5. Summary of Nonclinical Pharmacology and Toxicology Section of the Application

5. SUMMARY OF NONCLINICAL PHARMACOLOGY AND TOXICOLOGY SECTION OF THE APPLICATION

A complete listing of the preclinical pharmacology and toxicology studies conducted and previously submitted under IND applications [redacted] (filed by GlaxoWellcome Inc.) and [redacted] (filed by Triangle Pharmaceuticals, Inc.) is provided in Item 5, Nonclinical Pharmacology and Toxicology.

5.1. Brief Summary of Preclinical Pharmacology

Emtricitabine is intended to be taken as a single oral 200 mg dose once daily for extended periods, which translates into an adult dose of about 3-4 mg/kg/day. At the recommended clinical dose, systemic exposure ($AUC_{0\rightarrow24}$) in subjects with HIV infection averaged 10 hr*μg/mL and peak plasma concentration (C_{max}) was approximately 2 μg/mL.

The aim of emtricitabine treatment is to block HIV replication without adversely affecting the host; thus, primary pharmacologic activity targets the virus, and any secondary pharmacologic activity in patients is considered undesirable. Primary pharmacodynamic studies are described in Item 7, Microbiology, and summarized in Section 7 of this Application Summary. Secondary pharmacodynamic and safety pharmacology studies are summarized here.

Potential pharmacologic effects of emtricitabine on the nervous, cardiovascular, respiratory, gastrointestinal, urinary, and coagulation systems have been characterized in a variety of *in vitro* and *ex vivo* studies utilizing receptors and tissues from rats, guinea pigs, rabbits, and cats and single-dose *in vivo* studies in mice, rats, and dogs. These studies revealed that emtricitabine had no undesirable effects on any organ system at doses much greater (on a mg/kg basis) than the recommended clinical dose or at concentrations much greater than those produced in patients at the recommended clinical dose.

The secondary and safety pharmacology program is summarized below:

Text Table 5.1.
Secondary and Safety Pharmacology Program

Organ System Evaluated	Dose Route	Species
General Pharmacologic Effects	oral	mouse, rat
Nervous System	*in vitro*	rat brain
	ex vivo	rat stomach, guinea pig ileum
	oral	mouse, rat
Cardiovascular System	*in vitro*	rat heart, rat liver, dog heart
	ex vivo	rat heart, guinea pig heart, rabbit aorta, cat heart
	oral	rat
	intravenous	dog
Respiratory System	*ex vivo*	guinea pig trachea
	oral	mouse, rat
	intravenous	dog
Gastrointestinal System	*ex vivo*	rat stomach, guinea pig ileum
	oral	mouse
Urinary System	oral	rat
Coagulation System	*in vitro*	rabbit platelets

Patients will take emtricitabine orally, so emtricitabine was given orally in most *in vivo* pharmacology studies to mimic the intended clinical dose route. Cardiovascular effects also were studied in dogs dosed intravenously.

Patients will take emtricitabine once a day. All *in vivo* pharmacology studies used a single dose with variable postdose evaluation periods. In oral *in vivo* pharmacology studies, emtricitabine was suspended in 0.5% aqueous methylcellulose. All studies included appropriate vehicle control groups.

5.2. Secondary Pharmacodynamics and Safety Pharmacology

Because emtricitabine's primary pharmacodynamic activity is against HIV, any pharmacodynamic activity in the host was considered undesirable and potentially adverse. Consequently, studies that investigated potential host pharmacologic effects are discussed together. The following tables summarize the secondary pharmacodynamic and safety pharmacology studies according to the model employed (i.e., *in vitro*, *ex vivo*, or *in vivo*).

