ± 0.39 )	326	420 ± 104 ( 3.26 ± 0.51 )	104	253 ± 9 ( 13.54 ± 0.92 )	9	143 ± 23
Kidney	785 ± 261 ( 5.52 ± 1.43 )	261	2533 ± 579 ( 6.11 ± 1.29 )	579	657 ± 168 ( 5.09 ± 0.99 )	168	72 ± 12 ( 3.83 ± 0.43 )	12	15 ± 9
Adrenal gland	273 ± 444 ( 1.66 ± 2.54 )	444	244 ± 50 ( 0.61 ± 0.22 )	50	146 ± 46 ( 1.13 ± 0.28 )	46	33 ± 3 ( 1.76 ± 0.10 )	3	N. D.
Spleen	88 ± 104 ( 0.56 ± 0.58 )	104	165 ± 34 ( 0.40 ± 0.10 )	34	121 ± 23 ( 0.94 ± 0.09 )	23	37 ± 5 ( 1.97 ± 0.21 )	5	18 ± 2
Pancreas	206 ± 277 ( 1.30 ± 1.55 )	277	196 ± 27 ( 0.47 ± 0.04 )	27	136 ± 30 ( 1.06 ± 0.13 )	30	31 ± 5 ( 1.64 ± 0.18 )	5	17 ± 4
Fat	15 ± 8 ( 0.10 ± 0.04 )	8	82 ± 54 ( 0.19 ± 0.10 )	54	24 ± 6 ( 0.19 ± 0.06 )	6	14 ± 4 ( 0.73 ± 0.25 )	4	10 ± 4
Brown fat	32 ± 6 ( 0.22 ± 0.02 )	6	135 ± 22 ( 0.32 ± 0.01 )	22	62 ± 6 ( 0.49 ± 0.03 )	6	33 ± 3 ( 1.79 ± 0.24 )	3	14 ± 5
Skeletal muscle	17 ± 4 ( 0.12 ± 0.01 )	4	70 ± 13 ( 0.17 ± 0.01 )	13	102 ± 10 ( 0.80 ± 0.02 )	10	19 ± 2 ( 1.03 ± 0.07 )	2	N. D.
Skin	62 ± 7 ( 0.44 ± 0.07 )	7	209 ± 39 ( 0.50 ± 0.04 )	39	107 ± 28 ( 0.83 ± 0.17 )	28	31 ± 5 ( 1.65 ± 0.36 )	5	16 ± 6
Bone marrow	35 ± 3 ( 0.25 ± 0.06 )	3	170 ± 21 ( 0.41 ± 0.04 )	21	121 ± 12 ( 0.95 ± 0.12 )	12	39 ± 6 ( 2.08 ± 0.33 )	6	30 ± 5
Aorta	595 ± 985 ( 3.58 ± 5.65 )	985	1498 ± 1663 ( 3.32 ± 3.31 )	1663	130 ± 57 ( 1.00 ± 0.34 )	57	28 ± 9 ( 1.49 ± 0.36 )	9	N. D.
Mesenteric lymph node	189 ± 86 ( 1.50 ± 1.17 )	86	1110 ± 726 ( 2.48 ± 1.29 )	726	302 ± 73 ( 2.34 ± 0.37 )	73	35 ± 4 ( 1.86 ± 0.21 )	4	16 ± 3
Testis	15 ± 2 ( 0.11 ± 0.04 )	2	70 ± 18 ( 0.17 ± 0.03 )	18	82 ± 6 ( 0.64 ± 0.04 )	6	31 ± 4 ( 1.63 ± 0.18 )	4	5 ± 2
Epididymis	134 ± 210 ( 0.82 ± 1.19 )	210	134 ± 25 ( 0.33 ± 0.07 )	25	113 ± 21 ( 0.88 ± 0.09 )	21	32 ± 11 ( 1.71 ± 0.59 )	11	8 ± 2

Tissue	Radioactivity concentration (ng eq. of TPI/g or ml.)					
	15 min	1 hr	6 hr	24 hr	72 hr	
Prostate gland	48 ± 15 ( 0.34 ± 0.11 )	295 ± 241 ( 0.67 ± 0.46 )	381 ± 243 ( 3.12 ± 2.27 )	39 ± 7 ( 2.06 ± 0.22 )		11 ± 3
Stomach	33574 ± 7824 ( 244.31 ± 78.88 )	4560 ± 2491 ( 11.26 ± 6.86 )	2047 ± 1060 ( 16.24 ± 9.13 )	48 ± 16 ( 2.57 ± 0.95 )		18 ± 4
Jejunum	50790 ± 45909 ( 342.80 ± 276.73 )	13863 ± 13781 ( 29.32 ± 27.47 )	2546 ± 833 ( 19.98 ± 6.01 )	52 ± 24 ( 2.79 ± 1.39 )		22 ± 12
Ileum	4531 ± 4454 ( 29.18 ± 23.93 )	50183 ± 54380 ( 116.67 ± 109.35 )	8174 ± 3081 ( 64.39 ± 25.99 )	180 ± 76 ( 9.58 ± 4.08 )		50 ± 26
Cecum	890 ± 765 ( 6.58 ± 5.51 )	2829 ± 3116 ( 6.09 ± 6.23 )	33635 ± 25673 ( 272.81 ± 219.03 )	665 ± 515 ( 34.20 ± 23.79 )		16 ± 6
Colon	23347 ± 43132 ( 156.62 ± 289.45 )	2950 ± 2688 ( 6.57 ± 5.64 )	26953 ± 6058 ( 210.55 ± 37.79 )	578 ± 178 ( 30.63 ± 8.22 )		16 ± 2
Urinary bladder	6406 ± 10290 ( 38.86 ± 58.78 )	6182 ± 4761 ( 15.00 ± 12.64 )	5231 ± 2286 ( 42.57 ± 21.92 )	217 ± 146 ( 11.26 ± 6.91 )		11 ± 8

Data are expressed as the mean values ± S.D. of four animals.

Figures in parentheses are expressed as the ratio of concentration in tissue relative to plasma.

N.D. : Not detected

(Excerpted from Applicant's submission)

[<sup>14</sup>C-TPI]TAS-102 or <sup>14</sup>C-TPI was spiked into fresh plasma obtained from male animals and healthy human volunteers, and the in vitro protein binding ratio was determined. <sup>14</sup>C-FTD was 1.9-4%, 4.1-6.4%, 3.1-5.5%, 3-6.8%, and 1.3-7.1% protein bound in rats, mice, dogs, monkeys, and humans, respectively.

Following a single oral administration of [<sup>14</sup>C-TPI]TAS-102 or <sup>14</sup>C-TPI to non-fasted rats, the majority of radioactivity was excreted in the feces. Similar results were seen in rats fasted for 16 hours (data not shown). The absorption extent of TPI in the digestive tract was determined to be ~15-19%.

**Table 26: Excretion of Radioactivity following a single oral administration of [<sup>14</sup>C-TPI]TAS-102 or <sup>14</sup>C-TPI to non-fasted rats**

Time (hr)	Excretion of radioactivity (% of dose)							
	<sup>14</sup> C-TPI]TAS-102				<sup>14</sup> C-TPI			
	50mg TAS-102/kg [(50mg FTD+23.6mg TPI)/kg]				23.6mg/kg			
	Urine	Feces	Expired air	Total	Urine	Feces	Expired air	Total
0-4	5.4 ± 2.6	—	0.1 ± 0.1	—	5.4 ± 1.2	—	0.1 ± 0.1	—
8	7.8 ± 0.8	0.1 ± 0.1	0.1 ± 0.0	7.9 ± 0.8	9.3 ± 0.9	0.0 ± 0.0*	0.2 ± 0.1	9.4 ± 0.9
24	13.6 ± 1.6	77.2 ± 2.2	0.3 ± 0.1	91.1 ± 1.4	14.6 ± 2.0	79.8 ± 6.8	0.5 ± 0.1	94.8 ± 6.5
48	14.2 ± 1.8	83.2 ± 2.1	0.4 ± 0.1	97.8 ± 1.3	15.5 ± 2.2	85.0 ± 2.8	0.5 ± 0.1	101.0 ± 0.6
72	14.2 ± 1.8	83.4 ± 2.1	0.4 ± 0.1	97.9 ± 1.3	15.5 ± 2.2	85.1 ± 2.8	0.6 ± 0.1	101.2 ± 0.7
96	14.2 ± 1.8	83.4 ± 2.1	0.4 ± 0.1	98.0 ± 1.4	15.6 ± 2.2	85.2 ± 2.7	0.6 ± 0.1	101.3 ± 0.7
120	14.3 ± 1.8	83.4 ± 2.1	0.4 ± 0.1	98.0 ± 1.4	15.6 ± 2.2	85.2 ± 2.7	0.6 ± 0.1	101.3 ± 0.7
144	14.3 ± 1.8	83.4 ± 2.1	0.4 ± 0.1	98.0 ± 1.4	15.6 ± 2.2	85.2 ± 2.7	0.6 ± 0.1	101.3 ± 0.7
168	14.3 ± 1.8	83.4 ± 2.1	0.4 ± 0.1	98.0 ± 1.4	15.6 ± 2.2	85.2 ± 2.7	0.6 ± 0.1	101.3 ± 0.7
Carcass (168hr)				0.0 ± 0.0				0.0 ± 0.0

Data are expressed as the mean values ± S.D. of four animals.

\* : Feces sample was not obtained by two of four animals.

— : Not determined

(Excerpted from Applicant's submission)

Following single IV administration of [<sup>14</sup>C-TPI]TAS-102 to non-fasted rats, excretion of radioactivity in the urine, feces, and expired air was 96.8%, 2.9%, and 0.2% of the dose at 168 hrs post-dose, respectively. Excretion of radioactivity in the bile was 0.2% following oral administration of [<sup>14</sup>C-TPI]TAS-102 to bile duct-cannulated rats at 48 hrs post-dose; excretion of radioactivity in the urine and feces was 23.8% and 67.9% of the dose, respectively.

Metabolite analysis was conducted using HPLC. Following single oral administration of [<sup>14</sup>C-TPI]TAS-102 to non-fasted rats, unchanged TPI was the major plasma component up to 4 hrs post-dose. The metabolite HTP1 accounted for ~58% of the radioactivity in the plasma at 8 hours post-dose (see Table 27). Similar results were seen in fasted rats (data not shown).

**Table 27: Metabolites detected in plasma following single oral administration of [<sup>14</sup>C-TPI]TAS-102 to non-fasted rats**

Metabolite	% of radioactivity in plasma						
	15 min	30 min	1 hr	2 hr	4 hr	6 hr	8 hr
TPI	83.4 ± 7.0	89.0 ± 3.1	89.8 ± 2.6	75.5 ± 10.5	48.1 ± 5.9	30.3 ± 3.7	19.5 ± 2.6
HTP1	2.5 ± 0.5	1.8 ± 0.8	3.7 ± 1.3	15.8 ± 5.0	39.5 ± 2.2	44.2 ± 2.5	57.9 ± 6.4
Others	12.3 ± 6.5	7.2 ± 3.4	5.5 ± 1.6	7.4 ± 5.5	9.6 ± 6.9	21.7 ± 3.4	18.8 ± 5.2
Recovery (%)*	98.2 ± 0.6	98.0 ± 1.0	99.0 ± 0.3	98.6 ± 0.5	97.2 ± 2.0	96.2 ± 1.9	96.2 ± 0.9
Unextractable fraction (%)	1.8 ± 0.6	2.0 ± 1.0	1.0 ± 0.3	1.4 ± 0.5	2.8 ± 2.0	3.8 ± 1.9	3.8 ± 0.9

Plasma was analyzed by HPLC.

Data are expressed as the mean values ± S.D. of four animals.

\* : Recovery of radioactivity from plasma

(Excerpted from Applicant's submission)

The metabolic profile of [<sup>14</sup>C-TPI]TAS-102 in urine and feces is shown in Table 28. Following single oral administration of [<sup>14</sup>C-TPI]TAS-102, TPI was the major component of urine and feces up to 24 hrs post-dose. The metabolites HTU1 and HTF1 were detected in rat urine and feces, respectively. Given that HTP1, HTU1, and HTF1 exhibited the same retention time on HPLC chromatograms, the Applicant concluded that these metabolites were identical. Single oral administration of [<sup>14</sup>C-TPI]TAS-102 or <sup>14</sup>C-TPI resulted in similar excretion and metabolic profiles, suggesting that FTD had little effect on the metabolism of TPI.

**Table 28: Metabolites detected in urine and feces following single oral administration of [<sup>14</sup>C-TPI]TAS-102 to non-fasted rats**

Metabolite	% of radioactivity in urine			Metabolite	% of radioactivity in feces
	0-4 hr	4-8 hr	8-24 hr		
TPI	92.4 ± 2.2	82.9 ± 6.6	49.3 ± 5.0	TPI	77.1 ± 2.7
HTU1	5.2 ± 2.9	12.4 ± 7.5	46.9 ± 4.8	HTF1	18.0 ± 4.1
Others	2.3 ± 0.7	4.6 ± 2.1	3.8 ± 0.5	Others	1.7 ± 0.2
Recovery (%)*	100.0 ± 0.0	99.9 ± 0.1	100.0 ± 0.0	Recovery (%)*	96.8 ± 1.4
Unextractable fraction (%)	0.0 <sup>U</sup> ± 0.0	0.1 ± 0.1	0.0 <sup>U</sup> ± 0.0	Unextractable fraction (%)	3.2 ± 1.4

Urine was analyzed by HPLC.

Data are expressed as the mean values ± S.D. of four animals.

\* : Recovery of radioactivity from urine

<sup>U</sup> : < 0.05 %

Feces was analyzed by HPLC.

Data are expressed as the mean values ± S.D. of four animals.

\* : Recovery of radioactivity from feces

(Excerpted from Applicant's submission)

## AE-4140: Pharmacokinetic Study of TAS-102 Structural Analysis of Metabolite in Rats After Oral Administration of <sup>14</sup>C-TPI

### Methods

Drug, lot, specific activity (SA), TPI, 030317  
 % purity: <sup>14</sup>C-TPI, CP-2943, 7.31 MBq/mg, ≥ 98%  
 Dose 23.6 mg/kg (96 MBq/kg)  
 Frequency, ROA, dose volume Single, oral, 5 mL/kg  
 Formulation/Vehicle: 0.5% HPMC

Species/Strain: 7 week old male Sprague-Dawley rats  
 Number/Group: 4/group

The Applicant structurally analyzed the in vivo metabolites of TPI following single oral administration of 23.6 mg/kg <sup>14</sup>C-TPI to male rats. Consistent with Study #AE-2350-2G, HPLC demonstrated that the TPI metabolites detected in rat plasma (HTP1), urine (HTU1), and feces (HTF1) were identical. LC/MS analysis identified the structure of HTP1/HTU1/HTF1 to be 6-hydroxymethyluracil (6-HMU).

### AE-6930-G: Pharmacokinetic Evaluation of TAS-102 using [<sup>14</sup>C]FTD or [<sup>14</sup>C]TPI in cynomolgus monkeys

#### Methods

Drug, lot, specific activity (SA), FTD, 065022; TPI, 7A0104  
 % purity: <sup>14</sup>C-FTD, CP-3228, 6.69 MBq/mg, ≥ 98%  
<sup>14</sup>C-TPI, CP-3229, 7.41 MBq/mg, ≥ 96%  
 Dose 10 mg/kg (10 mg/kg FTD + 4.71 mg/kg TPI; 3 MBq/kg)  
 Frequency, ROA, dose volume Single, oral, 5 mL/kg  
 Formulation/Vehicle: 0.5% HPMC  
 Species/Strain: 3-4 year old male cynomolgus monkeys  
 Number/Group: 3/group

The Applicant examined the absorption, metabolism, and excretion of TAS-102 following single oral administration of [<sup>14</sup>C-FTD]TAS-102 or [<sup>14</sup>C-TPI]TAS-102 to fasting male cynomolgus monkeys. Radioactivity was measured by LSC. The PK parameters following single administration of 10 mg/kg [<sup>14</sup>C-FTD]TAS-102 or 10 mg/kg [<sup>14</sup>C-TPI]TAS-102 are shown in Table 29.

**Table 29: Pharmacokinetic Parameters following single administration of [<sup>14</sup>C-FTD]TAS-102 or [<sup>14</sup>C-TPI]TAS-102 to fasting cynomolgus monkeys**

Time (hr)	Radioactivity concentration (ng eq. of FTD/mL)		Time (hr)	Radioactivity concentration (ng eq. of TPI/mL)	
	Blood	Plasma		Blood	Plasma
0.5	2172 ± 1772	4120 ± 3446	0.5	45 ± 30	78 ± 55
1	8711 ± 7021	16113 ± 12799	1	134 ± 45	224 ± 80
2	7712 ± 5978	13871 ± 11369	2	332 ± 118	526 ± 183
3	5957 ± 4169	10912 ± 8466	3	444 ± 151	618 ± 240
4	4058 ± 2500	7178 ± 4858	4	402 ± 139	517 ± 191
6	2030 ± 774	3374 ± 1464	6	157 ± 56	126 ± 39
8	1330 ± 351	2138 ± 684	8	78 ± 24	57 ± 13
12	1082 ± 239	1628 ± 399	12	37 ± 11	33 ± 2
24	926 ± 173	1316 ± 205	24	20 ± 11	22 ± 10
C <sub>max</sub> (ng eq./mL)	11179 ± 5347	20205 ± 10061	C <sub>max</sub> (ng eq./mL)	444 ± 151	618 ± 240
T <sub>max</sub> (hr)	2.0 ± 1.0	2.0 ± 1.0	T <sub>max</sub> (hr)	3.0 ± 0.0	3.0 ± 0.0
AUC <sub>0-last</sub> (ng eq.-hr/mL)	49600 ± 19500 (0-24 hr)	83900 ± 37300 (0-24 hr)	AUC <sub>0-last</sub> (ng eq.-hr/mL)	2470 ± 800 (0-24 hr)	2950 ± 880 (0-24 hr)
AUC <sub>0-∞</sub> (ng eq.-hr/mL)	99400 ± 24000	144000 ± 37200	AUC <sub>0-∞</sub> (ng eq.-hr/mL)	2660 ± 850	3220 ± 860
t <sub>1/2</sub> (hr)	38.8 ± 15.6 (8-24 hr)	33.7 ± 21.5 (8-24 hr)	t <sub>1/2</sub> (hr)	7.1 ± 1.7 (4 or 8-24 hr)	9.1 ± 3.2 (4 or 8-24 hr)

Data are expressed as the mean values ± S.D. of three animals.  
 Figures in parentheses represent the time ranges for calculation.

Data are expressed as the mean values ± S.D. of three animals.  
 Figures in parentheses represent the time ranges for calculation.

(Excerpted from Applicant's submission)

Following a single oral administration of [<sup>14</sup>C-FTD]TAS-102 to cynomolgus monkeys, excretion of radioactivity in the urine and feces up to 168 hrs post-dose was 79.4% and

3.8% of the dose, respectively. Following a single oral administration of [<sup>14</sup>C-TPI]FTD-TPI to cynomolgus monkeys, excretion of radioactivity in the urine and feces up to 168 hrs post-dose was 27.3% and 68.1% of the dose, respectively.