Text Table 5.2.
In Vitro and *Ex Vivo* Secondary Pharmacology Studies

Study Type	Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Receptor Binding: *In vitro* Receptor Binding Potential	The effects of FTC on the specific binding of various radioactively labeled ligands was studied in 19 different receptor binding assays. Tissues were obtained in all but two assays from Sprague Dawley rats. A heart preparation was obtained from beagle dogs for use as a calcium release channel binding assay, and platelets isolated from New Zealand White rabbits were used for a platelet activating factor assay.	FTC had no pharmacologically significant binding affinity at the adenosine (A_1 and A_2), adrenergic (alpha$_1$, alpha$_2$, and beta), angio-tensin II, benzodiazepine, calcium channel (dihydropyridine and phenylalkylamine), calcium release channel (ryanodine), cholinergic (M_1 and M_2), dopamine$_2$, GABA$_a$ gated chloride channel (TBPS), glutamate, neurotensin, platelet activating factor, and serotonergic ($5HT_{1A}$ and $5HT_2$) receptors.	6338	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: *In vitro* Peripheral Autonomic Receptors (Muscle Function)	Isolated muscle preparations were used to assess effects of FTC on autonomic function and peripheral receptors, *in vitro*. Cholinergic (guinea pig ileum), adrenergic (rabbit aorta, guinea pig atria, and trachea), histaminergic (guinea pig atria), and serotonergic (rat fundus), as well as tissue responsiveness to arachodonic acid (rat fundus), bradykinin (guinea pig ileum), and angiotensin II (rabbit aorta) were tested.	FTC (10 µM or 100 µM) had little or no direct effect on various isolated muscle preparations and had no major inhibitory effects on the contractile responses to acetylcholine, norepinephrine, serotonin, isoproterenol, arachidonic acid, histamine, bradykinin and angiotensin II.	6337	IND [redacted] Serial No. 000 [redacted]
Cardiovascular Effects: *In vitro* Isolated Cardiac Muscle Function	Isolated perfused rat heart: Male CD (SD) rat hearts were connected to a Langendorff perfusion apparatus. The effects of a 100 µM FTC perfusate solution for 30 min on heart rate and spontaneous ventricular arrhythmias were studied. Papillary and atrial muscle of the cat: Male cat papillary muscle strips of left atrial muscle were attached to Plexiglas® holders containing platinum electrodes and mounted in an organ bath containing Krebs solution. The inotropic effects of	No effects of FTC were seen in isolated perfused rat hearts. Minor positive inotropism was observed (12±6% ↑ in developed tension) on cat papillary muscle preparations after 30 min at 100 µM FTC. Only negligible positive inotropism was observed (2±3% increase	6323	IND [redacted] Serial No. 000 [redacted]

Text Table 5.2.
In Vitro and *Ex Vivo* Secondary Pharmacology Studies

Study Type	Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
	a 100 µM FTC preparation were studied in atrial and papillary muscle. Spontaneously-beating guinea-pig paired atria: Male albino Hartley guinea-pig paired atria were suspended in organ baths containing Krebs-Ringer solution and the force of contractions monitored. The chronotropic and inotropic effects of a 100 µM FTC solution were studied.	in developed tension) on cat atrial preparations. No chronotropic or inotropic effects of FTC on the spontaneously-beating guinea-pig heart.		
Intestinal Effects: *In vitro* Isolated Guinea Pig Ileum	Strips of ileum from 6 Duncan Hartley guinea pigs were isolated and individually suspended in a tissue bath containing Kreb's solution. Contractions were recorded through an isotonic transducer and graph-recorder. The response of each strip to reference concentrations of acetylcholine, histamine and barium chloride was determined and the strips were challenged with FTC at concentrations of 10, 30 and 100 µM.	FTC did not elicit any significant agonist effect at any of the test concentrations, nor did it alter contractile responses induced by acetylcholine, histamine or barium chloride.	477	IND [redacted] Serial No. 000 [redacted]

Text Table 5.1 (continued) ***In Vivo*** **Secondary Pharmacology Studies**

Study Type	Species/ Strain	Sex and No./Grp [Total No.]	Route/ Dose Groups/Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
General Effects: Body Temperature	Rat/Wistar	5 M [20]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Rectal temperature was taken at 0, 15, 30, 60, and 120 min post-dose.	FTC had no effect on body temperature at the doses tested.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Modified Irwin Screen with Toxicity Observations	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, 100 mg/kg. 0.5, 1, 2, and 24 hr, postdosing observation period and daily for 7 days.	No effects. FTC was well tolerated at all doses.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Modified Irwin Screen with Toxicity Observations	Mouse/CD-1	4 M [24]	PO, single dose FTC at 0, 100, 250, 500, 750, or 1000 mg/kg and gross behavioral effects followed for 7 days	No effects. FTC was well tolerated in mice at doses up to 1000 mg/kg.	6319	IND [redacted] Serial No. 000 [redacted]
	Rat/CD (SD)	4 M [16]	PO, single dose FTC at 0, 250, 500, or 1000 mg/kg and gross behavioral effects followed for 7 days	No effects. FTC was well tolerated in rats at doses up to 1000 mg/kg.		
Neuropharmacology: Spontaneous Locomotor Activity	Mouse/ICR	8 M [32]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Spontaneous movement measured for 3 min. prior to and at 15 min. intervals for 2 hr postdosing.	No effects at any of the doses tested.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Motor Incoordination	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg Ability to remain on a 15 rpm revolving rubber covered rod was tested.	No effect was found on motor incoordination at any dose.	477	IND [redacted] Serial No. 000 [redacted]

Text Table 5.1 (continued) ***In Vivo*** **Secondary Pharmacology Studies**

Study Type	Species/ Strain	Sex and No./Grp [Total No.]	Route/ Dose Groups/Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Neuropharmacology: Hexobarbital Potentiation (Sleeping Time)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. 70 mg/kg hexobarbital given i.p. 1 hr post FTC dose and sleeping time measured.	FTC had no effect on hexobarbital-induced sleeping time at the doses tested.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Anticonvulsant Activity (Maximal Electroshock)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Effect of FTC on the pattern of induced tonic or clonic convulsions was studied at 1 hr post-dose.	There was no effect of FTC on either mortality or the numbers of mice exhibiting tonic or clonic convulsions.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Anticonvulsant Activity (Metrazole)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. 100 mg/kg metrazole (pentylenetetrazole) given i.p. 1 hr post FTC dose and clonic and tonic convulsions and mortality measured.	There were no effects of FTC on the number of mice exhibiting clonic/tonic convulsions and mortality at any dose.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Proconvulsant Activity (Electroshock)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. At 1 hr after dosing, a sub-threshold electroshock was applied and clonic/tonic convulsions and mortality measured.	There were no effects of FTC on the number of mice exhibiting clonic/tonic convulsions and mortality at any dose.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Proconvulsant Activity (Metrazole)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. 70 mg/kg metrazole (pentylenetetrazole) given i.p. 1 hr post FTC dose and clonic and tonic convulsions measured.	There were no effects of FTC on the number of mice exhibiting clonic/tonic convulsions and mortality at any dose.	477	IND [redacted] Serial No. 000 [redacted]

Text Table 5.1 (continued) ***In Vivo*** **Secondary Pharmacology Studies**

Study Type	Species/ Strain	Sex and No./Grp [Total No.]	Route/ Dose Groups/Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Neuropharmacology: Analgesic Activity (Tail Flick)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Before and at 60 min after dosing, a beam of radiant heat was focused onto the tail until a flick response was elicited, or until 15 sec elapsed.	No animals exhibited an increase in latency of the tail flick response at any dose.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Analgesic Activity (PQ Writhing)	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Phenylquinone (PQ) was given i.p. (2 mg/kg) at 1 hr post FTC dose and the number of writhes exhibited for 5-10 min recorded.	There was no attenuation of PQ-induced writhing in mice at the doses tested.	477	IND [redacted] Serial No. 000 [redacted]
Neuropharmacology: Body Temperature	Rat/Wistar	5 M [20]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Rectal temperature was taken at 0, 15, 30, 60, and 120 min post-dose.	FTC had no effect on body temperature at the doses tested.	477	IND [redacted] Serial No. 000 [redacted]
Cognitive Effects: Conditioned Avoidance	Rat/ Long Evans	6 F [6], ovariecto-mized	IP, dose escalated FTC at 0, 30, or 100 mg/kg. Rats were trained to the conditioned avoidance task of avoiding foot shock in response to an audio-visual cue, then given FTC and the ability to escape shock measured.	FTC had no effect on the conditioned avoidance response at the doses tested.	6320	IND [redacted] Serial No. 000 [redacted]
Cardiovascular Effects: Cardiovascular Function	Rat/ Wistar	5 M [20]	PO, single dose FTC at 0, 5, 10, or 50 mg/kg. The carotid artery was cannulated and mean arterial pressure (MAP) and heart rate determined.	FTC had no effects on the MAP at any dose.	477	IND [redacted] Serial No. 000 [redacted]