**Table 30: Excretion of Radioactivity following a single oral dose of [<sup>14</sup>C-FTD]TAS-102 or [<sup>14</sup>C-TPI]TAS-102 to fasted cynomolgus monkeys**

Time (hr)	Excretion of radioactivity (% of dose)			Time (hr)	Excretion of radioactivity (% of dose)		
	Urine	Feces	Total		Urine	Feces	Total
0 - 6	67.5 ± 7.9	-	-	0 - 6	18.0 ± 3.9	-	-
12	74.4 ± 4.7	-	-	12	23.4 ± 5.7	-	-
24	77.6 ± 6.1	2.1 ± 1.1	79.7 ± 7.0	24	25.6 ± 5.5	52.3 ± 14.3	77.9 ± 10.3
48	78.3 ± 6.3	3.1 ± 0.5	81.3 ± 6.5	48	26.8 ± 5.6	66.4 ± 6.8	93.1 ± 1.5
72	78.6 ± 6.2	3.3 ± 0.3	82.0 ± 6.2	72	27.0 ± 5.5	67.5 ± 6.5	94.5 ± 1.0
96	78.9 ± 6.1	3.5 ± 0.3	82.4 ± 6.1	96	27.1 ± 5.5	67.7 ± 6.6	94.8 ± 1.1
120	79.1 ± 6.0	3.6 ± 0.2	82.7 ± 5.9	120	27.2 ± 5.4	67.9 ± 6.6	95.1 ± 1.2
144	79.2 ± 6.0	3.7 ± 0.2	83.0 ± 5.8	144	27.2 ± 5.4	68.0 ± 6.6	95.2 ± 1.2
168	79.4 ± 6.0	3.8 ± 0.2	83.2 ± 5.8	168	27.3 ± 5.4	68.1 ± 6.6	95.4 ± 1.2
Cage washing (168 hr)			0.8 ± 0.7	Cage washing (168 hr)			1.6 ± 0.6

Data are expressed as the mean values ± S.D. of three animals.

-: Not determined

Data are expressed as the mean values ± S.D. of three animals.

-: Not determined

(Excerpted from Applicant's submission)

Metabolites in plasma and urine were analyzed using liquid chromatography in combination with mass spectrometry and radio-HPLC. Following a single oral administration of [<sup>14</sup>C-FTD]TAS-102 to cynomolgus monkeys, unchanged FTD accounted for 56% of radioactivity in the plasma at 1 hr post-dose and declined over time. FTY was the major metabolite of FTD and accounted for up to 34% of radioactivity in the plasma up to 2 hrs post-dose. The minor metabolites F-Peak 1, F-Peak 2 (hydrolyzed FTY), and F-Peak 3 (≤8.5% of radioactivity; glucuronide of FTD) were also detected in plasma. A similar metabolic profile was observed in the urine, with FTY accounting for up to 54% up radioactivity in the urine up to 24 hrs post-dose.

**Table 31: Metabolites detected in plasma and urine following single oral dose of [<sup>14</sup>C-FTD]TAS-102 to cynomolgus monkeys**

Metabolites	% of radioactivity in plasma				Metabolites	% of radioactivity in urine		
	1 hr	2 hr	6 hr	12 hr		0-6 hr	6-12 hr	12-24 hr
F-Peak 1	N.D.	N.D.	1.6	0.8	F-Peak 1	N.D.	3.7	4.2
F-Peak 2	1.4	2.0	3.2	1.1	F-Peak 2	2.8	7.6	5.9
FTY	26.1	34.2	15.4	5.6	FTY	43.0	53.7	51.7
F-Peak 3	5.3	7.5	8.5	7.6	F-Peak 3	2.5	5.2	4.5
FTD	56.2	41.7	12.6	2.7	FTD	41.4	18.8	11.4
Others	8.5	9.1	16.3	0.0	Others	5.8	7.6	18.5
Recovery (%) <sup>1)</sup>	97.5	94.5	57.6	17.8	Recovery (%) <sup>1)</sup>	95.5	96.6	96.2

Data were obtained from the pooled sample of three monkeys.

1): Recovery of radioactivity from plasma

N.D.: Not detected

Data were obtained from the pooled sample of three monkeys.

1): Recovery of radioactivity from urine

N.D.: Not detected

(Excerpted from Applicant's submission)

Following a single oral administration of [<sup>14</sup>C-TPI]TAS-102 to cynomolgus monkeys, unchanged TPI accounted for the majority of radioactivity in plasma (≤81%) up to 6 hrs post-dose. T-Peak 3 and 5 were also detected in plasma, and T-Peak 3 accounted for 52% of radioactivity at 12 hrs post-dose. Unchanged TPI accounted for up to 86% of radioactivity in the urine up to 12 hrs post-dose. T-Peak 1, 2, 3 (uracil), 4, and 6 (imino-oxidated TPI) were also detected in urine, with T-Peak 3 accounted for 28% of radioactivity in the urine at 24 hrs post-dose.

**Table 32: Metabolites detected in plasma and urine following single oral dose of [<sup>14</sup>C-TPI]TAS-102 to cynomolgus monkeys**

Metabolites	% of radioactivity in plasma				Metabolites	% of radioactivity in urine		
	1 hr	2 hr	6 hr	12 hr		0-6 hr	6-12 hr	12-24 hr
T-Peak 3	N.D.	N.D.	9.7	51.8	T-Peak 1	N.D.	N.D.	1.3
TPI	49.3	67.9	80.5	44.0	T-Peak 2	N.D.	N.D.	3.3
T-Peak 5	4.6	3.8	6.1	N.D.	T-Peak 3	N.D.	5.3	27.8
Others	46.1	27.6	2.4	4.2	TPI	85.7	77.2	44.7
Recovery (%) <sup>1)</sup>	100.5	99.3	98.7	108.3	T-Peak 4	1.0	N.D.	2.5
					T-Peak 6	1.1	1.5	2.5
					Others	9.8	13.0	12.5
					Recovery (%) <sup>1)</sup>	97.6	97.0	94.6

Data were obtained from the pooled sample of three monkeys.  
1): Recovery of radioactivity from plasma  
N.D.: Not detected

Data were obtained from the pooled sample of three monkeys.  
1): Recovery of radioactivity from urine  
N.D.: Not detected

**12DA18: Metabolite Analysis for FTD and TPI Using Cryopreserved Human Hepatocytes**

The Applicant evaluated the in vitro metabolism of FTD and TPI using cryopreserved human hepatocytes. <sup>14</sup>C-FTD (lot # CP-3228) or <sup>14</sup>C-TPI (lot # CP-3229) were incubated with six lots of human hepatocytes for 3 hrs. FTD was metabolized primarily to FTY; uracil-5-carboxylic acid (5-CU) and 5-carboxy-2'-deoxyuridine (5-Car-dUrd; 5-CdUrd) were also detected. 5-CdUrd was detected following incubation with PBS(+) in the absence of hepatocytes, suggesting it was produced nonenzymatically. The Applicant did not examine the in vitro metabolism of <sup>14</sup>C-FTD in hepatocytes isolated from animals for comparison.

**Table 33: In vitro metabolism of <sup>14</sup>C-FTD in cryopreserved human hepatocytes**

	Lot	% of total peak			
		FTD	FTY	5-Car-dUrd	5-CU
0 hr	789	100.0	0.0	0.0	0.0
	886	98.7	0.8	0.6	0.0
	HC4-6	99.6	0.0	0.5	0.0
	719	98.9	0.4	0.8	0.0
	HC3-7	98.7	0.2	1.2	0.0
	HC4-5	98.9	0.4	0.8	0.0
Control	789	96.9	0.4	2.8	0.0
	886	96.2	0.2	3.7	0.0
	HC4-6	97.0	0.0	3.1	0.0
	719	96.7	0.3	3.2	0.0
	HC3-7	96.3	0.2	3.5	0.0
	HC4-5	96.2	0.2	3.7	0.0
<sup>14</sup> C-FTD	789	51.2	44.5	2.3	2.1
	886	14.1	78.9	1.6	5.5
	HC4-6	0.0	92.3	0.0	7.8
	719	9.0	84.2	1.1	5.9
	HC3-7	20.6	73.4	1.8	4.3
	HC4-5	0.0	92.1	0.5	7.5
<sup>14</sup> C-FTD + TPI 100 µmol/L	789	92.4	4.9	2.7	0.0
	886	58.0	37.9	2.1	2.2
	HC4-6	82.7	14.4	2.3	0.7
	719	83.2	13.9	2.5	0.5
	HC3-7	88.7	7.9	3.1	0.4
	HC4-5	70.4	25.9	2.4	1.3
<sup>14</sup> C-FTD + TPI 30 µmol/L	789	92.3	5.2	2.6	0.0
	886	55.5	40.0	2.5	2.1
	HC4-6	81.5	15.4	2.5	0.7
	719	81.1	15.7	2.5	0.7
	HC3-7	88.8	7.9	3.1	0.3
	HC4-5	69.3	27.2	2.3	1.2

0 hr : 0 hr incubation of <sup>14</sup>C-FTD in human hepatocytes. Control : 3 hr of <sup>14</sup>C-FTD in PBS(+). Except for both 0 hr and control, 3 hr incubation of <sup>14</sup>C-FTD in human hepatocytes was conducted. The values represent the means (n=2) of individual data. <sup>14</sup>C-FTD : 5 µg/mL.

(Excerpted from Applicant's submission)

In contrast, TPI was not substantially metabolized in human hepatocytes (data not shown). The Applicant also assessed the contribution of thymidine phosphorylase to the metabolism of FTD by calculating the effect of TPI on the metabolism of FTD. Incubation with 30  $\mu\text{M}$  and 100  $\mu\text{M}$  TPI inhibited  $^{14}\text{C}$ -FTD metabolism by 78.1% and 79.5%, respectively, indicating that FTD is primarily metabolized by thymidine phosphorylase (see Table 34). Inhibition of thymidine metabolism by TPI was used as a positive control (data not shown).

**Table 34: Effect of TPI on metabolism of  $^{14}\text{C}$ -FTD in cryopreserved human hepatocytes**

	% inhibition						Mean	S.D.
	Lot : 789	Lot : 886	Lot : HC4-6	Lot : 719	Lot : HC3-7	Lot : HC4-5		
$^{14}\text{C}$ -FTD +TPI 100 $\mu\text{mol/L}$	90.2	53.5	85.3	84.6	90.0	73.2	79.5	14.1
$^{14}\text{C}$ -FTD +TPI 30 $\mu\text{mol/L}$	89.9	50.4	84.0	82.2	90.1	72.0	78.1	15.1

(Excerpted from Applicant's submission)

### 12DB03: Metabolic study of $^{14}\text{C}$ -FTD using human liver microsomes

The Applicant evaluated the in vitro metabolism of  $^{14}\text{C}$ -FTD using human liver microsomes. The microsomes were incubated with  $^{14}\text{C}$ -FTD (lot # CP-3228) at 37°C for up to 20 min with (Groups A and C) or without (Groups B and D) NADPH, and FTD metabolites were analyzed using radio-HPLC. TPI was added to samples in Groups C and D. The metabolic activity of coumarin was included as a positive control to confirm that CYPs had metabolic activity in the test system. No FTD metabolites were detected in the presence or absence of NADPH in Groups A-D, suggesting that FTD is not metabolized by CYP enzymes. The in vitro metabolism of  $^{14}\text{C}$ -FTD using animal liver microsomes was not examined.

### 11DA34: Study of Blood Cell Distribution of FTD and TPI Using Blood of Human, Monkey, and Rat

The Applicant investigated the blood cell distribution of FTD and TPI in human (n=3), cynomolgus monkey (n=3), and Sprague-Dawley rat (n=5) blood. 0.5, 5, and 50  $\mu\text{g/mL}$   $^{14}\text{C}$ -FTD (lot #CP-3228) and 10, 100, and 1000 ng/mL  $^{14}\text{C}$ -TPI (lot #CP-3229) were spiked into blood and incubated at 37 °C for 5 minutes. These concentrations were chosen based on the Cmax on Day 12 following administration of 35 mg/m<sup>2</sup> TAS-102 twice daily in the Japanese Phase 1 study. Radioactivity was measured using LSC. The remaining spiked blood was centrifuged to obtain plasma samples, and radioactivity was again measured using LSC. The blood/plasma concentrations ratios (Rb) of FTD and TPI were <1 in rat, monkey, and human blood, indicating that FTD and TPI were preferentially distributed to plasma rather than blood cells in all three species.

**Table 35: Blood/plasma concentration ratio of FTD and TPI in rat, monkey, and human blood**

FTD concentration (µg/mL)	Rb			TPI concentration (ng/mL)	Rb		
	Rat	Monkey	Human		Rat	Monkey	Human
0.5	0.713 ± 0.026	0.644 ± 0.010	0.611 ± 0.068	10	0.865 ± 0.076	0.680 ± 0.032	0.661 ± 0.033
5	0.701 ± 0.026	0.628 ± 0.025	0.596 ± 0.041	100	0.793 ± 0.016	0.634 ± 0.015	0.598 ± 0.044
50	0.788 ± 0.042	0.678 ± 0.028	0.619 ± 0.018	1000	0.776 ± 0.065	0.638 ± 0.014	0.581 ± 0.055

Values are shown as the mean ± SD (N=3)  
Rb: Blood/plasma concentration ratio (Cb/Cp)

Values are shown as the mean ± SD (N=3)  
Rb: Blood/plasma concentration ratio (Cb/Cp)

(Excerpted from Applicant's submission)

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

Previously reviewed under IND 57674 by Dr. Sandip K. Roy (1999).

### 6.2 Repeat-Dose Toxicity

**Study title:** A 13-Week Oral Repeated Dose Toxicity Study of TAS-102 in Rats with a 9-Week Recovery Period

Study no.: 07CA07

Study report location: Electronic

Conducting laboratory and location:

(b) (4)

Date of study initiation: 05/22/2007

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TAS-102 (combined form of FTD; Lot #: 056020, Purity: 100.2% and TPI; Lot #: 040602, Purity: 99.9%, Molar ratio of 1:0.5)

### Key Study Findings

- Whitish and fractured incisors at the 221 mg/kg dose level
- Decreased WBCs, lymphocytes, and reticulocytes in females at ≥74 mg/kg and in males at 221 mg/kg dose levels
- Decreased thymus weights and increased ovarian weights at the 221 mg/kg dose level
- Slight decreases in dentin thickness and odontoblast, flattened ameloblast and papillary cells of the incisors at 221 mg/kg dose level

- Slight increase apoptotic bodies in the GI tract at  $\geq 74$  mg/kg dose levels
- Slight to moderate thymus atrophy
- Slight fatty infiltration in bone marrow at  $\geq 74$  mg/kg dose levels

## Methods

Doses: See table below  
 Frequency of dosing: Once daily x13 weeks  
 Route of administration: Oral gavage  
 Formulation/Vehicle: 5 mg/mL Hydroxypropyl Methylcellulose (HPMC) Solution  
 Species/Strain: Rat (Sprague-Dawley)  
 Age: 6 week old  
 Weight: Males: 169 to 199 g; Females: 136 to 179 g

Dose Group	TAS-102 Doses		Dose Volume mL/kg	Number of rats					
	mg/kg	mg/m <sup>2</sup>		Main groups		Recovery groups		TK groups	
	[FTD+TPI]	FTD+TPI		♂	♀	♂	♀	♂	♀
1	0 [0+0]	0	5	12	12	6	6	3	3
2	7.4 [5+2.4]	44.4	5	12	12	--	--	9	9
3	22.1 [15+7.1]	132.6	5	12	12	6	6	9	9
4	74 [50+24]	444	5	12	12	6	6	9	9
5	221 [150+71]	1326	5	12	12	6	6	9	9

## Observations and Results

### Mortality

There were no treatment-related mortalities during the study.

### Clinical Signs

Discoloration of the incisors (whitish incisors) was observed in all the rats (18 males, 18 females), broken incisors in 15 males and 7 females, malocclusion in one male and 3 females, and diarrhea in 3 males, at the 221 mg/kg dose level of TAS-102. At the 74 mg/kg dose level, broken incisors were noted in one female and one male while whitish incisors were noted in one female. Whitish incisors, broken deciduous incisors, and malocclusion persisted during the recovery period.

### Body Weights

At the 221 mg/kg dose level of TAS-102, decreases in body weight gain occurred in females from Week 7 and in males from Week 9 of dosing. At the end of the dosing period, the decrease in body weight gain was 14% in males and females compared to control groups.

It is not clear whether the decrease in body weight gain was a direct effect of TAS-102 or an indirect effect as a consequence of reduced food intake subsequent to broken incisors. Decreased body weight did not reverse during the recovery period, but was not progressive.

### Feed Consumption

At the 221 mg/kg dose level, there was a significant decrease in food consumption during Week 8 (-18%) and Week 11 (-18%) in males compared to controls while in females, the decrease in food consumption was during Week 7 (-18%) and Week 10 (-14%) compared to controls.

Again, it is not clear whether the decrease in food consumption was a direct effect of TAS-102 or an indirect effect as a consequence of broken incisors.

### Ophthalmoscopy

There were no consistent remarkable treatment-related findings.

### Hematology

Parameter	Sex	0	7.4 mg/kg	22.1 mg/kg	74 mg/kg	221 mg/kg
Terminal/Recovery						
<b>WBC</b> 10 <sup>3</sup> /μL	M	9.7	--	--	--	↓36%
	F	8.0	↓19%	↓14%	↓29%	↓43%
<b>RBC</b> 10 <sup>3</sup> /μL	M	8.7	--	--	↓5%	↓11%
	F	7.6	--	--	--	↓4%
<b>Lymphocytes</b> 10 <sup>3</sup> /μL	M	7.5	↓4%	↓2%	↓6%	↓39%
	F	5.6	↓14%	↓13%	↓28%	↓41%
<b>Reticulocytes</b> 10 <sup>6</sup> /μL	M	134.4	↑8%	↑10%	↓3%	↓11%
	F	134.7	↓18%	↓4%	↓13%	↓16%

### Clinical Chemistry

There were no consistent remarkable changes in clinical chemistry parameters.

### Urinalysis

There were no remarkable TAS-102-related changes in urinalysis.

### Gross Pathology

Macroscopic Signs	Group Size:	0		7.4 mg/kg		22.1 mg/kg		74 mg/kg		221 mg/kg	
		M	F	M	F	M	F	M	F	M	F
		12/6	12/6	12	12	12/6	12/6	12/6	12/6	12/6	12/6
Grade		Terminal/Recovery									
<b>Skin</b>											
Loss of fur, focal	Present	1	3	1	2	1	1/1	1/1	2/1	1	3

Macroscopic Signs	Group Size:	0		7.4 mg/kg		22.1 mg/kg		74 mg/kg		221 mg/kg	
		M	F	M	F	M	F	M	F	M	F
		12/6	12/6	12	12	12/6	12/6	12/6	12/6	12/6	12/6
Grade	Terminal/Recovery										
<b>Incisors</b>											
Whitish	Present	--	--	--	--	--	--	--	--	12/6	12/5
Fractured	Present	--	1	--	--	--	--	1	--	5/2	2
<b>Thymus</b>											
Dark red, focal	Present	--	--	--	--	--	2	--	--	--	1
Small	Present	--	--	--	--	--	--	--	--	--	1

## Organ Weights

The mean thymus weights, both absolute and relative to body weight, decreased in male and female rats at the 221 mg/kg dose level. In males, the decrease in absolute thymus weight was 38% relative to control (272.6 g). In females, the decrease in absolute thymus weight was 39% relative to control (301 g). There was an increase in the absolute weights of ovaries by 13% compared to control (45.3 g) at the 221 mg/kg dose level.