Text Table 5.1 (continued) ***In Vivo*** **Secondary Pharmacology Studies**

Study Type	Species/ Strain	Sex and No./Grp [Total No.]	Route/ Dose Groups/Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Cardiovascular Effects: Cardiovascular Function	Rat/CD (SD)	8 M (16)	PO, single dose FTC at 0 or 250 mg/kg. Systolic blood pressure (SBP) and heart rate were determined in conscious normotensive animals by the tail cuff method.	There were no changes in SBP or heart rate following FTC.	6321	IND [redacted] Serial No. 000 [redacted]
Cardiovascular Effects: Cardiovascular Function	Dog/ Beagle	4 M (4)	IV, dose escalation of 1, 2.5, 5, 10, and 20 mg/kg. Standard cardiovascular and respiratory parameters were determined in anesthetized, surgically instrumented, spontaneously breathing dogs. Changes in MAP induced by carotid occlusion, norepinephrine, acetyl choline, and vagal nerve stimulation were also studied.	FTC had no effect on cardiovascular or respiratory parameters, nor did it produce changes in responses to carotid occlusion, norepinephrine, acetyl choline, or vagal nerve stimulation.	6322	IND [redacted] Serial No. 000 [redacted]
Digestive Effects: Gastrointestinal Motility	Mouse/ICR	10 M [40]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. Mice were given an oral suspension of 5% charcoal in 10% tragacanth gum arabic at 1 hr post FTC dose. At 0.25 hr later, the intestines were removed and intestinal transit quantified.	FTC had no effect on intestinal motility at any dose.	477	IND [redacted] Serial No. 000 [redacted]

Text Table 5.1 (continued) ***In Vivo*** **Secondary Pharmacology Studies**

Study Type	Species/ Strain	Sex and No./Grp [Total No.]	Route/ Dose Groups/Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Renal Effects: Renal Function	Rat/ Long Evans	6 M [24]	PO, single dose FTC at 0, 10, 30, or 100 mg/kg. A 15 mL/kg volume of saline was given followed by FTC at 10 mL/kg and urine collected for 6 hr. Urine volume, Na^+, K^+, and Cl^- concentrations, and pH were measured.	FTC had no effect on the measured renal function parameters at any dose.	477	IND [redacted] Serial No. 000 [redacted]

5.2.1. Discussion and Conclusions

The results of the secondary and safety pharmacology program suggest that emtricitabine is without undesirable effects on any organ system at systemic (AUC) exposures ~10 to >50 fold human exposure at the recommended clinical dose (rodent exposures for 100 and 1000 mg/kg doses, respectively, as compared to human AUC). *In vivo* cardiovascular studies, including evaluation of lead II electrocardiograms collected from anesthetized dogs given a cumulative dose of 38.5 mg/kg emtricitabine administered intravenously over a 1-hour period, were negative for effects on cardiovascular function. *In vitro* tests for inotropic and chronotropic activity, including the Langendorff preparation using rat hearts, were also negative for secondary pharmacologic effects of emtricitabine.

5.3. Preclinical Toxicology

5.3.1. Brief Summary of Preclinical Toxicology

The general systemic toxicity, genotoxicity, reproductive toxicity, cytotoxicity, bone marrow toxicity, and mitochondrial toxicity of emtricitabine have been characterized in a variety of *in vitro* and *in vivo* studies. As applicable, the studies were performed in accordance with International Conference on Harmonization guidances and in compliance with Good Laboratory Practice regulations. Potential carcinogenicity is being characterized in 2-year studies initiated in mice and rats in April 2001. There have been no unusual observation in the 2-year studies to date. The studies mentioned above revealed that emtricitabine was well tolerated for up to a year at doses producing systemic exposures much greater than those produced in patients at the recommended clinical dose. Toxicity was limited to animals in the high dose groups and to mild reversible anemia in mice (3000 mg/kg/day for 6 months) and soft feces in female monkeys (2000 mg/kg/day for 1 month). Emtricitabine was not genotoxic, did not adversely affect reproduction or embryofetal development, was relatively non-cytotoxic to human cells including bone marrow progenitor cells, and did not produce mitochondrial toxicity.

The toxicology program is summarized in the following table:

Text Table 5.3.
Summary of the Emtricitabine Toxicology Program

Study Type and Duration	Dose Route	Species
Single-Dose General Toxicity	IV and oral	mouse, rat
Repeat-Dose General Toxicity		
2 week	oral	mouse
1 month	oral	mouse, monkey
3 month	oral	mouse, rat, monkey
6 month	oral	mouse
1 year	oral	monkey
Genotoxicity	*in vitro*	*Salmonella* bacteria, mouse lymphoma cells
	oral	mouse micronucleus test
Carcinogenicity (dosing started April 2001)	oral	mouse, rat
Reproductive Toxicity	oral	mouse, rat, rabbit
Fertility, early development	oral	mouse, rat
Embryofetal development	oral	mouse, rabbit
Peri-/post-natal effects	oral	mouse
Cytotoxicity	*in vitro*	Various human cell lines
		Freshly isolated human peripheral blood mononuclear cells
Bone Marrow Toxicity	*in vitro*	human bone marrow progenitor cells
Mitochondrial Toxicity	*in vitro*	human HepG2 cells
		human MT2 cells
	in vivo	liver, heart, and skeletal muscle from mice and monkeys in 3-month studies examined by EM
		Neurophysiologic studies in monkeys in 1-, 3-, and 12-month studies

Patients will take emtricitabine orally, so emtricitabine was given orally in all toxicity studies in order to mimic the intended clinical dose route. Two intravenous single-dose toxicity studies also were done.

Patients will take emtricitabine once-a-day. Multidose toxicology studies employed either once- or twice-daily dosing (specified for each study), but doses are expressed as the total daily dose in all reports. With twice-daily dosing, equal amounts were given with 5-6 hours between doses. Emtricitabine was given as the free base in toxicology studies, so no adjustment for salt form was necessary.

Patients will take emtricitabine continuously without "drug holidays," so animals in the multidose toxicology studies were dosed seven days per week.

In most of the toxicology studies emtricitabine was suspended in 0.5% aqueous methylcellulose because the concentrations necessary to achieve the high doses given in the studies exceeded the aqueous solubility of emtricitabine. All studies included appropriate vehicle control groups.

Unless otherwise noted, plasma concentrations of emtricitabine were measured in all toxicology studies in order to evaluate systemic exposures and toxicokinetic parameters.

5.3.2. Single-Dose (Acute) Toxicity Studies

The following table summarizes all of the single-dose (acute) toxicology studies conducted for the emtricitabine program.

Text Table 5.4.
Summary of Single-Dose (Acute) Preclinical Toxicology Studies

Study Type, Species/ Strain	No./Sex/Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
Acute Mice, Quanming Rats, Wistar	Mouse IV: 10 (20) Mouse PO: 10 (20) Rat PO: 10 (20)	Intravenous/ single dose PO (gavage)/ single dose PO (gavage)/ single dose	700 1050 1400	There were no deaths, no abnormal reactions during the 14-day observation period, and no abnormal changes in feed consumption or body weight.	6112	IND [redacted] Serial No. 082 [redacted]
Acute CD-1 Mice	5/sex (10)	PO (gavage)/ single dose	4,000	There were no deaths, no clinical symptoms of toxicity, no treatment-related changes in body weight, and no gross pathological findings.	6339	IND [redacted] Serial No. 000 [redacted]
Acute CD-1 Mice	5/sex (10)	Intravenous/ single dose	200	There were no deaths, no clinical symptoms of toxicity, no treatment-related changes in body weight, and no gross pathological findings.	6340	IND [redacted] Serial No. 000 [redacted]
Acute CD Rats	5/sex (10)	PO (gavage)/ single dose	4,000	There were no deaths, no clinical symptoms of toxicity, no treatment-related changes in body weight, and no gross pathological findings.	6341	IND [redacted] Serial No. 000 [redacted]
Acute CD Rats	5/sex (10)	Intravenous/ single dose	200	There were no deaths, no clinical symptoms of toxicity, no treatment-related changes in body weight, and no gross pathological findings.	6342	IND [redacted] Serial No. 000 [redacted]

5.3.3. Repeat-Dose (Subchronic and Chronic) Toxicity Studies

The following table summarizes all of the repeat-dose (subchronic and chronic) toxicology studies conducted for the emtricitabine program.