## Histopathology

Adequate Battery

Peer Review

## Histological Findings

Microscopic Signs	Group Size:	0		7.4 mg/kg		22.1 mg/kg		74 mg/kg		221 mg/kg	
		M	F	M	F	M	F	M	F	M	F
		12/6	12/6	3	2	12/1	12/1	12/6	12/3	12/6	12/6
Grade	Terminal/Recovery										
<b>Incisors</b>											
↓dentin thickness	Slight	--	--	--	--	--	--	--	--	12	11
↓Odontoblast	Slight	--	--	--	--	--	--	--	--	12	12
Disarrangement, odontoblast	Slight	--	--	--	--	--	--	9	8	3/1	2
	Mod	--	--	--	--	--	--			9	10
Enamel matrix residue	Slight	--	--	--	--	--	--	--	--	12	12
Flatten, ameloblast and papillary cells	Slight	--	--	--	--	--	--	--	--	12/1	10
Osteodentin	Slight	--	--	--	--	--	--	7	5	4	3
	Mod	--	--	--	--	--	--			8	9
<b>Duodenum</b>											
Increased apoptotic bodies, crypt	Slight	--	--	--	--	--	--	5	5	12	10

Microscopic Signs		0		7.4 mg/kg		22.1 mg/kg		74 mg/kg		221 mg/kg	
		M	F	M	F	M	F	M	F	M	F
	Group Size:	12/6	12/6	3	2	12/1	12/1	12/6	12/3	12/6	12/6
	Grade	Terminal/Recovery									
<b>Jejunum</b>											
Increased apoptotic bodies, crypt	Slight	--	--	--	--	--	--	3	6	11	10
<b>Ileum</b>											
Increased apoptotic bodies, crypt	Slight	--	--	--	--	--	--	2	3	11	8
<b>Thymus</b>											
Atrophy	Slight		--	--	--	--	--	--	--	4	2
	Mod		--	--	--	--	--	--	--		2
Hemorrhage, focal	Slight	2	--	--	--	1	2	2	--	3/2	4
<b>Bone marrow</b>											
Fatty infiltration	Slight	--	--	--	--	--	--	1	1	6	4
<b>Lung</b>											
Mineralization	Slight	--	--	--	--	--	--	--	--	1	--

## Toxicokinetics

The  $C_{max}$  and  $AUC_{0-24h}$  for FTD, FTY (a metabolite of FTD) and TPI increased with increasing doses of TAS-102. Although the values seem to be higher in females, there were no remarkable differences between males and females for the  $T_{max}$ ,  $C_{max}$  or  $AUC_{0-24h}$  for FTD, FTY and TPI. In Week 13 of administration, the  $AUC_{0-24h}$  values for FTD and FTY were slightly higher than those after the first administration, suggesting that they tended to accumulate after repeated administration (Table 36 Table 37 Table 38)

**Table 36: TK Parameters for FTD**

Dose level of TAS-102 (mg/kg)	TK parameters for FTD	First administration (Day 0)		Week 13 of administration	
		Male	Female	Male	Female
7.4 [FTD: 5] [TPI: 2.4]	$T_{max}$ (h)	0.5	0.5	0.5	0.5
	$C_{max}$ (ng/mL)	298	377	612	783
	$AUC_{0-24h}$ (ng·h/mL)	240	275	468	566
22.1 [FTD: 15] [TPI: 7.1]	$T_{max}$ (h)	0.5	0.5	0.5	0.5
	$C_{max}$ (ng/mL)	1430	2380	2360	3310
	$AUC_{0-24h}$ (ng·h/mL)	1110	1560	1800	2550
74 [FTD: 50] [TPI: 24]	$T_{max}$ (h)	0.5	0.5	0.5	0.5
	$C_{max}$ (ng/mL)	4700	4210	5580	6340
	$AUC_{0-24h}$ (ng·h/mL)	5510	5380	6530	7800
221 [FTD: 150] [TPI: 71]	$T_{max}$ (h)	0.5	0.5	0.5	0.5
	$C_{max}$ (ng/mL)	7400	11200	8630	13200
	$AUC_{0-24h}$ (ng·h/mL)	16100	16700	23100	21700

(Excerpted from Applicant's submission)

**Table 37: TK Parameters for FTY**

Dose level of TAS-102 (mg/kg)	TK parameters for FTY	First administration (Day 0)		Week 13 of administration	
		Male	Female	Male	Female
7.4	T <sub>max</sub> (h)	0.5	0.5	0.5	0.5
[FTD: 5]	C <sub>max</sub> (ng/mL)	2540	3090	3660	4820
[TPI: 2.4]	AUC <sub>0-24h</sub> (ng·h/mL)	3920	2750	5280	4370
22.1	T <sub>max</sub> (h)	0.5	0.5	0.5	0.5
[FTD: 15]	C <sub>max</sub> (ng/mL)	8000	11400	9880	13100
[TPI: 7.1]	AUC <sub>0-24h</sub> (ng·h/mL)	12900	11200	13500	15000
74	T <sub>max</sub> (h)	1.0	1.0	0.5	1.0
[FTD: 50]	C <sub>max</sub> (ng/mL)	13700	12700	13200	15900
[TPI: 24]	AUC <sub>0-24h</sub> (ng·h/mL)	34200	27600	34800	33100
221	T <sub>max</sub> (h)	1.0	1.0	2.0	1.0
[FTD: 150]	C <sub>max</sub> (ng/mL)	25400	27000	21500	28000
[TPI: 71]	AUC <sub>0-24h</sub> (ng·h/mL)	93900	100000	137000	172000

(Excerpted from Applicant's submission)

**Table 38: TK Parameters for TPI**

Dose level of TAS-102 (mg/kg)	TK parameters for TPI	First administration (Day 0)		Week 13 of administration	
		Male	Female	Male	Female
7.4	T <sub>max</sub> (h)	1.0	1.0	1.0	0.5
[FTD: 5]	C <sub>max</sub> (ng/mL)	42.0	77.8	74.4	94.3
[TPI: 2.4]	AUC <sub>0-24h</sub> (ng·h/mL)	124	151	181	196
22.1	T <sub>max</sub> (h)	1.0	0.5	0.5	0.5
[FTD: 15]	C <sub>max</sub> (ng/mL)	140	160	177	311
[TPI: 7.1]	AUC <sub>0-24h</sub> (ng·h/mL)	414	369	424	492
74	T <sub>max</sub> (h)	1.0	0.5	0.5	0.5
[FTD: 50]	C <sub>max</sub> (ng/mL)	435	583	431	853
[TPI: 24]	AUC <sub>0-24h</sub> (ng·h/mL)	1290	1320	1080	1380
221	T <sub>max</sub> (h)	1.0	1.0	1.0	0.5
[FTD: 150]	C <sub>max</sub> (ng/mL)	879	998	721	1260
[TPI: 71]	AUC <sub>0-24h</sub> (ng·h/mL)	3360	3060	4580	4100

(Excerpted from Applicant's submission)

**Study title:** A 13-Week Oral Repeated Dose Toxicity Study of TAS-102 in  
Cynomolgus Monkeys with a 9-week Recovery Period

Study no.: B-6227

Study report location: Electronic

Conducting laboratory and location:



(b) (4)

Date of study initiation: 07/30/2007  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: TAS-102 (combined form of FTD; Lot #: 202016, Purity: 100.1% and TPI; Lot #: 7A0104, Purity: 100.2%, Molar ratio of 1:0.5)

### Key Study Findings

- One female animal at the 29.42 mg/kg dose level was sacrificed preterminally on Day 85, due to deteriorating general condition and undernourished state likely associated with effects of TAS-102 on the lymphatic-hematopoietic system and digestive tract.
- Major organs of toxicity include the hematopoietic system and gastrointestinal tract

### Methods

Doses: See table below  
 Frequency of dosing: Once daily x13 weeks  
 Route of administration: Oral  
 Dose volume: 5 mL/kg  
 Formulation/Vehicle: 0.5% Hydroxypropylmethylcellulose (HPMC) Solution  
 Species/Strain: Cynomolgus monkeys (b) (4)  
 Age: 3 to 5 years old  
 Weight: Males: 3.77 to 6.88 kg; Females: 2.81 to 4.37 kg

Dose Group	TAS-102 Doses		Dose Volume mL/kg	Number of Animals			
	mg/kg	mg/m <sup>2</sup>		Main groups		Recovery groups	
	[FTD+TPI]	FTD+TPI		♂	♀	♂	♀
1	0 [0+0]	0	5	3	3	2	2
2	1.839 [1.25+0.589]	22.068	5	3	3	0	0
3	7.355 [5+2.355]	88.26	5	3	3	2	2
4	29.42 [20+9.42]	353.04	5	3	3	2	2
5	20 (FTD)	240	5	3	3	2	2

### Observations and Results

#### Mortality

One female (#4103) at the 29.42 mg/kg dose level was sacrificed moribund on Day 85. Clinical signs included frequent soft and watery stool, lateral position and hypothermia.

## Clinical Signs

Soft/Watery stool persisted in males at the 29.42 mg/kg dose level up to Week 6 and throughout the treatment period in females. Soft/Watery stool was absent in recovery animals.

**Table 39: Incidence of Clinical Signs**

Dose of TAS-102	0		1.839 mg/kg		7.355 mg/kg		29.42 mg/kg		FTD 20 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Sex										
Number of animals	5	5	3	3	5	5	5	5	5	5
Vomiting	--	--	--	1	1		1		2	
Soft/Watery stool	--	--	--	--	--	--	2	2	--	--
Bloody stool (positive occult blood)	--	--	--	--	--	--	1	--	--	--
Decreased locomotor activity	--	--	--	--	--	--	1	--	--	--

## Body Weights

Body weight loss was remarkable in monkeys treated with TAS-102 at the 29.42 mg/kg dose level.

**Table 40: Body Weight changes in TAS-102-treated Monkeys**

TAS-102 Dose (mg/kg)		Body Weight	
		Week 0 (g)	Week 13 Change
0	M	5.71	7.5%
	F	3.42	5%
1.839	M	5.51	4.9%
	F	3.48	17.5%
7.355	M	5.61	1.6%
	F	3.32	↓0.3%
29.42	M	5.9	↓6.9%
	F	3.33	↓12.3%
FTD 20	M	6.0	4.7%
	F	3.34	6.6%

## Feed Consumption

Mean food consumption was remarkably decreased in males from Weeks 4-7, with the lowest values during Week 6 (27% of control values of 150 g/day in animals at the 29.42 dose level). Food consumption returned to control levels from week 8 through the study period. The reason for the decrease in food consumption during this period is not entirely clear. There were no remarkable changes in food consumption in female animals during the treatment period.

## Ophthalmoscopy

No remarkable ophthalmologic changes were observed during treatment with TAS-102.

## ECG

There were no remarkable changes in ECG parameters in any monkey following administration of TAS-102 up to a dose of 29.42 mg/kg or FTD alone at a dose of 20 mg/kg.

## Hematology

Decreased hematologic parameters as seen in Table 41, including RBC, and WBC, are suggestive of bone marrow suppression.

**Table 41: Changes in Hematologic Parameters Relative to Control Values**

Parameter	Sex	TAS-102 (mg/kg)								FTD (mg/kg)	
		0		1.839		7.355		29.42		20	
		Week									
		6	13	6	13	6	13	6	13	6	13
Terminal/Recovery											
RBC 10 <sup>4</sup> /μL	M	573	588	-4%	-7%	-7%	13%	-9%	-16%	--	--
	F	577	507	-135	-10%	-8%	-11%	-14%	-15%	-8%	-8%
Hemoglobin g/dL	M	13.8	14.3	-3%	-6%	-3%	-6%	-9%	-11%	3%	4%
	F	13.3	13.3	-6%	-3%	-2%	-2%	-9%	-7%	-3%	-4%
Hematocrit %	M	44.3	45	-6%	-6%	-5%	-8%	-8%	-8%	3%	6%
	F	43.9	42.3	-9%	--	-3%	--	-12%	-8%	-3%	-2%
Neutrophils 10 <sup>2</sup> /μL	M	62.3	53.1	37.1%	15%	22%	33%	-49%	-11%	7%	41%
	F	54.4	59.3	-20%	-6%	-27%	23%	-30%	-27%	-36%	-16%
WBC 10 <sup>2</sup> /μL	M	120	114	30%	-17%	4%	-8%	-42%	-38%	4%	13%
	F	115.8	117.6	7%	9%	-16%	-15%	-28%	-29%	-15%	-8%
Monocytes 10 <sup>2</sup> /μL	M	3.3	4	61%	13%	3%	-3%	-21%	-25%	24%	10%
	F	2.9	3.2	17%	3%	-31%	-22%	-24%	-47%	14%	3%
Lymphocytes 10 <sup>2</sup> /μL	M	51.1	42.9	13%	-41%	-17%	-36%	-34%	-60%	9%	-7%
	F	54	50.9	36%	27%	-4%	-7%	-26%	-29%	5%	-5%
Reticulocytes 10 <sup>9</sup> /μL	M	87.6	59.7	-12%	-4%	-41%	-29%	-38%	-3%	-8%	12%
	F	63.1	57.9	13%	13%	-10%	--	-41%	-43%	8%	6%

## Clinical Chemistry

There were no remarkable TAS-102-related clinical chemistry findings.

## Urinalysis

There were no remarkable TAS-102-related findings in the urine

**Gross Pathology**

There were no treatment-related gross pathological observations.

**Organ Weights**

There were no treatment-related changes in organ weights.

**Histopathology**

Adequate Battery

Peer Review

Histological Findings

**Table 42: Histopathological Findings**

Microscopic Signs	Grade	TAS-102 (mg/kg)								FTD (mg/kg)	
		0		1.839		7.355		29.42		20	
		M	F	M	F	M	F	M	F	M	F
		Group Size:	3/2	3/2	3/2	3/2	3/2	3/2	3/2	1/2/2	3/2
<b>Adrenal</b>		<b>Pre-terminal/Terminal/Recovery</b>									
Mineralization, corticomedullary	Minimal							1			
Hypertrophy, cortical cell, focal	Minimal					1					1
Hypertrophy, cortical cell, diffuse	Mild								1		
<b>Bone+bone marrow, femoral</b>											
Hypocellularity, bone marrow	Mild								1		
<b>Bone+bone marrow, sternal</b>											
Hypocellularity, bone marrow	Mild								1		
<b>Eye</b>											
Erosion	Minimal									1	
<b>Heart</b>											
Hemorrhage, focal	Minimal					1/1				1	1
Myocarditis, focal	Minimal	--	1	--	3		2/1	--	1	--	1
<b>Intestine, duodenum</b>											
Cell infiltration, lymphocytic	Minimal	1						2		2	

Microscopic Signs	Grade	TAS-102 (mg/kg)								FTD (mg/kg)		
		0		1.839		7.355		29.42		20		
		M	F	M	F	M	F	M	F	M	F	
		Group Size:	3/2	3/2	3/2	3/2	3/2	3/2	3/2	1/2/2	3/2	3/2
Pre-terminal/Terminal/Recovery												
<b>Intestine, jejunum</b>												
Atrophy, mucosal	Mimimal									1		
Cell infiltration, lymphocytic	Mimimal						2		1		1	
<b>Intestine, ileum</b>												
Atrophy, mucosal	Minimal								1			
<b>Intestine, cecum</b>												
Regeneration, mucosal	Mod	--	--	--	--	--	--	--	1	--	--	
Hemorrhage, focal	Minimal	--	--	--	--	--	--	--	1	--	--	
Edema	Mild	--	--	--	--	--	--	--	1	--	--	
Cell infiltration, lymphocytic	Minimal	--	1	--	2	1	--	2	--	--	1/2	
	Mild	2	2	--	1	1	3	--	2	--	2	
Ulcer	Mild	--	--	--	--	--	--	--	1	--	--	
Cell infiltration, inflammatory	Minimal	--	--	--	--	--	--	--	1	--	--	
Necrosis, single cell, mucosal	Minimal	--	--	--	--	--	--	--	1	--	--	
<b>Intestine, colon</b>												
Regeneration, mucosal	Mod	--	--	--	--	--	--	--	1	--	--	
Hemorrhage, focal	Minimal	--	--	--	--	--	--	--	1	--	--	
Edema	Mild	--	--	--	--	--	--	--	1	--	--	
Cell infiltration, lymphocytic	Minimal	--	3	--	1	--	2	--	1	--	2	
	Mild	--	--	--	2	--	1	--	1	--	1	
<b>Intestine, rectum</b>												
Regeneration, mucosal	Minimal	--	--	--	--	--	--	--	1	--	--	
Cell infiltration, lymphocytic	Minimal	--	2	--	2	--	2	--	1	--	3	
	Mild	--	--	--	1	--	1	--	1	--	--	
Cell infiltration, Inflammatory	Minimal	--	--	--	--	--	--	--	1	--	--	
	Mild	--	--	--	--	--	--	--	1	--	--	
Necrosis, single cell, mucosal	Minimal	--	--	--	--	--	--	--	1	--	--	
<b>Kidney</b>												
Mineralization, papillary	Minimal	1	--	1	--	2	--	2/2	2	1/1	2	
<b>Larynx</b>												
Hemorrhage, focal	Mild	--	--	--	--	--	--	1	--	--	--	
<b>Liver</b>												
Microgranuloma	Minimal	1	2	--	2	2	3/2	3	2	2	2	
	Mild	--	--	--	1	--	--	--	--	--	--	
Necrosis	Minimal	--	--	--	2	--	--	--	--	--	--	

Microscopic Signs	Group Size:	TAS-102 (mg/kg)								FTD (mg/kg)	
		0		1.839		7.355		29.42		20	
		M	F	M	F	M	F	M	F	M	F
		3/2	3/2	3/2	3/2	3/2	3/2	3/2	1/2/2	3/2	3/2
Grade	Pre-terminal/Terminal/Recovery										
<b>Lymph node, mesenteric</b>											
Atrophy	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Lymph node, submandibular</b>											
Atrophy	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Medulla oblongata</b>											
Gliosis	Minimal	--	--	--	--	--	--	--	1	--	--
Hemorrhage, focal	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Ovary</b>											
Atrophy	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Pancreas</b>											
Atrophy, Acinar focal	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Spinal cord</b>											
Gliosis	Minimal	--	--	--	--	--	--	--	1	--	--
Hemorrhage, focal	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Spleen</b>											
Atrophy	Minimal	--	--	--	--	--	--	--	1	--	--
	Mild	--	--	--	--	--	--	--	1	--	--
<b>Stomach</b>											
Atrophy, mucosal	Minimal	--	--	--	--	--	--	--	1	--	--
Regeneration	Minimal	--	--	--	--	--	--	--	1	--	--
Cell infiltration, lymphocytic	Minimal	--	2	--	1	--	2/1	--	2/2	--	2/2
	Mild	--	--	--	1	--	1	--	--	--	1/1
Cell infiltration, inflammatory	Minimal	--	--	--	--	--	--	--	1	--	--
Necrosis, single cell, mucosal	Minimal	--	--	--	--	--	--	--	1	--	--
<b>Testis</b>											
Immature	Minimal	--	1	1	--	1	--	3/2	--	--	--
	Mild	--	--	1	--	--	--	--	--	--	--
<b>Thymus</b>											
Atrophy	Minimal	--	--	--	2	1	1/1	--	--	--	2
	Mild	--	3/2	2	1	1	--	--	1/2	1/2	--
	Mod	2/1	--	1	--	1	1	1	1	1	1
	Severe	1	--	--	--	2	1	2	1	1	--
<b>Uterus</b>											
Atrophy	Mild	--	--	--	--	--	--	--	1	--	--
<b>Vagina</b>											
Atrophy	Mild	--	--	--	--	--	--	--	1	--	--

Other than gliosis in the medulla oblongata and spinal cord, toxicities seen in the female that was euthanized early (shown in bold type) at the 29.42 mg/kg dose level, most of the toxicities were also seen in terminally sacrificed animals.