Text Table 5.5.
Summary of Repeat-Dose (Subchronic and Chronic) Preclinical Toxicology Studies

Study Type, Species/ Strain	No./Sex/Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
2-Week Mice/CD-1	Main Study: 15 (120) Clinical Pathology: 5 (40) Satellite (TK): 8 (48) Recovery: 5 (40) for 2 wks	PO (gavage)/ 2-wks TK on days 2 and 14 Pre-study clinical pathology on 5 (40), 5 (40) on Day 14, and 5 (40) at recovery.	0 (vehicle), 120, 600, 3000 (divided dose, 6 hr apart)	120 mg/kg/d: No effects. 600 mg/lkg/d: No effects. 3000 mg/kg/d: No effects on any of the measured parameters, including body weights, feed consumed, ophthalmology, clinical pathology, organ weights, or histopathology. TK: Mean plasma levels at 3000 mg/kg/day at 45 min post dose on days 2 and 14 were 318 and 389 µg/mL for males, respectively, and 358 and 405 µg/mL for females. The mid and low doses were proportionally lower.	466	IND [redacted] Serial No. 000 [redacted]
2-Week Mice/CD-1	8 (32)	PO (gavage)/ 2 wks	0 (vehicle), 120 (divided dose, 6 hr apart)	120 mg/kg/d: No effects.	9996	IND [redacted] Serial No. 253 [redacted]
4-Week Mice/CD-1	Main Study: 14 (112) Clinical Pathology: 10(80) Satellite (TK): 38 (228) Recovery: 4 (32) for 13 days	PO (gavage)/ 4-wks TK during Days 3 and 32 Clinical pathology pre-study and Days 31 and 32	0 (vehicle), 120, 600, 3000 (divided dose, 6 hr apart)	120 mg/kg/d: No effects. 600 mg/lkg/d: No effects. 3000 mg/kg/d: Increased MCV, MCH, and RDW. Increased relative spleen weight, heart, pituitary, and ovary weights, and decreased testes and thymus weights. No other treatment-related effects were seen. Additionally, there were no ultrastructural changes (TEM) in heart or skeletal muscle. TK: Mean high dose exposures ($AUC_{0\rightarrow\infty}$) in males and females were 1798 and 1709 hr*µg/mL (Day 3) and 1934 and 1514 hr*µg/mL (Day 32), respectively.	6318 6318.02	IND [redacted] Serial No. 000 [redacted] IND [redacted] Serial No. 007 [redacted]

Text Table 5.5.
Summary of Repeat-Dose (Subchronic and Chronic) Preclinical Toxicology Studies

Study Type, Species/ Strain	No./Sex/Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
4-Week Mice/CD-1	10 (80)	PO (gavage)/ 4 wks	0 (vehicle), 133, 400, 1200 (divided dose, 6 hr apart)	133 mg/kg/d: No effects. 400 mg/kg/d: No effects. 1200 mg/kg/d: Increased hematocrit, hemoglobin, and MCV. Increased male kidney weight without associated histopathologic kidney findings.	11362	IND [redacted] Serial No. 335 [redacted]
6-Month Mice/CD-1	Main Study: 30 (240) Satellite (TK): 40 (240)	PO (gavage)/ up to 26 wks Interim sac: Wk 13 for 10 (80) Terminal sac: Wk 26 for 15 (120) Recovery sac: Wk 30 for 5 (40)	0 (vehicle) 167, 500, 1500 (once daily dose)	167 mg/kg/d: no observed effect 500 mg/kg/d: no observed effect 1500 mg/kg/d: Increased mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) after 26 wks of treatment in males and females. Histopathology showed no effects related to treatment with emtricitabine. TK: Except for females at 167 mg/kg/d, mice at all doses had measurable emtricitabine in pre-dose plasma samples (lower limit of quantitation = 0.1 µg/mL). T_{max} was 30-60 minutes postdose. Systemic exposure ($AUC_{0\rightarrow24}$) increased proportionally as the dose increased. After 3 months' dosing, $AUC_{0\rightarrow24}$ at 1500 mg/kg/d was 513 and 570 h*µg/mL in males and females, respectively. After 6 months' dosing, corresponding $AUC_{0\rightarrow24}$ was 732 and 899 h*µg/mL, respectively.	3932 3932.02 (TK)	IND [redacted] Serial No. 040 [redacted] IND [redacted] Serial No. 276 [redacted]