### Toxicokinetics

On Day 1, and Weeks 6 and 13 of administration of TAS-102, the  $C_{max}$  and  $AUC_{0-24h}$  of FTD, FTY and TPI in plasma increased with the increase in the dose level in both males and females. There were no clear sex differences on any examination day.  $T_{max}$  was comparable among dose levels on each day of examination.

For the concentration of FTD in plasma, the  $C_{max}$  and  $AUC_{0-24h}$  in the TAS-102 groups were higher than in the FTD only group in both males and females, though the level of FTD was the same in both groups (20 mg/kg), supporting the role of TPI in increasing levels of FTD exposure. The  $C_{max}$  and  $AUC_{0-24h}$  of FTY in plasma in TAS-102 groups were lower than in the FTD group in both males and females. On Day 1 of administration, TPI (at a concentration of 0.505 ng/mL) was detected in plasma of 1 female (5105, 1 hour after dosing) in the FTD group, but it was only slightly higher than the lower limit of quantification (0.5 ng/mL). FTD, FTY, and TPI were not detected in plasma of control group animals on any examination day.

**Table 43: TK Parameters for FTD**

Dose (mg/kg)	<u>0 (Control)</u>		<u>TAS-102 1.839</u>		<u>TAS-102 7.355</u>		<u>TAS-102 29.42</u>		<u>FTD 20</u>	
Day 1										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>
$T_{max}$ (h)	NC	NC	1.2	1.7	1.3	1.3	2.4	1.5	1.7	0.5
$C_{max}$ (ng/mL)	NC	NC	966	471	6610	3670	14500	25400	537	581
$AUC_{0-24h}$ (ng·h/mL)	NC	NC	1090	866	12900	8080	50200	55900	783	621
Week 6										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>
$T_{max}$ (h)	NC	NC	0.7	0.8	0.8	1.2	1.8	0.9	0.8	0.6
$C_{max}$ (ng/mL)	NC	NC	2390	967	9940	5980	18700	23600	622	450
$AUC_{0-24h}$ (ng·h/mL)	NC	NC	2900	1080	18600	12900	65100	52100	870	433
Week 13										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 4</u>	<u>M: 5</u>	<u>F: 5</u>
$T_{max}$ (h)	NC	NC	0.7	0.5	0.8	1.0	1.4	0.8	0.8	1.0
$C_{max}$ (ng/mL)	NC	NC	3390	1090	10600	5100	19000	16700	465	400
$AUC_{0-24h}$ (ng·h/mL)	NC	NC	3200	1670	17100	11800	59900	45800	675	433

NC: Not Calculated. [The concentrations of FTD in the control group were less than the quantification limit.]

(Excerpted from Applicant's submission)

**Table 44: TK Parameters for FTY**

Dose (mg/kg)	<u>0 (Control)</u>		<u>TAS-102 1.839</u>		<u>TAS-102 7.355</u>		<u>TAS-102 29.42</u>		<u>FTD 20</u>	
Day 1										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>
<u>T<sub>max</sub> (h)</u>	NC	NC	1.2	0.8	1.3	1.1	2.4	1.4	1.8	0.9
<u>C<sub>max</sub> (ng/mL)</u>	NC	NC	793	754	2630	2590	4910	6050	11200	17600
<u>AUC<sub>0-24h</sub> (ng·h/mL)</u>	NC	NC	1660	1600	7680	6850	26700	20800	31400	35300
Week 6										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>
<u>T<sub>max</sub> (h)</u>	NC	NC	0.8	0.7	0.9	1.2	2.4	1.0	1.2	1.0
<u>C<sub>max</sub> (ng/mL)</u>	NC	NC	974	725	1750	1470	2720	3210	11200	17300
<u>AUC<sub>0-24h</sub> (ng·h/mL)</u>	NC	NC	1630	1410	4230	4090	19100	14900	37700	34700
Week 13										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 4</u>	<u>M: 5</u>	<u>F: 5</u>
<u>T<sub>max</sub> (h)</u>	NC	NC	0.7	0.7	1.1	0.8	1.6	1.8	1.6	1.1
<u>C<sub>max</sub> (ng/mL)</u>	NC	NC	1120	802	1890	1620	2380	2500	8840	15300
<u>AUC<sub>0-24h</sub> (ng·h/mL)</u>	NC	NC	1550	1420	4110	4820	18100	18200	35100	35800

NC: Not Calculated. [The concentrations of FTY in the control group were less than the quantification limit.]

(Excerpted from Applicant's submission)

**Table 45: TK Parameters for TPI**

Dose (mg/kg)	<u>0 (Control)</u>		<u>TAS-102 1.839</u>		<u>TAS-102 7.355</u>		<u>TAS-102 29.42</u>		<u>FTD 20</u>	
Day 1										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>
<u>T<sub>max</sub> (h)</u>	NC	NC	2.3	2.7	2.0	2.0	2.8	1.6	NC	1.0 <sup>a)</sup>
<u>C<sub>max</sub> (ng/mL)</u>	NC	NC	74.4	32.4	124	166	371	892	NC	0.505 <sup>a)</sup>
<u>AUC<sub>0-24h</sub> (ng·h/mL)</u>	NC	NC	252	118	534	747	1860	3220	NC	0.379 <sup>a)</sup>
Week 6										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 5</u>
<u>T<sub>max</sub> (h)</u>	NC	NC	1.7	1.7	1.8	2.0	1.8	1.8	NC	NC
<u>C<sub>max</sub> (ng/mL)</u>	NC	NC	58.5	20.7	154	104	331	345	NC	NC
<u>AUC<sub>0-24h</sub> (ng·h/mL)</u>	NC	NC	202	107	596	528	2010	1770	NC	NC
Week 13										
<u>Number of animals</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 3</u>	<u>F: 3</u>	<u>M: 5</u>	<u>F: 5</u>	<u>M: 5</u>	<u>F: 4</u>	<u>M: 5</u>	<u>F: 5</u>
<u>T<sub>max</sub> (h)</u>	NC	NC	1.7	2.0	1.8	2.4	2.0	2.0	NC	NC
<u>C<sub>max</sub> (ng/mL)</u>	NC	NC	87.7	28.0	194	82.4	441	418	NC	NC
<u>AUC<sub>0-24h</sub> (ng·h/mL)</u>	NC	NC	257	140	664	525	2220	2090	NC	NC

NC: Not Calculated. [The concentrations of TPI in the control group were less than the quantification limit.]

a): The data of the animal numbered 5105.

(Excerpted from Applicant's submission)

## Dosing Solution Analysis

Dosing solution analysis was appropriately conducted and the concentration was within acceptable limits.

## 7 Genetic Toxicology

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

**Study title:** Reverse mutation test of TAS-102 in bacteria

Study no.: 00CA11

Study report location: Electronic

Conducting laboratory and location:



Date of study initiation: June 21, 2000

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TAS-102 (combined form of FTD; Lot #: 807010, Purity: 100% and TPI; Lot #: 970904, Purity: 99.2%, Molar ratio of 1:0.5)

#### Key Study Findings

- TAS-102 was positive for reverse mutagenicity

#### Methods

Strains: *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E. coli* WP2uvrA

Concentrations in definitive study: 46.0, 91.9, 184, 368, 735, 1470, 2940, and 5880 µg/plate of TAS-102 (total of FTD + TPI), [31.3, 62.5, 125, 250, 500, 1000, 2000, and 4000 µg/plate (as the amount of FTD)] without or with

Formulation/Vehicle: Physiological saline

Basis of concentration selection: Based on preliminary study

Incubation & sampling time: 48-49 hours incubation

The Applicant evaluated the potential for mutagenicity of TAS-102 using the Ames test by the preincubation method in five tester strains: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2uvrA. Physiological saline was the vehicle control. Briefly 0.1 mL of bacterial suspension was put into a small tube containing 0.1 mL of each dosing solution and 0.5 mL of S9 mix or 0.5 mL of 0.1 M sodium phosphate buffered solution (pH 7.4) and the mixture was cultured at 37°C for 20 minutes with shaking. Then the mixture was added to 2 mL of top agar warmed to about 45°C, placed on the top of the minimum glucose agar plate culture medium and cultured at 37°C for 48 to 49 hours. The test was performed twice to confirm the reproducibility of the results and two plates in each dose level were used in both tests.

The number of revertant colonies on the plate was counted by a colony analyzer and the mean value was calculated.

Positive control articles used were: *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG), 9-aminoacridine (9AA), 2-nitrofluorene (2NF), and 2-aminoanthracene (without S9) and (2AA) (with S9). All the positive control articles were dissolved in DMSO.

Bacterial strain	Without S9 mix		With S9 mix	
	Article	Concentration (µg/plate)	Article	Concentration (µg/plate)
TA100	ENNG	3	2AA	1
TA98	2NF	1	2AA	0.5
TA1535	ENNG	5	2AA	2
TA1537	9AA	80	2AA	2
WP2uvrA	ENNG	2	2AA	10

When the mean value of the number of revertant colonies per plate in the treatment of a test article increased more than twice that in the vehicle control group and there was dose-dependency in the increment, the Applicant concluded that the test was positive. Importantly, the biological validity of the result under the test condition was taken into consideration in the final analysis.

## Results

As shown in Table 46, in TA1535 the number of revertant colonies increased dose-dependently (from 184 to 1470 µg/plate), and was twice or more that in the vehicle control group at ≥184 µg/plate without and with S9 mix. In TA100, although there was dose-dependent increase, the number of revertant colonies was not twice or more that in the vehicle control group with or without S9 mix. In WP2uvrA, the number of revertant colonies increased dose-dependently (from 46 to 735 µg/plate), and was twice or more that in the vehicle control group at ≥368 µg/plate without S9 mix. In WP2uvrA with S9 mix, although there was dose-dependent increase (from 368 to 1470 µg/plate), the number of revertant colonies was not twice or more that in the vehicle control group. In TA98 and TA1537, the increase (if any) in the number of revertant colonies was neither dose-dependent nor twice or more that in the vehicle control group without and with S9 mix. These results suggest that TAS-102 induced base pair substitution type mutation. No growth inhibition was observed in any tester strain up to the highest concentration of TAS-102.

In each of the positive control groups, the number of revertant colonies was more than twice that in the vehicle control group indicating that the present test was performed under appropriate conditions.

**Table 46: Results of bacterial reversion test of TAS-102 (main test)**

S9 mix	Chemical	Dose (µg/plate)	Dose as FTD (µg/plate) <sup>a)</sup>	No. of Revertants (Colony number/plate)					
				Base pair substitution			Frameshift		
				TA100	TA1535	WP2uvrA	TA98	TA1537	
S9mix (-)	Saline	0	0	122	10	29	24	8	
				130 (126) <sup>b)</sup>	13 (12)	28 (29)	27 (26)	9 (9)	
	TAS-102	46.0	31.3	131	17	24	29	10	
				130 (131)	12 (15)	33 (29)	24 (27)	7 (9)	
		91.9	62.5	160	18	34	23	14	
				174 (167)	19 (19)	35 (35)	22 (23)	8 (11)	
		184	125	181	23	38	23	9	
				149 (165)	30 (27)	41 (40)	24 (24)	8 (9)	
		368	250	170	34	52	19	7	
				180 (175)	26 (30)	63 (58)	28 (24)	13 (10)	
		735	500	176	35	61	20	5	
				199 (188)	36 (36)	63 (62)	14 (17)	6 (6)	
		1470	1000	198	41	66	20	5	
				200 (199)	37 (39)	51 (59)	27 (24)	7 (6)	
		2940	2000	212	42	61	26	4	
225 (219)	33 (38)			62 (62)	25 (26)	4 (4)			
5880	4000	239-)	30-)	63-)	25-)	4-)			
		202-)	(221)	37-)	(34)	65-)	(64)	22-)	(24)
S9mix (+)	Saline	0	0	161	11	35	33	6	
				141 (151)	12 (12)	30 (33)	38 (36)	12 (9)	
	46.0	31.3	129	20	40	43	12		
			121 (125)	18 (19)	40 (40)	48 (46)	10 (11)		
	91.9	62.5	148	19	36	30	10		
			144 (146)	14 (17)	38 (37)	37 (34)	6 (8)		
	184	125	158	30	40	42	16		
			152 (155)	21 (26)	32 (36)	22 (32)	12 (14)		
	368	250	170	28	35	29	14		
			203 (187)	28 (28)	37 (36)	23 (26)	11 (13)		
	735	500	207	32	47	28	11		
			205 (206)	43 (38)	48 (48)	27 (28)	12 (12)		
	1470	1000	207	46	52	31	5		
			223 (215)	39 (43)	52 (52)	32 (32)	6 (6)		
	2940	2000	207	37	45	42	4		
233 (220)			31 (34)	55 (50)	36 (39)	4 (4)			
5880	4000	227-)	31-)	52-)	21-)	4-)			
		225-)	(226)	37-)	(34)	46-)	(49)	27-)	(24)
Positive control	S9mix(-)	Chemical	ENNG	ENNG	ENNG	2NF	9AA		
		Dose(µg/plate)	3	5	2	1	80		
		Colony No./ Plate	624	494	567	148	398		
	S9mix(+)	Chemical	2AA	2AA	2AA	2AA	2AA		
		Dose(µg/plate)	1	2	10	0.5	2		
		Colony No./ Plate	612	154	268	171	73		
		588 (600)	160 (157)	333 (301)	146 (159)	88 (81)			

<sup>a)</sup> The dose was expressed as FTD; <sup>b)</sup> Mean of 2 plates.

-), There was no cytotoxicity.

Abbreviation: ENNG, N-ethyl-N'-nitro-N-nitrosoguanidine; 2NF, 2-nitrofluorene;

9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

(Excerpted from Applicant's submission)

The results of the reverse mutagenicity test were reproduced in a confirmatory test (Table 47).

**Table 47: Results of bacterial reversion test of TAS-102 (confirmative test)**

S9 mix	Chemical	Dose (µg/plate)	Dose as FTD (µg/plate) <sup>a)</sup>	No. of Revertants (Colony number/plate)				
				Base pair substitution			Frameshift	
				TA100	TA1535	WP2uvrA	TA98	TA1537
S9mix (-)	Saline	0	0	117	11	27	20	13
				137 (127) <sup>b)</sup>	9 (10)	24 (26)	23 (22)	14 (14)
	TAS-102	46.0	31.3	121	15	41	20	13
				152 (137)	10 (13)	35 (38)	31 (26)	23 (18)
		91.9	62.5	138	20	30	20	14
				150 (144)	10 (15)	30 (30)	22 (21)	14 (14)
		184	125	163	23	41	16	20
				148 (156)	18 (21)	39 (40)	19 (18)	16 (18)
		368	250	174	29	52	15	12
				187 (181)	34 (32)	54 (53)	17 (16)	14 (13)
735	500	190	26	75	19	13		
		221 (206)	30 (28)	83 (79)	16 (18)	8 (11)		
1470	1000	219	27	77	14	14		
		193 (206)	20 (24)	85 (81)	18 (16)	9 (12)		
2940	2000	210	29	80	17	12		
		230 (220)	39 (34)	88 (84)	14 (16)	8 (10)		
5880	4000	166-)	17-)	92-)	22-)	11-)		
		224-) (195)	33-) (25)	79-) (86)	14-) (18)	6-) (9)		
S9mix (+)	Saline	0	0	131	7	39	28	19
				124 (128)	7 (7)	43 (41)	33 (31)	22 (21)
	46.0	31.3	161	17	43	29	20	
			151 (156)	14 (16)	31 (37)	35 (32)	22 (21)	
	91.9	62.5	167	13	34	24	15	
			139 (153)	20 (17)	31 (33)	21 (23)	18 (17)	
	184	125	160	20	38	25	17	
			200 (180)	18 (19)	43 (41)	35 (30)	24 (21)	
	368	250	194	21	48	18	17	
			193 (194)	17 (19)	53 (51)	27 (23)	20 (19)	
735	500	201	27	62	18	14		
		206 (204)	30 (29)	62 (62)	20 (19)	14 (14)		
1470	1000	192	35	69	21	12		
		230 (211)	33 (34)	73 (71)	17 (19)	12 (12)		
2940	2000	216	39	65	15	18		
		202 (209)	48 (44)	74 (70)	23 (19)	16 (17)		
5880	4000	216-)	29-)	59-)	31-)	14-)		
		209-) (213)	28-) (29)	48-) (54)	20-) (26)	12-) (13)		
Positive control	S9mix(-)	Chemical	ENNG	ENNG	ENNG	2NF	9AA	
		Dose(µg/plate)	3	5	2	1	80	
	Colony No./ Plate	697	842	399	152	280		
		698 (698)	843 (843)	364 (382)	155 (154)	379 (330)		
	S9mix(+)	Chemical	2AA	2AA	2AA	2AA	2AA	
		Dose(µg/plate)	1	2	10	0.5	2	
Colony No./ Plate	427	145	596	102	142			
	432 (430)	189 (167)	620 (608)	100 (101)	122 (132)			

<sup>a)</sup>, The dose was expressed as FTD; <sup>b)</sup>, Mean of 2 plates.

-), There was no cytotoxicity.

Abbreviation: ENNG, N-ethyl-N'-nitro-N-nitrosoguanidine; 2NF, 2-nitrofluorene;

9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

(Excerpted from Applicant's submission)

Given the reproducibility of the test, under the test conditions, the results suggest that TAS-102 was positive for mutagenicity.

**Study title: A Reverse Mutation Test of FTD in Bacteria**

Study no.: 06CA06  
Study report location: Electronic  
Conducting laboratory and location:  (b) (4)

Date of study initiation: March 29, 2006  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: FTD, Lot #: 056020; and Purity, 100.2%

**Key Study Findings**

- FTD was positive for mutagenicity

**Methods**

Strains: *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E.coli* WP2uvrA, without or with S9 mix  
Concentrations in definitive study: 78.1, 156, 313, 625, 1250, 2500, 5000  
Basis of concentration selection: Based on preliminary study  
Formulation/Vehicle: Physiological saline  
Incubation & sampling time: 48-49 hours incubation

The Applicant conducted the test by the preincubation method. *S typhimurium* TA98, TA100, TA1535, TA1537, and *E coli* WP2uvrA were treated with FTD in the presence and absence of metabolic activation and the number of revertants was determined. The same test was repeated twice or three times to confirm the reproducibility of the test results. For all the bacterial strains, seven dose levels of FTD, i.e., 78.1, 156,313,625, 1250, 2500, and 5000 µg/plate were selected. Physiological saline was used as the vehicle control. The commercial positive control set was used for the positive controls. According to the Applicant, the dose levels of positive control solutions were selected in accordance with the *Guidelines for Genotoxicity Studies on Drugs* established by the (Japanese) Ministry of Health and Welfare and *Mutagenicity Test on the Industrial Safety and Health Law* (Chemical Substances Investigation Division, Industrial Safety and Health Department, the Ministry of Labor: 1991).

**Study Validity**

When the mean number of revertants per plate showed a more than 2-fold increase compared with the vehicle control and a dose dependency was observed, the treatment

tested was judged to be positive. A final assessment was conducted considering the biological validity of the study conditions used.

	In the absence of metabolic activation		In the presence of metabolic activation	
TA98	AF-2	0.1 µg/plate	2AA	0.5 µg/plate
TA100	AF-2	0.01 µg/plate	2AA	1 µg/plate
TA1535	NaN3	0.5 µg/plate	2AA	2 µg/plate
TA1537	9AA	80 µg/plate	2AA	2 µg/plate
WP2uvrA	AF-2	0.01 µg/plate	2AA	10 µg/plate

## Results

As shown in Table 48, in TA100, TA1535, and WP2uvrA, the revertant colonies increased dose-dependently and were more than twice that in the vehicle control in the presence or absence of metabolic activation. In TA1537, the increase (if any) in revertant colonies was not dose-dependent in the presence or absence of metabolic activation. Although the first experiment with TA98, showed a dose-dependent increase of more than 2-fold in the number of revertant colonies relative to the vehicle control group in the presence or absence of metabolic activation, the increase was not reproduced in 2nd (Table 49) or 3rd (not shown) experiment. Given the lack of reproducibility of the increase of revertant colonies, the result of TA98 was judged as negative. The positive control group showed a more than 2-fold increase relative to each vehicle control group in the number of revertants, indicating that the study was conducted in an appropriate manner. None of the bacterial strains tested exhibited bacterial growth inhibition in the FTD treatment groups.

Under the conditions of this experiment, FTD has the potential to induce bacterial mutations.

**Table 48: Bacterial Reverse Mutation Test (1st experiment)**

Name of test article: FTD		Exp.No.: 06CA06				
Metabolic activation	Dose (µg/plate)	No. of revertants (colonies/plate)				
		Base pair substitution type			Frame shift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9mix (-)	Saline	113 97 ( 105 ) <sup>a)</sup>	6 11 ( 9 )	24 21 ( 23 )	14 9 ( 12 )	11 9 ( 10 )
	78.1	141 140 ( 141 )	25 29 ( 27 )	26 21 ( 24 )	22 13 ( 18 )	5 9 ( 7 )
	156	162 159 ( 161 )	34 34 ( 34 )	23 46 ( 35 )	7 25 ( 16 )	3 8 ( 6 )
	313	216 202 ( 209 )	29 38 ( 34 )	43 42 ( 43 )	32 25 ( 29 )	10 17 ( 14 )
	625	261 271 ( 266 )	72 50 ( 61 )	74 86 ( 80 )	29 24 ( 27 )	13 5 ( 9 )
	1250	339 376 ( 358 )	86 85 ( 86 )	135 118 ( 127 )	26 25 ( 26 )	6 10 ( 8 )
	2500	386 400 ( 393 )	90 87 ( 89 )	176 139 ( 158 )	23 25 ( 24 )	8 12 ( 10 )
	5000	370 -) 374 -) ( 372 )	80 -) 93 -) ( 87 )	166 -) 161 -) ( 164 )	16 -) 22 -) ( 19 )	4 -) 5 -) ( 5 )
	Saline	115 121 ( 118 )	13 11 ( 12 )	25 26 ( 26 )	18 18 ( 18 )	11 14 ( 13 )
	78.1	172 160 ( 166 )	27 28 ( 28 )	36 47 ( 42 )	29 28 ( 29 )	8 8 ( 8 )
	156	189 222 ( 206 )	34 46 ( 40 )	56 51 ( 54 )	26 40 ( 33 )	14 21 ( 18 )
	313	255 224 ( 240 )	48 57 ( 53 )	61 58 ( 60 )	28 45 ( 37 )	18 26 ( 22 )
	625	312 352 ( 332 )	71 79 ( 75 )	78 59 ( 69 )	40 40 ( 40 )	23 14 ( 19 )
	1250	402 388 ( 395 )	104 111 ( 108 )	78 111 ( 95 )	47 47 ( 47 )	18 17 ( 18 )
2500	450 472 ( 461 )	122 130 ( 126 )	111 95 ( 103 )	25 42 ( 34 )	11 10 ( 11 )	
5000	431 -) 481 -) ( 456 )	133 -) 117 -) ( 125 )	96 -) 97 -) ( 97 )	31 -) 37 -) ( 34 )	9 -) 11 -) ( 10 )	
Name of Positive Control		AF-2	NaN <sub>3</sub>	AF-2	AF-2	9AA
(µg/plate)		0.01	0.5	0.01	0.1	80
S9mix (-)	Colonies	625	356	162	612	598
	/plate	620 ( 623 )	389 ( 373 )	169 ( 166 )	559 ( 586 )	482 ( 540 )
Name of Positive Control		2AA	2AA	2AA	2AA	2AA
(µg/plate)		1	2	10	0.5	2
S9mix (+)	Colonies	653	202	489	231	205
	/plate	643 ( 648 )	220 ( 211 )	425 ( 457 )	210 ( 221 )	184 ( 195 )

Abbreviation: AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; NaN<sub>3</sub>, Sodium azide;

9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

<sup>a)</sup>, Mean of 2 plates; -), There was no cytotoxicity.

(Excerpted from Applicant's submission)

**Table 49: Bacterial Reverse Mutation Test (2nd experiment)**

Name of test article: FTD		Exp.No.: 06CA06				
Metabolic activation	Dose (µg/plate)	No. of revertants (colonies/plate)				
		Base pair substitution type			Frame shift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9mix (-)	Saline	88	8	20	16	13
		102 ( 95 ) <sup>a)</sup>	11 ( 10 )	18 ( 19 )	21 ( 19 )	7 ( 10 )
	78.1	124	19	32	21	7
		141 ( 133 )	26 ( 23 )	46 ( 39 )	16 ( 19 )	7 ( 7 )
	156	154	24	35	9	6
		168 ( 161 )	31 ( 28 )	47 ( 41 )	11 ( 10 )	9 ( 8 )
	313	210	50	75	18	7
		178 ( 194 )	62 ( 56 )	67 ( 71 )	32 ( 25 )	12 ( 10 )
	625	293	55	93	22	10
		274 ( 284 )	62 ( 59 )	102 ( 98 )	32 ( 27 )	13 ( 12 )
	1250	305	80	133	28	12
		315 ( 310 )	76 ( 78 )	139 ( 136 )	27 ( 28 )	8 ( 10 )
	2500	418	101	186	21	4
		391 ( 405 )	98 ( 100 )	191 ( 189 )	25 ( 23 )	6 ( 5 )
5000	345 -)	71 -)	186 -)	27 -)	6 -)	
	333 -) ( 339 )	110 -) ( 91 )	168 -) ( 177 )	23 -) ( 25 )	4 -) ( 5 )	
S9mix (+)	Saline	115	12	34	21	9
		108 ( 112 )	12 ( 12 )	30 ( 32 )	38 ( 30 )	16 ( 13 )
	78.1	147	33	41	31	12
		153 ( 150 )	24 ( 29 )	38 ( 40 )	32 ( 32 )	9 ( 11 )
	156	147	50	33	31	14
		207 ( 177 )	41 ( 46 )	69 ( 51 )	33 ( 32 )	9 ( 12 )
	313	253	65	66	33	5
		265 ( 259 )	66 ( 66 )	73 ( 70 )	39 ( 36 )	5 ( 5 )
	625	295	88	82	41	12
		322 ( 309 )	111 ( 100 )	91 ( 87 )	32 ( 37 )	7 ( 10 )
	1250	383	112	103	41	10
		375 ( 379 )	108 ( 110 )	97 ( 100 )	29 ( 35 )	11 ( 11 )
	2500	529	116	100	34	9
		469 ( 499 )	116 ( 116 )	146 ( 123 )	34 ( 34 )	18 ( 14 )
5000	489 -)	134 -)	124 -)	33 -)	9 -)	
	450 -) ( 470 )	138 -) ( 136 )	95 -) ( 110 )	29 -) ( 31 )	10 -) ( 10 )	
Name of Positive Control		AF-2	NaN <sub>3</sub>	AF-2	AF-2	9AA
(µg/plate)		0.01	0.5	0.01	0.1	80
S9mix (-)	Colonies /plate	549	452	145	530	553
		525 ( 537 )	453 ( 453 )	133 ( 139 )	567 ( 549 )	500 ( 527 )
Name of Positive Control		2AA	2AA	2AA	2AA	2AA
(µg/plate)		1	2	10	0.5	2
S9mix (+)	Colonies /plate	699	241	594	196	138
		699 ( 699 )	230 ( 236 )	614 ( 604 )	203 ( 200 )	122 ( 130 )

Abbreviation: AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide; NaN<sub>3</sub>, Sodium azide;

9AA, 9-aminoacridine; 2AA, 2-aminoanthracene.

<sup>a)</sup>, Mean of 2 plates: -), There was no cytotoxicity.

(Excerpted from Applicant's submission)

**Table 50: The historical data in bacterial reverse mutation test**

In the absence of metabolic activation					
	TA100	TA1535	TA98	TA1537	WP2 <i>uvrA</i>
Vehicle control and spontaneous control	102 ± 18.8 <sup>a1</sup>	13 ± 6.0	19 ± 5.7	13 ± 3.9	27 ± 8.0
Positive control	AF-2	NaN <sub>3</sub>	AF-2	9AA	AF-2
Dose (µg/plate)	0.01	0.5	0.1	80	0.01
Mean±S.D.	473 ± 41.9	448 ± 34.5	506 ± 81.6	506 ± 243.6	152 ± 13.7
In the presence of metabolic activation					
Chemical	TA100	TA1535	TA98	TA1537	WP2 <i>uvrA</i>
Vehicle control and spontaneous control	110 ± 18.3	14 ± 7.2	26 ± 6.1	17 ± 4.0	31 ± 8.4
Positive control	2AA	2AA	2AA	2AA	2AA
Dose (µg/plate)	1	2	0.5	2	10
Mean±S.D.	513 ± 180.4	170 ± 50.2	116 ± 60.2	124 ± 38.9	580 ± 120.4

<sup>a1</sup>, Mean±S.D.

Abbreviation: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide: AF-2, 9-aminoacridine: 9AA

9AA, 9-aminoacridine; Sodium azide, NaN<sub>3</sub>; 2-aminoanthracene, 2AA

These data were collected from 1999 in 2006

(Excerpted from Applicant's submission)

**Study title:** Bacterial Reverse Mutation Study of TPI

Study no.: B050590

Study report location: Electronic

Conducting laboratory and location:



Date of study initiation: May 31, 2005

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TPI Lot #: 970904; Purity: 99.2%

**Key Study Findings**

- TPI did not induce bacterial gene mutations

## Methods

Strains:	<i>S. typhimurium</i> TA100, TA1535, TA98, and TA1537 and <i>E. scherichia coli</i> WP2uvrA.
Concentrations in definitive study:	156, 313, 625, 1250, 2500, and 5000 µg/plate ithout or with S9 mix
Basis of concentration selection:	Based on preliminary study
Formulation/Vehicle:	Water for injection (DW)
Incubation & sampling time:	48 hours incubation

Mutagenicity of the test substance, TPI, was assessed in a bacterial reverse mutation assay using *S. typhimurium* TA100, TA98, TA1535, and TA1537 and *E. coli* WP2uvrA. The test was conducted by the pre-incubation method using TPI at concentrations of: 156, 313, 625, 1250, 2500, and 5000 µg/plate in the presence or absence of S9 mix.

The positive control substances are recommended in the Guidelines on Genotoxicity Tests of Pharmaceuticals and the selected concentrations are widely used in bacterial reverse mutation assays.

Test strain	Positive control and dose (µg/plate)			
	Without S9 mix		With S9 mix	
TA100	AF-2	0.01	2-AA	1
TA1535	NaN <sub>3</sub>	0.5	2-AA	2
TA98	AF-2	0.1	2-AA	0.5
TA1537	9-AA	80	2-AA	2
WP2uvrA	AF-2	0.01	2-AA	10

## Results

As shown in Table 51, for all test strains, there was no concentration-dependency and the number of revertant colonies in the test substance-treated groups was less than twice that in the corresponding negative (vehicle) control regardless of the presence or absence of S9 mix. The reproducibility of the preliminary test results was confirmed.

The number of revertant colonies in the negative (vehicle) control and positive control groups of the present study were within acceptable ranges of historical data at the testing facility (Table 52). The positive controls used in the assays in the presence or absence of S9 mix showed positive responses to the respective test strains, as evidenced by the number of revertant colonies being greater than 2-fold of the respective negative (vehicle) control value, thus confirming the validity of the present study.

**Table 51: Results of the Bacterial Reverse Mutation Study of TPI (Main test)**

With (+) or without (-) S9 mix	Dose level (µg / plate)	Number of revertants (number of colonies / plate)				
		Base-pair change type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9 mix (-)	Negative control	96 104 ( 100 )	12 13 ( 13 )	38 27 ( 33 )	24 16 ( 20 )	11 15 ( 13 )
	156	109 104 ( 107 )	13 15 ( 14 )	35 32 ( 34 )	19 16 ( 18 )	15 10 ( 13 )
	313	101 108 ( 105 )	15 9 ( 12 )	34 32 ( 33 )	17 24 ( 21 )	9 11 ( 10 )
	625	96 116 ( 106 )	12 13 ( 13 )	26 30 ( 28 )	17 19 ( 18 )	14 9 ( 12 )
	1250	109 108 ( 109 )	11 11 ( 11 )	26 32 ( 29 )	21 18 ( 20 )	10 13 ( 12 )
	2500	98 103 ( 101 )	10 14 ( 12 )	35 37 ( 36 )	24 18 ( 21 )	13 11 ( 12 )
	5000	104 114 ( 109 )	14 11 ( 13 )	29 35 ( 32 )	16 17 ( 17 )	15 12 ( 14 )
S9 mix (+)	Negative control	103 123 ( 113 )	12 13 ( 13 )	29 35 ( 32 )	26 33 ( 30 )	18 15 ( 17 )
	156	111 113 ( 112 )	11 12 ( 12 )	39 38 ( 39 )	23 22 ( 23 )	11 17 ( 14 )
	313	104 108 ( 106 )	13 11 ( 12 )	37 36 ( 37 )	23 23 ( 23 )	20 21 ( 21 )
	625	108 126 ( 117 )	11 11 ( 11 )	44 38 ( 41 )	30 27 ( 29 )	16 13 ( 15 )
	1250	113 101 ( 107 )	14 12 ( 13 )	30 39 ( 35 )	26 22 ( 24 )	16 20 ( 18 )
	2500	111 124 ( 118 )	13 14 ( 14 )	34 33 ( 34 )	22 24 ( 23 )	21 19 ( 20 )
	5000	118 113 ( 116 )	11 16 ( 14 )	38 38 ( 38 )	22 32 ( 27 )	21 19 ( 20 )
Positive control S9 mix (-)	Name	AF-2	NaN <sub>3</sub>	AF-2	AF-2	9-AA
	Dose (µg/plate)	0.01	0.5	0.01	0.1	80
Positive control S9 mix (+)	Name	2-AA	2-AA	2-AA	2-AA	2-AA
	Dose (µg/plate)	1	2	10	0.5	2
Positive control S9 mix (+)	Name	1437	213	929	422	191
	Number of colonies / plate	1375 ( 1406 )	209 ( 211 )	951 ( 940 )	399 ( 411 )	170 ( 181 )

(Note) Negative control: Water for injection (Mean)

Bacterial or fungous contamination was not observed in the sterility test group.  
 Microbial toxicity was not observed in any test strains regardless of the presence or absence of S9 mix.  
 Precipitation was not observed on the agar plates in any doses regardless of the presence or absence of S9 mix.

(Excerpted from Applicant's submission)

**Table 52: Historical ranges of negative (solvent) and positive control values**

## 1. Negative (solvent) control

Test strains	S9	Number of tests <sup>1</sup>	Number of revertant colonies/plate	
			Mean $\pm$ SD	Acceptable range <sup>2</sup>
TA100	S9 –	81	106 $\pm$ 9	88 – 124
	S9 +	85	114 $\pm$ 11	92 – 136
TA1535	S9 –	75	11 $\pm$ 2	7 – 15
	S9 +	75	12 $\pm$ 2	8 – 16
WP2 <sub>uvrA</sub>	S9 –	12	31 $\pm$ 6	19 – 43
	S9 +	12	36 $\pm$ 6	24 – 48
TA98	S9 –	75	19 $\pm$ 3	13 – 25
	S9 +	104	25 $\pm$ 3	19 – 31
TA1537	S9 –	75	12 $\pm$ 2	8 – 16
	S9 +	75	17 $\pm$ 3	11 – 23

## 2. Positive control

Test strains	S9	Chemical and dose ( $\mu$ g/plate)	Number of tests <sup>1</sup>	Number of revertant colonies/plate	
				Mean $\pm$ SD	Acceptable range <sup>3</sup>
TA100	S9 –	AF-2 0.01	82	638 $\pm$ 107	317 – 1276
	S9 +	2-AA 1	86	1197 $\pm$ 169	690 – 2394
TA1535	S9 –	NaN <sub>3</sub> 0.5	76	472 $\pm$ 62	286 – 944
	S9 +	2-AA 2	76	197 $\pm$ 22	131 – 394
WP2 <sub>uvrA</sub>	S9 –	AF-2 0.01	13	222 $\pm$ 43	93 – 444
	S9 +	2-AA 10	13	1475 $\pm$ 256	707 – 2950
TA98	S9 –	AF-2 0.1	76	671 $\pm$ 69	464 – 1342
	S9 +	2-AA 0.5	105	368 $\pm$ 45	233 – 736
TA1537	S9 –	9-AA 80	76	224 $\pm$ 65	29 – 448
	S9 +	2-AA 2	76	192 $\pm$ 25	117 – 384

1. Data from Jan. 9, 2004 to Dec. 9, 2004 in our laboratory.

2. Mean $\pm$ 2SD.

3. The minimum value is mean–3SD, and the maximum value is larger one, mean+3SD or double the mean number.

(Excerpted from Applicant's submission)

Thus, under the conditions of this study, TPI did not induce bacterial gene mutations.

## 7.2 *In Vitro* Assays in Mammalian Cells

### Study title: Chromosomal aberration test on TAS-102 in CHL cells

Study no.: 00CA12

Study report location: Electronic

Conducting laboratory and location: (b) (4)

(b) (4)

Date of study initiation: June 21, 2000  
 GLP compliance: Yes  
 QA statement: Yes  
 Drug, lot #, and % purity: TAS-102 (combined form of FTD; Lot #: 807010, Purity: 100% and TPI; Lot #: 970904, Purity: 99.2%, Molar ratio of 1:0.5)

### Key Study Findings

- Significant structural aberrations were observed in CHL cells treated with varying concentrations of TAS-102 without or with S9 mix compared to vehicle control.
- TAS-102 was positive for clastogenicity in vitro

### Methods

Cell line: Chinese Hamster Lung (CHL)  
 Concentrations in definitive study: 34.0, 68.0, 136, 272, 544, 1088, 2175, and 4350 µg/mL (23.1, 46.3, 92.5, 185, 370, 740, 1480, and 2960 µg/mL as the amount of FTD) short treatment without or with S9 mix  
 0.0230, 0.0461, 0.0922, 0.184, 0.369, 0.738, 1.48, and 2.95 µg/mL (0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 µg/mL as the amount of FTD) 24-hour and 48-hour continuous treatment  
 Basis of concentration selection: Preliminary study  
 Positive control: Mitomycin C (MMC) without S9  
 Cyclophosphamide (CPA) with S9 mix  
 Formulation/Vehicle: MMC: Water for injection; CPA; Physiological solution  
 Incubation & sampling time: Short: 6 hours

The chromosomal aberration test of TAS-102 using mammalian (CHL) cells in culture was performed using a short treatment method (6-hour treatment) to examine the clastogenicity of TAS-102.