Text Table 5.5.
Summary of Repeat-Dose (Subchronic and Chronic) Preclinical Toxicology Studies

Study Type, Species/ Strain	No./Sex/Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
6-Month Mice/CD-1	Main Study: 35 (280) Satellite (TK): 28 (168)	PO (gavage)/ up to 26 wks Interim sac: Wk 13 for 10 (80) Terminal sac: Wk 26 for 20 (160) Recovery sac: Wk 29 for 5 (40)	0 (vehicle), 120, 600, 3000 (once daily dose)	120 mg/kg/d: no observed effect after 3 or 6 months. 600 mg/kg/d: no observed effect after 3 or 6 months. 3000 mg/kg/d: After 3 and 6 months there were decreased RBC values (reversible), increased MCV (reversible), MCH and RDW values, and increased urine quantity (reversible),. After 6-months of dosing, increased absolute and relative thyroid weights in females (reversible) were observed. There were no other notable changes observed, including ultrastructural (TEM) changes in liver, cardiac or skeletal muscle. TK: Wk 13 high dose exposures ($AUC_{0\rightarrow24}$) for males and females were 1735 and 1432 hr*µg/mL, respectively. Corresponding Wk 25 exposures were 2024 and 1548 hr*µg/mL.	6361 (3-mo) 6384 (6-mo)	IND [redacted] Serial No. 006 [redacted] IND [redacted] Serial No. 010 [redacted]
3-Month Rat/CD	Main Study: 10 (80) Satellite (TK): 18 (108)	PO (gavage)/ 3 months	0 (vehicle), 120, 600, 3000	120 mg/kg/d: No observed effects. 600 mg/kg/d: No observed effects. 3000 mg/kg/d: Lower pituitary and thyroid gland weights without associated histopathologic findings. TK: Emtricitabine was rapidly absorbed, with females absorbing it more rapidly than males. Mean $t_{½}$ was 2.4-3.8 h on Day 2 and 3.6-6.5 h on Day 90. Day 90 C_{max} was 20-50% greater in females than males C_{max} was greater on Day 90 than Day 2 in both sexes (5-30% in males, 20-60% in females) $AUC_{0\rightarrow24}$ was the same on Days 2 and 90 in males but 20-40% greater on Day 90 than Day	10922	IND [redacted] Serial No. 335 [redacted]

Text Table 5.5.
Summary of Repeat-Dose (Subchronic and Chronic) Preclinical Toxicology Studies

Study Type, Species/ Strain	No./Sex/Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
				2 in females. Dose-normalized C_{max} tended to decrease with increasing dose but dose-normalized $AUC_{0\rightarrow24}$ remained relatively constant, suggesting a linear relationship between systemic exposure and daily dose in rats from 120 to 3000 mg/kg/day.		
4-Week Monkey/ cynomolgus	5 (40)	PO (gavage)/ 4 wks	0 (vehicle) 80, 400, 2000 (divided dose, 6 h apart)	80 mg/kg/d: no observed effects 400 mg/kg/d: no observed effects 2000 mg/kg/d: Increased soft feces in females. No other treatment-related effects were seen including body weight, clinical pathology, urinalysis, ophthalmology, neurophysiology, electrocardiography, organ weights, histopathology, neuropathology and electron microscopy (heart and skeletal muscle). TK: Male and female Days 3 and 27 exposures ($AUC_{0\rightarrow24}$) at the high dose were dose proportional, with Day 3 values at 1261 and 1529 hr*µg/mL, respectively, and corresponding Day 27 values at 1459 and 1462 hr*µg/mL.	6317 6317.02	IND [redacted] Serial No. 000 [redacted] IND [redacted] Serial No. 007 [redacted]