### Results

Results in Table 53 show that following a short treatment of CHL cells with TAS-102 in the absence of S9 mix, the incidence of structural aberration was significantly increased in the groups treated with TAS-102 at 68.0 to 544 µg/mL, compared with the vehicle

control group (0.0%). Similarly, following short treatment with TAS-102 at concentrations  $\geq 544 \mu\text{g/mL}$  in the presence of S9 mix, the incidence of structural aberration was significantly increased compared with the vehicle control group (0.0%). Given the statistically significant increase in number of aberrant cells following short treatment with TAS-102, the Applicant did not conduct further observations of chromosomal specimens following longer treatment.

The incidence of polyploid cells ranged from 0.0% to 2.0% following short treatment with TAS-102 in the presence and absence of S9 mix which was not statistically significant compared to the vehicle control group (0.5%). Chromosomal structural aberration increased markedly in the MMC treated group without S9 mix and in the CPA treated group with S9 mix, indicating that the present study was performed appropriately.

**Table 53: Chromosomal aberration test on TAS-102 in CHL cell**

Chemical	Dose ( $\mu\text{g/mL}$ )	Dose as FTD ( $\mu\text{g/mL}$ ) <sup>a)</sup>	S9 mix	Treatment time <sup>b)</sup>	No. of cells observed	Aberrant cell Number						TA (%)	TAG (%)	Poly-ploid(%)
						gap	ctb	cte	csb	cse	frg			
Saline	0	0	-	6(18)	200	0	0	0	0	0	0	0.0	0.0	0.5
TAS-102	68.0	46.3	-	6(18)	200	6	41	31	1	0	4	27.5*	29.0	0.5
TAS-102	136	92.5	-	6(18)	200	6	53	36	1	0	7	34.5*	35.0	0.0
TAS-102	272	185	-	6(18)	200	7	67	36	2	0	22	47.5*	48.5	1.0
TAS-102	544	370	-	6(18)	200	2	53	35	1	0	15	36.5*	37.0	0.5
MMC	0.1	-	-	6(18)	200	1	31	125	1	0	0	68.0*	68.0	0.5
Saline	0	0	+	6(18)	200	0	0	0	0	0	0	0.0	0.0	0.5
TAS-102	544	370	+	6(18)	200	3	9	10	0	1	3	9.0*	10.0	2.0
TAS-102	1088	740	+	6(18)	200	1	11	11	0	0	1	8.0*	8.5	0.5
TAS-102	2175	1480	+	6(18)	200	3	6	8	0	0	0	5.0*	6.5	1.0
TAS-102	4350	2960	+	6(18)	200	2	14	10	0	0	5	11.5*	12.0	1.0
CPA	10	-	+	6(18)	200	1	15	134	0	0	1	69.0*	69.0	0.0

Abbreviation: ctb, chromatid break; cte, chromatid exchange; csb, chromosomal break; cse, chromosomal exchange;

frg, fragmentation; TAG, total cells which have chromosomal aberrants including gap;

TA, total cells which have chromosomal aberrants excluding gap; MMC, mitomycin C; CPA, cyclophosphamide monohydrate.

<sup>a)</sup>Dose was calculate as FTD <sup>b)</sup>Treatment time (expression time)

\* Significant difference from the vehicle control (Fisher's exact test,  $p < 0.05$ ).

(Excerpted from Applicant's submission)

The cell density was  $\geq 76.0\%$  in the groups treated with TAS-102 at  $34.0 \mu\text{g/mL}$  to  $4350 \mu\text{g/mL}$  ( $23.1 \mu\text{g/mL}$  to  $2960 \mu\text{g/mL}$  as the amount of FTD) compared with the vehicle control group in short treatment without or with S9 mix (Table 54).

**Table 54: Effect of TAS-102 on cell growth in CHL cells**

Short treatment without S9mix.							Continuous treatment (24hrs)						
Chemical	Dose ( $\mu\text{g/mL}$ )	Dose as FTD ( $\mu\text{g/mL}$ ) <sup>a)</sup>	S9mix	Relative cell density(%) <sup>b)</sup>			Chemical ( $\mu\text{g/mL}$ )	Dose as FTD ( $\mu\text{g/mL}$ ) <sup>a)</sup>	Treatment Time	Relative cell density(%) <sup>b)</sup>			
				Dish 1	Dish 2	Mean				Dish 1	Dish 2	Mean	
Saline	0	0	-	100.0	100.0	100.0	Saline	0	0	24	100.0	100.0	100.0
TAS-102	34.0	23.1	-	92.0	91.0	91.5	TAS-102	0.023	0.0156	24	98.0	100.0	99.0
	68.0	46.3	-	95.0	102.0	98.5		0.0461	0.0313	24	96.0	90.0	93.0
	136	92.5	-	92.0	90.0	91.0		0.0922	0.0625	24	85.0	92.0	88.5
	272	185	-	84.0	75.0	79.5		0.184	0.125	24	81.0	91.0	86.0
	544	370	-	81.0	71.0	76.0		0.369	0.25	24	85.0	84.0	84.5
	1088	740	-	95.0	92.0	93.5		0.738	0.5	24	81.0	84.0	82.5
	2175	1480	-	93.0	86.0	89.5		1.48	1.0	24	75.0	71.0	73.0
	4350	2960	-	74.0	80.0	77.0		2.95	2.0	24	75.0	73.0	74.0

Short treatment with S9mix.							Continuous treatment (48hrs)						
Chemical	Dose ( $\mu\text{g/mL}$ )	Dose as FTD ( $\mu\text{g/mL}$ ) <sup>a)</sup>	S9mix	Relative cell density(%) <sup>b)</sup>			Chemical ( $\mu\text{g/mL}$ )	Dose as FTD ( $\mu\text{g/mL}$ ) <sup>a)</sup>	Treatment Time	Relative cell density(%) <sup>b)</sup>			
				Dish 1	Dish 2	Mean				Dish 1	Dish 2	Mean	
Saline	0	0	+	100.0	100.0	100.0	Saline	0	0	48	100.0	100.0	100.0
TAS-102	34.0	23.1	+	101.0	95.0	98.0	TAS-102	0.023	0.0156	48	114.0	110.0	112.0
	68.0	46.3	+	105.0	98.0	101.5		0.0461	0.0313	48	102.0	96.0	99.0
	136	92.5	+	103.0	88.0	95.5		0.0922	0.0625	48	103.0	100.0	101.5
	272	185	+	105.0	104.0	104.5		0.184	0.125	48	98.0	91.0	94.5
	544	370	+	94.0	79.0	86.5		0.369	0.25	48	82.0	78.0	80.0
	1088	740	+	89.0	95.0	92.0		0.738	0.5	48	72.0	71.0	71.5
	2175	1480	+	96.0	93.0	94.5		1.48	1.0	48	57.0	60.0	58.5
	4350	2960	+	95.0	87.0	91.0		2.95	2.0	48	57.0	58.0	57.5

<sup>a)</sup> Dose was calculate as FTD

<sup>b)</sup> The rate of cell density treated with TAS-102 compared with that treated with saline.

(Excerpted from Applicant's submission)

These results suggest that under the test conditions, TAS-102 was positive for clastogenicity in vitro.

### In Vitro Assays of FTD or TPI in Mammalian Cells

Results of tests for the clastogenicity of FTD using CHL/IU cells treated with 46.3, 92.5, 185, 370, 740, 1480, and 2960  $\mu\text{g/mL}$  of FTD for 6 hours (short-term treatment) in the presence and absence of S9 mix suggest that FTD is clastogenic.

An in vitro chromosomal aberration study of TPI (700, 1400, and 2800  $\mu\text{g/mL}$ ) using CHL/IU cells, did not show a significant increase in the incidence of chromosomally aberrant cells compared with the vehicle control value under any treatment conditions. Thus, under the conditions employed in this study TPI does not to have the ability to induce chromosomal aberrations.

### 7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

#### Study title: Micronucleus test of TAS-102

Study no: 01CA15

Study report location: Electronic

Conducting laboratory and location:



Date of study initiation: July 02, 2001

GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: TAS-102 (combined form of FTD; Lot #: 807010, Purity: 100% and TPI; Lot #: 970904, Purity: 99.2%, Molar ratio of 1:0.5)

## Key Study Findings

- TAS-102 was positive in in vivo micronucleus test

## Methods

Doses in definitive study: 74.1, 222, 667 and 2000 mg/kg (50.4, 151, 453 and 1360 mg/kg, respectively, as FTD).  
Frequency of dosing: Single administration  
Route of administration: Oral gavage  
Dose volume: 10 mL/kg  
Formulation/Vehicle: HPMC solution  
Species/Strain: ddY mice  
Number/Sex/Group: 6/group  
Basis of dose selection: Based preliminary study  
Negative control: HPMC solution  
Positive control: Mitomycin (MMC; 1 mg/kg)

TAS-102 was administered orally once in the morning to 8-week old male mice.

## Study Validity

The sampling time in the main test was set at 24 hours after dosing, based on observations from preliminary study showing that the frequency of micronucleated peripheral blood cells was highest at 48 hours after dosing in all TAS-102 groups. The sampling time in the positive control group was set at 24 hours after the single dose based on the data of the testing laboratory. The dosing time was designated as time 0 in all groups. The frequency of micronucleated polychromatic erythrocytes (MNPCE) was determined by screening 2000 total polychromatic erythrocytes (total PCE) per animal. A positive response was defined as a significant increase in the frequency of MNPCE as compared with that in the vehicle control group at a 5% level of significance.

## Results

In the groups treated with TAS-102 (74.1, 222, 667, and 2000 mg/kg), the frequencies of MNPCEs increased dose-dependently (0.66, 0.71, 1.93 and 1.83%, respectively), compared with the frequency of MNPCE in the vehicle control group (0.14%). Administration of TAS-102 had no remarkable effect on body weight in any of the groups, compared with the vehicle treated group (Table 55). There were no mortalities at any dose level.

In the positive control group (treated with MMC, 1 mg/kg) the frequency of MNPCE increased significantly confirming that the test was performed appropriately.

**Table 55: The frequency of MNPCE induced by TAS-102 in ddY mice.**

Chemical	Dose as TAS-102 (mg/kg)	Dose as FTD (mg/kg)	Number of animals	Frequency of MNPCE (%) (Mean ± S.D.)	Total PCE/ total RBC (%) (Mean ± S.D.)	Body weight gain (g) <sup>a</sup> (Mean ± S.D.)
HPMC	0	0	6	0.14 ± 0.06	67.6 ± 9.3	-0.1 ± 0.3
TAS-102	74.1	50.4	6	0.66 ± 0.33*	67.8 ± 5.3	-0.1 ± 0.6
	222	151	6	0.71 ± 0.21*	67.6 ± 6.7	0.5 ± 0.6
	667	453	6	1.93 ± 0.86*	61.4 ± 8.1	0.0 ± 0.6
	2000	1360	6	1.83 ± 0.34*	63.0 ± 6.2	-0.4 ± 0.6
MMC	1.0 <sup>b</sup>	-	6	3.02 ± 0.68*	64.9 ± 3.7	-0.3 ± 0.5

Abbreviation: PCE, polychromatic erythrocyte; MNPCE, PCE have micronuclei; RBC, red blood cell; HPMC, hydroxypropylmethylcellulose; MMC, mitomycin C.

\*, There was significant difference from solvent control group ( $p < 0.05$ , conditional binomial test)

<sup>a</sup>, The body weight gain from before administration to sacrifice; <sup>b</sup>, Dose of mitomycin C

HPMC = 5 mg/mL HPMC solution (solvent control), MMC = positive control.

(Excerpted from Applicant's submission)

The result of this study suggests that TAS-102 has the micronucleus-inducing potential in vivo.

#### Micronucleus test of FTD or TPI in Mice

FTD was administered orally once in the morning to 8-week old male mice at doses of 10, 100, 500, and 1000 mg/kg, under similar conditions as described for TAS-102 above. The frequencies of MNPCEs increased dose dependently (0.2, 0.7, 1.7, and 2.0% at FTD doses of 10, 100, 500, and 1000 mg/kg, respectively). There was a significant increase in MNPCEs in the positive control group, indicating that the test was performed appropriately.

There were no FTD-related changes in body weight and no mortalities during the study.

The result of this study suggests that FTD has micronucleus-inducing potential in mice.

The ability of TPI to induce micronucleated cells in the bone marrow was assayed in an in vivo micronucleus test with male ICR mice. TPI was administered orally by gavage at doses of 500, 1000, and 2000 mg/kg once daily for two consecutive days. HPMC solution (0.5%) was administered orally as negative control while cyclophosphamide monohydrate (40 mg/kg) was administered intraperitoneally once.

No significant increases in the incidence of MNPCEs were observed when the groups treated with TPI were compared with the negative control group. The individual incidences of MNPCEs in the negative control group were within the range of the historical data in the testing facility. A significant increase in the incidence of MNPCEs was observed in the positive control group, indicating the validity of the present study.

Under the conditions employed in the present study, TPI did not induce micronucleated erythrocytes in mouse bone marrow.

## 8 Carcinogenicity

The Applicant did not conduct carcinogenicity studies of TAS-102, citing ICH S9, which states that *Carcinogenicity studies are not warranted to support marketing for therapeutics intended to treat patients with advanced cancer*, as justification.

### 9.1 Fertility and Early Embryonic Development

**Study title:** Study of fertility and early embryonic development to implantation in rats treated orally with TAS-102- Administration to males

Study no.: R-908

Study report location: Electronic

Conducting laboratory and location:

 (b) (4)

Date of study initiation: 08/27/2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TAS-102 (combination of FTD and TPI 1:0.5), Lot # (FTD, 202016, Purity 100.1%; TPI, 030317, Purity 99.5%)

#### Key Study Findings

Administration of TAS-102 up to 662 mg/kg daily for 14 days before and during mating did not appear to have a remarkable effect on male fertility

#### Methods

Doses: 0, 74, 221, 662 mg/kg

Frequency of dosing: Once daily x2 weeks before mating, throughout the mating period

Route of administration: Oral

Formulation/Vehicle: 0.5% (w/v) Hydroxypropylmethylcellulose

Species/Strain: Sprague-Dawley rats/SPF

Number/Sex/Group: 20/group

## Study design:

Test group	Sex	Dose level (mg/kg)			Dose Volume (mL/kg)	N
		TAS-102	FTD	TPI		
Control	M	0	0	0	10	20
	F	-	-	-	-	20
Low Dose	M	74	50	24	10	20
	F	-	-	-	-	20
Mid Dose	M	221	150	71	10	20
	F	-	-	-	-	20
High Dose	M	662	450	212	10	20
	F	-	-	-	-	20

**Observations and Results****Mortality:**

There no mortalities during the study.

**Clinical Signs**

There were no deaths or treatment-related changes in the general condition of the animals compared to controls.

**Body Weight**

Administration of TAS-102 to male rats during the pre-mating period resulted in decreases in body weight gain of  $\leq 5\%$  at the 221 mg/kg dose level, compared to controls on Days 11 (432 g) to 15 (449 g) and 10-15% at the 661 mg/kg dose level, compared to controls from Day 8 (418 g).

**Feed Consumption**

Decrease in food consumption during the pre-mating period was  $\leq 10\%$  at  $\leq 221$  mg/kg and 15-26% at the 662 mg/kg dose level on Days 2-11, compared to controls. On Day 15, food consumption was similar to that in controls.

**Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)**

Administration of TAS-102 up to 662 mg/kg daily for 14 days before mating did not appear to have effect on time to copulation, copulation index or conception rate (Table 56), suggesting that TAS-102 up to 662 mg/kg had no remarkable effect on male fertility.

**Table 56: Mating and fertility of male rats**

Dose mg/kg	No. of males	Days until copulation Mean±S.D.	Copulation index (%) a)	Conception rate (%) b)
0	20	2.2±1.2	18/20( 90.0)	16/18( 88.9)
74	20	2.9±2.8	20/20(100.0)	20/20(100.0)
221	20	2.0±1.0	20/20(100.0)	20/20(100.0)
662	20	1.9±0.9	19/20( 95.0)	17/19( 89.5)

a): (No. of copulated animals / No. of mated animals) X 100

b): (No. of pregnant animals / No. of copulated animals) X 100

No significant difference in any treated groups from control group.

(Excerpted from Applicant's submission)

Mating untreated females with males treated with TAS-102 up to 662 mg/kg for 2 weeks prior to and during mating did not appear to affect implantation index (Table 57).

Although there was a decreased embryo viability index at the 662 mg/kg dose level, the decrease was not significantly different from the control value.

**Table 57: Findings at the Middle of Gestation in Dams**

Dose	Number of			Implantation index (%*)	Number of embryos		Embryo viability index (%**)
	Dams	Corpora lutea	Implantations		Dead	Live	
0	16	264	261	98.9	12	249	95.3
74	20	326	312	93.8	9	303	97.3
221	20	328	318	97.1	18	300	94.2
662	17	273	267	97.9	20	247	92.5

\*(Number of implantations/Number of corpora lutea) X 100

\*\* (Number of live embryos/Number of implantations) X 100

The results of this study suggest that treatment of male rats with TAS-102 up to 662 mg/kg for 2 weeks prior to and during mating did not have a remarkable effect on implantation or embryo viability.

## Fertility and Early Embryonic Development

**Study title:** Study of Fertility and Early Embryonic Development to Implantation in Rats Treated Orally With TAS-102-Administration to Females

Study no.: R-904

Study report location: Electronic

Conducting laboratory and location:

(b) (4)

Date of study initiation: June 18, 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TAS-102 (combination of FTD and TPI 1:0.5), Lot # (FTD, 202016, Purity 100.1%; TPI, 030317, Purity 99.5%)

## Key Study Findings

Administration of TAS-102 up to 221 mg/kg daily for 14 days to female rats before mating with untreated males did not appear to have effect on female fertility.

## Methods

Doses: 0, 22, 74, 221 mg/kg

Frequency of dosing: Once daily for 2 weeks pre-mating, during mating and up to 7 days of gestation.

Dose volume: 10 mL/kg

Route of administration: Oral

Formulation/Vehicle: 0.5% (w/v) hydroxypropylmethylcellulose solution

Species/Strain: Sprague-Dawley rats

Satellite groups:

Study design:

Test group	Sex	Dose level (mg/kg)			Dose Volume (mL/kg)	N
		TAS-102	FTD	TPI		
Control	M	-	-	-	-	20
	F	0	0	0	10	20
Low Dose	M	-	-	-	-	20
	F	22	15	7	10	20
Mid Dose	M	-	-	-	-	20
	F	74	50	24	10	20
High Dose	M	-	-	-	-	20
	F	221	150	71	10	20

Female rats were administered TAS-102 daily for 2 weeks, following which each female was paired with an untreated male for not more than 2 weeks. Copulation was confirmed by the presence of vaginal plugs or sperm in the vaginal smear the following morning. The starting day of mating was designated as Day 0 and the number of days until copulation was counted.

## Observations and Results

### Mortality

There were mortalities in the treated females

### Clinical Signs

No abnormalities were reported during treatment.

### Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Administration of TAS-102 up to 221 mg/kg daily for 14 days to female rats before mating did not affect time to copulation, copulation index, or conception rate (Table 58), suggesting that TAS-102 up to 221 mg/kg had no remarkable effect on female fertility.

**Table 58: Mating and Fertility of Female Rats**

Dose mg/kg	No. of females	Days until copulation Mean±S.D.	Copulation index (%) a)	Conception rate (%) b)
0	20	3.0±2.7	20/20(100.0)	19/20( 95.0)
22	20	3.0±2.9	20/20(100.0)	19/20( 95.0)
74	20	2.9±1.2	20/20(100.0)	20/20(100.0)
221	20	2.3±1.1	20/20(100.0)	20/20(100.0)

a): (No. of copulated animals / No. of mated animals) × 100

b): (No. of pregnant animals / No. of copulated animals) × 100

No significant difference in any treated groups from control group.

(Excerpted from Applicant's submission)

Corpora lutea and implantations increased in a dose related manner and there was no difference in the implantation index. At the 221 mg/kg dose level of TAS-102 a high number of dead embryos was reported, reducing the embryo viability index to 76% (Table 59), suggesting that continued administration of TAS-102 during first two weeks of gestation resulted embryo lethality. Whereas the 221 mg/kg dose level is 3-fold higher than 74 mg/kg, the embryo lethality at the 221 dose level was more than 6-fold that at 74 mg/kg.

**Table 59: Findings at the Middle of Gestation in Dams treated with TAS-102**

Dose	Number of			Implantation index (%*)	Number of embryos		Embryo viability index (%**)
	Dams	Corpora lutea	Implantations		Dead	Live	
0	19	315	296	94.5	18	278	93.2
22	19	333	317	95.5	20	297	93.5
74	20	344	325	94.9	13	312	96.2
221	20	376	367	97.7	88	279	76.3

\* (Number of implantations/Number of corpora lutea) X 100

\*\* (Number of live embryos/Number of implantations) X 100

## 9.2 Embryonic Fetal Development

**Study title:** Reproductive and Developmental Toxicity Study of TAS-102 Study for Effects on Embryo-Fetal Development in Rats by Oral Administration

Study no.: 04CA18

Study report location: electronic

Conducting laboratory and location:

(b) (4)

Date of study initiation: July 30, 2004

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: TAS-102 (combination of FTD and TPI 1:0.5), Lot # (FTD, 202016, Purity 100.1%; TPI, 030317, Purity 99.5%)

Rats were administered vehicle or TAS-102 by oral gavage for 11 days from Gestation Days 7 through 17 (period from implantation until hard palate closure) and underwent caesarean section on Gestation Day 21 to determine the effects of TAS-102 on dams and embryo-fetal development.

### Key Study Findings

- Increases in embryo-fetal lethality at 221 mg/kg with exposures similar to clinical exposure (human exposure at 35 mg/m<sup>2</sup> BID: 23697 ng•h/mL; rat exposure 22400 ng•h/mL),
- Decreased fetal weights, delayed ossification, and skeletal abnormalities including vertebrae, sternebrae and ribs.

### Methods

Doses: 0, 22, 74, 221 mg/kg

Frequency of dosing: Once daily x11 days from Gestation Day 7 to Day 17

Dose volume: 5 mL/kg bw

Route of administration: Oral gavage  
 Formulation/Vehicle: 0.5% (w/v) hydroxypropylmethylcellulose solution  
 Species/Strain: Sprague-Dawley rats

Study design:

Test Group	Dose Level TAS-102 (FTD+TPI) (mg/kg)	Dose volume (mL/kg)	Sex	N
Control	0	5	F	22
Low	22 (17+5)	5	F	22
Mid	74 (50+24)	5	F	22
High	221 (150+71)	5	F	22

Twenty-two female rats were confirmed to have copulated in each group. On Gestation Day 21 necropsy was performed to confirm whether each female rat was pregnant (Table 60). For all female rats confirmed to be pregnant, corpus luteum count and implantation count were determined and the uterus was sectioned to determine the sites and number of live fetuses or dead embryos and fetuses.

**Table 60: Pregnant and Non-pregnant Rats**

Test Group	Dose Level TAS-102 (FTD+TPI) (mg/kg)	Dose volume (mL/kg)	Sex	N	
				Preg	Nonpreg
Control	0	5	F	21	1
Low	22 (17+5)	5	F	20	2
Mid	74 (50+24)	5	F	21	1
High	221 (150+71)	5	F	20	2

Preg – Pregnant; Nonpreg – nonpregnant

## Observations and Results

### Mortality

Administration of TAS-102 did not result in mortalities at any dose level during the study.

### Clinical Signs

There were no remarkable clinical signs at TAS-102  $\leq$ 74 mg/kg dose levels. At the 221 mg/kg dose level, one dead fetus, expelled from one dam (animal #0467), was found on the tray for fecal and urine collection on the morning of Gestation Day 21.

### Body Weight

Remarkable time-dependent loss in body weight gain was seen from gestation Day 15 (Day 8 of treatment) to Day 21 in the 221 mg/kg dose level of TAS-102 compared to the control group. Loss in body weight gain did not correlate with decrease in food

consumption. There were no remarkable changes in body weights at the 22 and 74 dose levels of TAS-102 compared to the control group.

**Table 61: Decrease in Body Weight Gain at 221 mg/kg TAS-102 Dose Level**

Gestation Day	Control Body Weight (g)	221 mg/kg TAS-102
13	327	↓3%
15	340	↓6%
17	364	↓10%
19	397	↓15%
21	427	↓19%

### Feed Consumption

Remarkable changes in food consumption were observed from Gestation Day 9 (treatment Day 2) through Gestation Day 19 in the 221 mg/kg dose level of TAS-102 compared to the control group. Decrease in food consumption did not correlate with loss in body weight gain. There were no remarkable changes in food consumption at the 22 and 74 dose levels of TAS-102 compared to the control group.

**Table 62: Decrease Food consumption at 221 mg/kg TAS-102 Dose Level**

Gestation Day	Control Food Consumption (g)	221 mg/kg TAS-102
7	27	↓2%
9	27	↓6%
11	27	↓9%
13	28	↓10%
15	27	↓11%
17	29	↓14%
19	30	↓13%
21	27	↓4%

### Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

As seen in Table 63, there was a reduction in the number of live fetuses from the female rats at the 22 and 221 mg/kg dose levels of TAS-102, compared to the control group. It is unclear if the reduction in the number of live fetuses at the 22 mg/kg dose level is treatment-related, as there was no reduction at the 74 mg/kg dose level and the Applicant did not provide historical control data. The increase in post-implantation loss at the 221 mg/kg dose level does appear to be treatment-related. Treatment with TAS-102 resulted in lower weights of male and female live fetuses at the 74 and 221 mg/kg dose levels.

**Table 63: Summary of Caesarean Section Data (F0)**

Compound Dosage(mg/kg)	Control	TAS-102		
		22	74	221
No. of Dams Examined	21	20	21	19
No. of Corpora Lutea	331	309	351	302
Mean ± SD	15.8±1.6	15.5±2.9	16.7±2.6	15.9±1.7
No. of Implantation Sites	303	253	304	283
Mean ± SD	14.4±2.1	12.7±3.4	14.5±2.4	14.9±1.7
Implantation Rate(%) (Mean±SD)	91.6±10.2	81.8±18.1	87.6±14.6	93.9±6.5
Pre Implantation Loss(%) (Mean±SD)	8.4±10.2	18.2±18.1	12.4±14.6	6.1±6.5
Post Implantation Loss(%) (Mean±SD)	1.9±4.8	6.8±9.3	2.7±3.6	53.7±24.7**
No. of Dead-Resorbed Fetuses	5	19	9	152
Metrial Glands	4	12	8	80
Placental Remnants	1	6	1	70
Early	0	1	0	1
Late	0	0	0	0
Macerated	0	0	0	1
Dead	0	0	0	0
No. of Live Fetuses	298	234	295	131
Mean ± SD	14.2±2.3	11.7±3.1*	14.0±2.2	6.9±3.7**
Male/Female	151/147	104/130	148/147	78/53
Sex Ratio	1.03	0.80	1.01	1.47
Body Weight of Male (Mean±SD)	5.66±0.36	5.67±0.47	5.21±0.23**	3.24±0.39**
Female(Mean±SD)	5.40±0.34	5.33±0.42	4.93±0.21**	3.10±0.45**

(Excerpted from Applicant's submission)

**Offspring (Malformations, Variations, etc.)****Table 64: Summary of External Anomalies of Live Fetuses (F1)**

Anomaly	TAS-102 (mg/kg)			
	0	22	74	221
Number of fetuses examined	298 (21)	234 (20)	295 (21)	131 (17)
Number of fetuses with anomalies	2 [0.7%] (2)	2 [0.9%] (2)	0	44 [33.6%] (13)
Anasarca	0	0	0	2 [1.5%] (2)
Cleft palate	0	0	0	2 [1.5%] (1)
Ectrodactyly	0	0	0	2 [1.5%] (2)
Kinked tail	0	0	0	38 [29.0%] (11)

Value in square brackets [y.z%] is the percentage of fetuses with anomalies relative to the corresponding number of fetuses examined

Value in parenthesis (x) is number of litters

Kinked tails were the major abnormality in the embryos.

**Table 65: Summary of Visceral Anomalies of Live Fetuses (F1)**

	TAS-102 (mg/kg)			
	0	22	74	221
Number of fetuses examined	150(21)	118(20)	147(21)	67(17)
Number of fetuses with anomalies	7[4.7%](5)	6[5.1%](5)	10[6.8%](6)	36[53.7%](15)

	TAS-102 (mg/kg)			
	0	22	74	221
Malpositioned thymus (persistence in the neck)	1[0.7%](1)	0	1[0.7%](1)	5[7.5%](4)
Membranous ventricular septal defect	2[1.3%](1)	4[3.4%](3)	1[0.7%](1)	6[9.9%](5)
Interrupted aortic arch	0	0	0	2[3.0%](2)
Malpositioned subclavian branch	0	0	2[1.4%](2)	9[13.4%](4)
Retroesophageal subclavian	0	0	1[0.7%](1)	3[4.5%](3)
Supernumerary lung lobe	0	0	0	3[4.5%](2)
Convoluted ureter	3[2.0%](3)	2[1.7%](2)	6[4.1%](4)	4[6.0%](4)
Dilated ureter	0	0	0	2[3.0%](2)
Left umbilical artery				7[10.4%](5)

Value in square brackets [y.z%] is the percentage of fetuses with anomalies relative to the corresponding number of fetuses examined

Value in parenthesis (x) is number of litters

**Table 66: Summary of Skeletal Anomalies of Live Fetuses (F1)**

Drug Dose (mg/kg)	TAS-102 (mg/kg)			
	0	22	74	221
Number of fetuses examined	148(21)	116(20)	148(21)	64(17)
Number of fetuses with anomalies	16[10.8%](8)	16[13.8%](8)	44[29.7%](15)	61[95.3%](17)
Incomplete ossification of interparietal bone	0	0	1[0.7%](1)	2[3.1%](2)
Incomplete ossification of supraoccipital bone	0	0	1[0.7%](1)	2[3.1%](2)
<b>Sternebra</b>				
Bipartite ossification	0	0	0	21[32.8%](11)
Misaligned	0	1[0.9%](1)	1[0.7%](1)	7[10.9%](6)
Sternoschiasis	0	1[0.9%](1)	0	3[4.7%](2)
<b>Rib</b>				
Branched rib cartilage	0	0	0	2[3.1%](2)
Cervical	0	0	0	2[3.1%](2)
Full supernumerary	0	1[0.9%](1)	4[2.7%](2)	17[26.6%](13)
Fused	0	0	0	3[4.7%](3)
Fused rib cartilage	0	0	0	5[7.8%](4)
Short rib	0	1[0.9%](1)	0	4[6.3%](3)
Short supernumerary rib	14[9.5%](8)	10[8.6%](4)	37[25%](13)	31[48.4%](16)
<b>Cervical arch</b>				
Absent	0	0	0	2[3.1%](2)
Fused	0	0	0	11[17.2%](7)
Incomplete ossification of	0	0	0	9[14.1%](7)
Unossified	0	0	0	2[3.1%](1)
<b>Cervical centrum</b>				
Fused	0	0	0	7[10.9%](2)
Split cartilage of	0	1[0.9%](1)	0	5[7.8%](2)
<b>Thoracic centrum</b>				
Bipartite ossification of	2[1.4%](1)	0	3[2%](2)	12[18.8%](8)
Dumbbell ossification of	0	1[0.9%](1)	1[0.7%](1)	4[6.3%](2)
Incomplete ossification of	0	0	0	3[4.7%](3)

Drug Dose (mg/kg)	TAS-102 (mg/kg)			
	0	22	74	221
Split cartilage of	0	0	0	2[3.1%](2)
Supernumerary thoracic vertebra	0	1[0.9%](1)	4[2.7%](2)	18[28.1%](13)
Misshapen lumbar arch	0	0	0	2[3.1%](1)
Supernumerary lumbar vertebra	0	0	0	5[7.8%](4)
Misshapen sacral arch	0	1[0.9%](1)	0	3[4.7%](3)
<b>Caudal centrum</b>				
Fused	0	0	0	3[4.7%](3)
Misaligned	0	0	1[0.7%](1)	14[21.9%](9)
Misshapen	0	0	0	33[51.6%](14)

Table 67: Summary of Ossification of Live Fetuses (F1)

Compound Dosage (mg/kg)	Control	TAS-102		
		22	74	221
<b>No. of Vertebrae</b>				
Cervical	7.0±0.0 [148]	7.0±0.0 [116]	7.0±0.0 [148]	7.0±0.1 [ 64]
Thoracic	13.0±0.0 [148]	13.0±0.0 [116]	13.0±0.1 [148]	13.4±0.3 [ 64]**
Lumbar	6.0±0.0 [148]	6.0±0.2 [116]	6.0±0.0 [148]	6.0±0.4 [ 64]*
Sacro-Coccygeal	10.3±0.7 [148]	10.2±0.9 [116]	10.1±0.4 [148]	6.1±1.4 [ 64]**
<b>No. of Ribs</b>				
Right	13.0±0.0 [148]	13.0±0.0 [116]	13.0±0.1 [148]	13.2±0.3 [ 64]**
Left	13.0±0.0 [148]	13.0±0.0 [116]	13.0±0.1 [148]	13.3±0.3 [ 64]**
<b>No. of Sternebrae</b>	6.0±0.1 [148]	5.9±0.2 [116]	6.0±0.1 [148]	3.9±0.9 [ 64]**
<b>No. of Metacarpal</b>				
Right	4.0±0.0 [148]	4.0±0.1 [116]	4.0±0.0 [148]	3.7±0.4 [ 64]**
Left	4.0±0.0 [148]	4.0±0.0 [116]	4.0±0.0 [148]	3.6±0.4 [ 64]**
<b>No. of Metatarsal</b>				
Right	4.9±0.2 [148]	4.8±0.2 [116]	4.7±0.2 [148]	3.9±0.2 [ 64]**
Left	4.9±0.2 [148]	4.8±0.2 [116]	4.8±0.2 [148]	3.8±0.6 [ 64]**
<b>No. of Carpal Proximal Phalanges</b>				
Right	2.6±1.1 [148]	2.4±1.2 [116]	2.1±1.0 [148]	0.8±0.7 [ 64]**
Left	2.4±1.1 [148]	2.3±1.2 [116]	2.0±0.9 [148]	0.5±0.5 [ 64]**
<b>No. of Tarsal Proximal Phalanges</b>				
Right	1.1±1.1 [148]	0.7±0.7 [116]	0.2±0.5 [148]**	0.0±0.0 [ 64]**
Left	1.2±1.1 [148]	0.7±0.7 [116]	0.3±0.5 [148]**	0.0±0.0 [ 64]**

Mean±SD

[ ] : No. of Fetuses Examined

Significant difference from control (\* P&lt;0.05, \*\* P&lt;0.01)

STATS : Statistical method DN:Dunnnett(Non-parametric)

(Excerpted from Applicant's submission)

## 10 Special Toxicology Studies

### In vitro 3T3 NRU Phototoxicity test of FTD or TPI

#### Study #B110945 (FTD) or B110946 (TPI)

The Applicant assessed the potential of FTD or TPI to induce phototoxicity in cultured mammalian cells using Balb/3T3 clone A31 cells derived from mouse embryo.

Based on the results of a preliminary study, the main tests were conducted at FTD or TPI concentrations of 7.81, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL under non-irradiation and irradiation conditions. Neither FTD nor TPI inhibited cell growth 50% or more at any of the concentrations under any of the treatment conditions so that photo-irritation factors could not be calculated for either component of the drug. Instead mean photo effects were calculated and values of  $\leq 0.1$  were considered non-phototoxic in this system. Based on the Phototox ver2 computer software, the calculated mean photo effects were  $-0.062$  for FTD and  $0.035$  for TPI.

These results suggest a low potential for FTD or TPI to induce phototoxicity.

## 11 Integrated Summary and Safety Evaluation

### Pharmacology

The pharmacologic activity of FTD:TPI was examined in in vitro and in vivo assays. FTD uptake into the DNA of NUGC-3 human gastric cells and MCF-7 human breast cancer cells was observed following 4 and 24 hours of incubation with concentrations of FTD that have been achieved clinically at the recommended dose of 35 mg/m<sup>2</sup> FTD:TPI given twice daily (BID). FTD uptake was less than that of thymidine, but greater than that of the other nucleoside analogues tested, including [1-beta-D-arabinofuranosylcytosine (Ara-C), gemcitabine (dFdC), and 5-fluoro-2'-deoxyuridine (FdUrd). Incubation with FTD resulted in inhibition of thymidine synthase (TS) activity as measured by a decrease in the dTTP pool. Following 4 hrs of washout, FTD-induced reduction of the dTTP pool recovered more quickly compared to that induced by FdUrd, suggesting that FdUrd-induced TS inhibition is more persistent.

In in vitro proliferation studies, FTD inhibited the proliferation of various human cancer cell lines with IC<sub>50</sub> values of 0.214 µM to 24.4 µM; this activity was similar to that seen with 5-FU which inhibited the same cell lines with IC<sub>50</sub> values ranging from 3.18 to 14 µM. FTD inhibited proliferation of HCT-15 human colorectal cancer cells with an IC<sub>50</sub> of 10.7 µM, compared to 5-FU which exhibited an IC<sub>50</sub> value of 4.96 µM. The mean C<sub>max</sub> of FTD determined in patients with solid tumors at the recommended dose of FTD:TPI was ~4857 ng/mL (~16 µM).

The in vivo activity of FTD:TPI was examined in nude mouse models of human cancer. Twice daily treatment with 150 mg/kg/day FTD:TPI significantly prolonged the survival of nude mice implanted with KM20C human colon adenocarcinoma xenografts compared to controls, and to mice treated with CPT-11 or TS-1. Twice daily treatment with 150 mg/kg/day FTD:TPI induced statistically significant inhibition of COL-1 (*KRAS* wild-type) and cetuximab-resistant HCT-116 (*KRAS* mutant) human colon cancer xenograft growth (55.8% and 55.5% TGI, respectively) compared to controls, indicating that FTD:TPI exhibits similar anti-tumor activity against *KRAS* wild-type and mutant tumors. Further, FTD:TPI exhibited in vivo anti-tumor activity against MX-1 human breast cancer xenografts relatively insensitive to the oral fluoropyrimidine anticancer drug TS-1. A correlation analysis suggested that cell lines with higher FTD incorporation

into DNA in in vitro assays were more sensitive to in vivo treatment with FTD:TPI in xenograft models.

The Applicant provided data from the literature demonstrating the activity of TPI as a thymidine phosphorylase inhibitor. The thymidine phosphorylase inhibitor TPI alone did not have anti-tumor activity in xenograft models, but did result in increased FTD exposures when given in combination with FTD compared to administration of FTD alone in multiple in vivo studies.

The Applicant also evaluated the in vitro interactions between FTD and thymidine (dThd) analog-type antiviral drugs in HCT-116 and NUGC-3 human gastric cells. Treatment with >10  $\mu\text{M}$  zidovudine (AZT) enhanced FTD-induced inhibition of NUGC-3 and HCT116 cells following 4 hrs and 72 hrs of FTD incubation. Since the clinical  $C_{\text{max}}$  of AZT is  $\sim 2 \mu\text{M}$ , it is unlikely that AZT will reach high enough concentrations in humans to enhance the anti-tumor effects of FTD:TPI.

### **Safety Pharmacology**

Both in vitro and in vivo safety pharmacology studies were conducted to assess the effects of FTD:TPI on CNS, cardiovascular, and respiratory function. Single oral administration of FTD:TPI up to 640 mg/kg had no significant effect on general physical condition, CNS behavior, respiratory rate, tidal volume, or minute volume up to 24 hours post-dose in male Sprague-Dawley rats.

FTD:TPI inhibited in vitro hERG-mediated potassium current in stably transfected HEK293 cells by 1.8%, 1.8%, and 2.5% at 3, 30, and 300  $\mu\text{M}$ , respectively. Neither FTD nor TPI mediated hERG inhibition, and  $\text{IC}_{50}\text{s}$  were not achieved or calculated for either entity. In a monkey cardiovascular safety study, while there was a statistically significant 6% prolongation of the mean QTc interval 1 hr after administration of FTD:TPI at the 40 mg/kg dose level compared to vehicle, there was no effect at the high dose level, suggesting that the finding was not FTD:TPI related. In keeping with these findings, neither single nor repeated administration of FTD:TPI had clinically relevant effects on QT/QTc prolongation compared to placebo in humans. In male cynomolgus monkeys implanted with a telemetry device, single oral administration of FTD:TPI resulted in a statistically significant 9-12% decrease in diastolic and mean blood pressure 4 hrs post-dose at the 40 and 160 mg/kg dose levels compared to vehicle; however, diastolic and mean blood pressure values were slightly increased compared to baseline. Decreased blood pressure has not been reported in humans treated with FTD:TPI. Single oral administration of FTD:TPI up to 160 mg/kg did not have significant effects on any other cardiovascular parameters.

### **Pharmacokinetics**

The Applicant examined the effects of TPI on the PK parameters of FTD in male cynomolgus monkeys. FTD was rapidly metabolized to its metabolite FTY following oral administration, but co-administration of TPI with FTD increased FTD exposure  $\sim 104$ -fold. These data indicate that TPI inhibits the metabolism of FTD. The Applicant further

demonstrated that co-administration of FTD and TPI at a molar ratio of 1:0.5 resulted in a sufficient amount of TPI to inhibit the degradation of FTD in monkeys. Thus, the molar FTD:TPI ratio of 1:0.5 was utilized for FTD:TPI dosing in the clinic.

The Applicant evaluated the absorption, distribution, metabolism, and excretion of FTD:TPI following a single oral or IV administration of 50 mg/kg [ $^{14}\text{C}$ -FTD]FTD:TPI or  $^{14}\text{C}$ -FTD to male rats. Mean oral bioavailability was calculated to be ~31%. Mean  $\text{AUC}_{(0-\infty)}$  was ~1.6-fold higher in non-fasted (fed) rats compared to fasted rats following oral administration. In contrast, when 35 mg/m<sup>2</sup> FTD:TPI was administered as a single dose to 14 patients with solid tumors after a standardized high-fat diet, the AUC of FTD did not change, but the FTD C<sub>max</sub> decreased by ~40% compared to the fasting state. Since the Applicant believes that the increased FTD C<sub>max</sub> observed in the fasting state may lead to increased hematologic toxicity, FTD:TPI will be administered to patients within 1 hour following completion of morning and evening meals. Following oral administration of [ $^{14}\text{C}$ -FTD]FTD:TPI to rats,  $^{14}\text{C}$ -FTD-related radioactivity reached its maximum levels in the stomach and jejunum at 15 minutes, in the cecum and colon at 6 hours post-dose, and in the remaining tissues at 1 hour post-dose. Mean tissue radioactivity concentrations generally decreased with time. Consistent with clinical observations of GI toxicity (nausea, diarrhea, vomiting, and abdominal pain), tissue distribution of  $^{14}\text{C}$ -FTD was the highest in the stomach. Radioactivity concentrations were generally higher in plasma than in tissues at  $\leq 1$  hour post-dose except for the kidney, ileum, urinary bladder, stomach, and jejunum. Notably, there was some distribution of FTD:TPI to the brain. Radioactivity concentrations in the cerebrum and cerebellum were 7% of that in plasma at 1 hour post-dose.

$^{14}\text{C}$ -FTD exhibited variable in vitro plasma protein binding in dogs, rats, mice, monkeys, and humans ranging from ~38-97%. Approximately 97% of  $^{14}\text{C}$ -FTD was bound to human plasma protein, resulting in a free drug concentration of approximately 480 nM in humans at the recommended dose of FTD:TPI. In vitro studies demonstrated that FTD and TPI preferentially distributed to plasma rather than blood cells in rat, monkey, and human blood.

The Applicant also evaluated the in vivo metabolism of FTD:TPI. Following a single oral administration of [ $^{14}\text{C}$ -FTD]FTD:TPI to non-fasted rats, the major metabolite FTY accounted for 58-82% of the radioactivity in the plasma up to 6 hours post-dose. Similarly, FTD was mainly metabolized in humans to FTY via thymidine phosphorylase. FTY was also the main metabolite of FTD in rat urine. FTD and FTY were not detected in rat feces up to 24 hours post-dose, but the metabolite HFF1 accounted for 44.3% of radioactivity in the feces. Metabolite analysis further indicated that addition of TPI to FTD:TPI inhibits the metabolism of FTD to FTY. Following a single oral administration of [ $^{14}\text{C}$ -FTD]FTD:TPI or  $^{14}\text{C}$ -FTD to rats, the majority of radioactivity was excreted in the urine.

The Applicant assessed absorption, distribution, metabolism, and excretion following a single oral or IV administration of 50 mg/kg [ $^{14}\text{C}$ -TPI]FTD:TPI or 23.6 mg/kg  $^{14}\text{C}$ -TPI to male rats. Although TPI exposures by AUC were relatively comparable in fasted and

non-fasted rats, C<sub>max</sub> was ~1.4-fold higher and T<sub>max</sub> was achieved earlier in fasted compared to non-fasted rats, suggesting a higher absorption rate in fasted rats. Following oral administration of [<sup>14</sup>C-TPI]FTD:TPI to rats, the highest level of radioactivity up to 4 hrs post-dose as measured by whole body autoradiograms was in the intestinal contents followed by urine in bladder and gastric contents. Tissue distribution of <sup>14</sup>C-TPI was the highest in the jejunum, ileum, and stomach. Radioactivity concentrations in the cerebrum and cerebellum were ~3% and 5% of that in plasma at 1 hour post-dose, respectively. Mean tissue radioactivity concentrations generally decreased with time. <sup>14</sup>C-TPI exhibited low in vitro plasma protein binding in dogs, rats, mice, and monkeys (≤7%). Approximately 1.3-7.1% of <sup>14</sup>C-TPI was bound to human plasma protein.

Following single oral administration of [<sup>14</sup>C-TPI]FTD:TPI or <sup>14</sup>C-TPI to rats, the majority of radioactivity was excreted in the feces. Unchanged TPI was the major component in plasma up to 4 hrs post-dose and in urine and feces up to 24 hrs post-dose. The metabolites HTP1, HTU1, and HTF1 were detected in plasma, urine, and feces, respectively. HPLC demonstrated that these metabolites were identical, and LC/MS analysis indicated that the identity of HTU1 was 6-HMU. In keeping with this, 6-HMU was the only metabolite of TPI detected in human plasma and urine, albeit at low or trace levels. TPI was not substantially metabolized in vitro using human hepatocytes. Thus, adequate exposure to 6-HMU occurred in rats to account for potential TPI metabolite-mediated toxicity. TPI also inhibited in vitro <sup>14</sup>C-FTD metabolism up to 79.5% in human hepatocytes, indicating that FTD is primarily metabolized by thymidine phosphorylase.

The Applicant also evaluated the absorption, metabolism, and excretion of FTD:TPI following single oral administration of [<sup>14</sup>C-FTD]FTD:TPI or [<sup>14</sup>C-TPI]FTD:TPI to fasting male cynomolgus monkeys. FTD-related radioactivity was excreted primarily in the urine, whereas TPI-associated radioactivity was excreted primarily in the feces. Following single oral administration of [<sup>14</sup>C-FTD]FTD:TPI, FTY was the major metabolite in plasma and urine. Unchanged FTD, F-Peak 1, F-Peak 2 (hydrolyzed FTY), and F-Peak 3 (glucuronide of FTD) were also detected in plasma and urine. Following single oral administration of [<sup>14</sup>C-TPI]FTD:TPI, unchanged TPI was the major component in plasma and urine. T-Peak 3 (uracil) and T-Peak 5 were also detected in plasma, and T-Peak 1, 2, 3 (uracil), 4, and 6 (imino-oxidated TPI) were also detected in urine.

The in vitro metabolism of [<sup>14</sup>C]FTD was evaluated using human liver microsomes and cryopreserved human hepatocytes. FTD was not metabolized in vitro in the presence or absence of NADPH using human liver microsomes. The primary metabolite of [<sup>14</sup>C]FTD in human hepatocytes was FTY, and minor metabolites were 5-CU and 5-CdUrd. 5-CdUrd was also detected following incubation with PBS alone, however, suggesting it was produced nonenzymatically. These in vitro results were consistent with the metabolism of FTD seen in humans. In human plasma, trifluridine was metabolized to FTY, 5-CU, and 5-CdUrd, with FTY being the primary metabolite and 5-CU and 5-CdUrd being detected in plasma and urine at low or trace levels. The Applicant did not examine the in vitro metabolism of <sup>14</sup>C-FTD in rodent, dog, or monkey

hepatocytes or liver microsomes. Since FTY was the primary metabolite detected in rat and monkey plasma/urine following oral administration of [<sup>14</sup>C-FTD]FTD:TPI, adequate exposure to FTY occurred in animals to account for potential metabolite-mediated toxicity. 5-CU and 5-CdUrd accounted for ≤8% and ≤2% of <sup>14</sup>C-FTD metabolites in human hepatocytes, respectively, and addition of TPI changed these percentages to ≤2.2% and ≤3.1%, respectively. Although 5-CU and 5-CdUrd were not detected in rat or monkey plasma, urine, or feces in the PK studies conducted by the Applicant, Rogers et al. reported that 5-CU and 5-CdUrd were detected in urine following single IV administration of <sup>14</sup>C-FTD to monkeys (Rogers, Hartman, et al. 1969). Given these data, the trace amounts of 5-CU and 5-CdUrd detected in human plasma, and the advanced cancer indication, further metabolite evaluation is not warranted at this time.

The Applicant investigated placental and embryofetal transfer of FTD:TPI following a single oral administration of ([<sup>14</sup>C]FTD)FTD:TPI or ([<sup>14</sup>C]TPI)FTD:TPI at a FTD:TPI dose level of 50 mg/kg to fasted pregnant female rats on Day 18 of gestation. At 0.5 hr post-dose, [<sup>14</sup>C]FTD-related radioactivity in the fetus, fetal blood, and fetal tissues was 21%, 21%, and ≤28%, respectively, of that in the maternal plasma. Radioactivity concentrations in the placenta, fetus, fetal blood, and most fetal tissues peaked at 1 hr post-dose, and then generally decreased in parallel with maternal plasma. The percentage distribution of [<sup>14</sup>C]FTD-related radioactivity to the fetus was highest at 1 hr post-dose (0.08% of dosed radioactivity), indicating relatively low transfer of FTD to the fetus. At 0.5 hr post-dose, [<sup>14</sup>C]TPI-related radioactivity in the fetus, fetal blood, and fetal tissues was 6%, 10%, and ≤7%, respectively, of that in the maternal plasma. Radioactivity concentrations peaked at 4 hrs post-dose in the fetal membrane, amniotic fluid, fetus, and fetal tissues and at 0.5 hrs post-dose in the placenta and fetal blood; radioactivity concentrations appeared to decrease at a slower rate in the placenta, fetal membrane, and fetal tissues/fluids compared to maternal plasma. Overall, the data shows that both FTD and TPI were able to cross the placenta and suggests that FTD crosses at a higher rate than TPI.

Following a single oral administration of ([<sup>14</sup>C]FTD)FTD:TPI to lactating rats on PND 10, radioactivity was excreted into milk and peaked in milk and plasma at 1 hr post-dose. [<sup>14</sup>C]FTD-related radioactivity was higher in plasma than in milk, except for 8 and 12 hrs post-dose when the levels were similar. Following a single oral administration of ([<sup>14</sup>C]TPI)FTD:TPI to lactating rats on PND 10, radioactivity peaked in milk and plasma at 4 hr and 1 hr post-dose, respectively. Beginning at 2 hrs post-dose, [<sup>14</sup>C]TPI-related radioactivity was higher in milk than in plasma, resulting in a milk/plasma ratio of ~5 at 24 hrs post-dose. Thus, FTD:TPI was excreted in milk following administration of ([<sup>14</sup>C]FTD)FTD:TPI and ([<sup>14</sup>C]TPI)FTD:TPI.

### **General Toxicology**

The Applicant submitted two GLP-compliant 13-week toxicology studies in Sprague-Dawley rats and cynomolgus monkeys. Rats were administered 0, 7.4, 22.1, 74, or 221 mg/kg of FTD:TPI (containing 0, 5, 15, 50, or 150 mg/kg of FTD) once daily for 13 weeks. Monkeys were administered 1.839, 7.355, or 29.42 mg/kg of FTD:TPI

(containing 0, 1.25, 5, or 20 mg/kg FTD) and 20 mg/kg of FTD alone, once daily for 13 weeks.

In rats, whitish and fractured incisors were observed at the 221 mg/kg dose level. Histopathological findings included disarrangement of the odontoblasts and osteodentin in more than half of the rats at the 74 mg/kg dose level and all rats at the 221 mg/kg dose level. Furthermore, almost all the rats at the 221 mg/kg dose level had decreases in dentin thickness and odontoblasts, as well as enamel matrix residue and flattening of the ameloblasts/papillary cells in the incisors. Abnormality of the incisors was observed at  $\geq 221$  mg/kg during the 4-week recovery period. The Applicant noted that in the present 13-week study, the whitish incisors began at approximately the same time after the initiation of dosing as in the previous 4-week study. Since no effect on the incisors was observed in monkeys, the effects in rats is believed to be due to the continuous growth of teeth in rats, and may not be clinically relevant in an adult patient population.

The major organs of toxicity include the hematopoietic system, as evidenced by decreased hematologic parameters, fatty infiltration in the bone marrow, thymic atrophy and hemorrhage at the 221 mg/kg dose level. Atrophy of the mesenteric and mandibular lymph nodes and hypoplasia of the bone marrow were also reported in the 28-day toxicity study reviewed at the time of the original IND submission. Epithelial cell necrosis in the gastrointestinal tract also occurred in rats in the 28-day study at FTD doses  $\geq 150$  mg/kg/day.

Major organs of toxicity in the monkey were similar to those in the rat; the hematopoietic system and the gastrointestinal tract. The pathologist attributed the death of the one monkey in the 13-week study and mortalities in previous studies to effects on the lymphatic-hematopoietic system and digestive tract associated with deteriorating general condition and undernourished state. The toxicities seen in clinical studies, including hematologic toxicities ( $\downarrow$ WBC,  $\downarrow$ RBC); mild bone marrow hypocellularity, gastrointestinal tract toxicities (including minimal mucosal atrophy, and mucosal necrosis) were predictable from nonclinical toxicology studies.

As expected, the exposure in monkeys to FTD in FTD:TPI at the 29.42 mg/kg dose level (containing 20 mg/kg FTD) was much higher than when an equivalent dose of FTD (20 mg/kg) was directly administered to monkeys. Whereas in the monkey, FTD alone did not show a tendency to accumulate with repeated dosing, FTD in FTD:TPI showed mild accumulation. Thus, while on Day 1, the exposure to FTD at the 1.839 mg/kg dose level of FTD:TPI was similar to the exposure to FTD alone at the 20 mg/kg dose level; by Week 6 of repeated dosing, the exposure to FTD at the 1.839 mg/kg dose level of FTD:TPI was more than 2-fold the exposure to FTD at the 20 mg/kg dose level of FTD alone. FTD and its metabolite FTY also showed a tendency to accumulate in the rat following administration of FTD:TPI, though the Applicant did not include a direct comparison to FTD alone in any of the submitted rat studies.

In genetic toxicology studies, FTD:TPI and FTD were positive for mutagenicity and in vitro and in vivo clastogenicity. TPI was negative for genotoxicity.

### **Reproductive and Developmental Toxicology**

In reproductive studies, administration of FTD:TPI to males at doses up to 662 mg/kg for 14 days prior to and during mating with untreated females did not have a remarkable effect on male fertility. Similarly, administration of FTD:TPI up to 221 mg/kg daily for 14 days to female rats before mating with untreated males did not have a remarkable effect on female fertility, though at the high dose of 221 mg/kg (resulting in exposures that were similar to the clinical exposure based on the toxicokinetic data from the 13-week repeat dose toxicology study), there was a decrease in the number of viable embryos, suggesting an effect of FTD:TPI on early development.

When administered to female rats during organogenesis, FTD:TPI increased embryo-fetal lethality at exposures similar to clinical exposures at the clinically recommended dose (human exposure at 35 mg/m<sup>2</sup> BID: 23, 697 ng•h/mL; rat exposure 22,400 ng•h/mL). Other observations included decreased fetal weights at doses ≥74 mg/kg, and delayed ossification, as well as external (kinked tail, ectrodactyly, cleft palate, and anasarca), visceral (great vessel anomalies), and skeletal abnormalities at the 221 mg/kg dose level.

### **References**

Fukushima M, Suzuki N, Emura T, Yano S, Kazuno H, Tada Y, Yamada Y, Asao T, 2000, Structure and Activity of Specific Inhibitors of Thymidine Phosphorylase to Potentiate the Function of Antitumor 2'-Deoxyribonucleosides, *Biochemical Pharmacology*, 59: 1227-1236.

Rogers WI, Hartman AC, Palm PE, Okstein C, Kensler CJ, 1969, the Fate of 5-Trifluoromethyl-2'-deoxyuridine in Monkeys, Dogs, Mice, and Tumor-bearing Mice, *Cancer Research*, 29: 953-961.

Zambelli F, Pesole G, and Pavesi G, 2009, Pscan: finding over-represented transcription factor binding site motifs in sequences from co-regulated or co-expressed genes, *Nucleic Acids Res*, 37(Web Server issue):W247–W252.

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/s/  
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GABRIEL S KHASAR  
08/25/2015

EMILY M FOX  
08/25/2015

WHITNEY S HELMS  
08/25/2015

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA 207981**

**NDA/BLA Number: 207,981    Applicant: Taihi Oncology, Inc.    Stamp Date: 12/19/2014**

**Drug Name: Lonsurf                      NDA Type: 505(b)(1) NME**

On **initial** overview of the NDA/BLA application for filing:

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR  
NDA 207981**

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		NA	
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?		NA	The NDA is not to support Rx to OTC switch

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? \_\_\_\_\_ Yes\_**

If the NDA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

NA

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Sachia Khasar, PhD and Emily M. Fox, PhD 02/02/2015  
 \_\_\_\_\_  
 Reviewing Pharmacologist Date

Whitney S. Helms, PhD 02/02/2015  
 \_\_\_\_\_  
 Team Leader/Supervisor Date

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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GABRIEL S KHASAR  
02/02/2015

EMILY M FOX  
02/02/2015

WHITNEY S HELMS  
02/03/2015