Text Table 5.5.
Summary of Repeat-Dose (Subchronic and Chronic) Preclinical Toxicology Studies

Study Type, Species/ Strain	No./Sex/Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
3-Month Monkey/ cynomolgus	5 (40)	PO (gavage)/ 13 wks Recovery sac: Wk 16 for 2 (16)	0 (vehicle), 40, 200, 1000. (divided dose, 6 h apart)	40 mg/kg/d: no observed effects 200 mg/kg/d: no observed effects 1000 mg/kg/d: no adverse effects in clinical signs, appetite changes, body weight, ophthalmology, electrocardiography, neurophysiology, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, neurohistopathology and histopathology. Additionally, samples of liver skeletal and cardiac muscle from control and high dose animals were subjected to ultrastructural examinations. TK: Dose proportional exposures ($AUC_{0\rightarrow24}$) were observed for Days 3 and 87. High-dose male and female values were 661 and 465 hr*µg/mL, respectively. Corresponding Day 87 values were 805 and 548 hr*µg/mL.	6385	IND [redacted] Serial No. 007 [redacted]
1-Year Monkey/ cynomolgus	Groups 1 and 4: 8 (32) Groups 2 and 3: 4 (16) Total: (48)	PO (gavage)/ 52 wks Interim sac: Wk 13 for 2 (8) in Groups 1 and 4 Recovery sac: Wk 56 for 2 (8) in Groups 1 and 4	0 (vehicle) 50, 200, 500 (divided dose, 6 h between doses)	50 mg/kg/d: no observed effects 200 mg/kg/d: no observed effects 500 mg/kg/d = FTC was well tolerated, with only a small decrease (non-significant) in mean erythrocyte counts and a significant increase in MCH values in females only. TK: Dose proportional exposures ($AUC_{0\rightarrow24}$) were observed. No sex differences noted. Wks 26 and 52 mean exposures at the high dose were 332 and 256 hr*µg/mL, respectively. Wks 1 and 13 values were similar.	9061 (3-mo) 3262 (6-mo)	Serial No. 080 [redacted]

5.3.4. Reproductive Toxicity Studies

The following table summarizes the reproductive toxicity studies conducted for the emtricitabine program.

Text Table 5.6.
Summary of Reproductive Toxicity Studies

Study Type, Species/ Strain	No./Sex/ Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
Fertility & Early Embryonic Development (Seg I) Mouse/ Crl:CD-1®BR	20 (80) treated females 20 (80) treated males	PO (gavage) M-≥4 wks prior to mating, and 10 full wks F-2 wks prior to and through cohabitation, then until gd 6	0 (control), 250, 500, 1,000 (divided dose, 6 h between doses)	250 mg/kg/d: no observed effect. 500 mg/kg/d: no observed effect. 1,000 mg/kg/d: no effect, fertility normal. Early embryonic development, inspected on gd-13, was normal. Conclusion: FTC had no effect on male or female fertility or on early embryonic development in treated females.	4436	IND [redacted] Serial 048 [redacted]
Male Fertility (Draft Report) Rat/Sprague Dawley	25 (100) treated males 25 (100) untreated pregnant females	PO (gavage) 73 days prior to mating and during mating. C-section on naïve untreated females on gd 13	0 (control), 150, 750, 3,000 (once daily)	150 mg/kg/d: no observed effect. 750 mg/kg/d: no observed effect. 3,000 mg/kg/d: The only effect was the clinical observation of post-dosing salivation. Sperm concentration and motility, organ weights, and histopathology were all normal. Conclusion: FTC had no effect on male fertility or early fetal development in naïve females.	6358	IND [redacted] Serial 082 [redacted]
Dose-Range Finding (for Seg II) Mouse/ Crl:CD-1®BR	5 (20) females	PO (gavage) Gestation days 6-15 C-section on gd-18	0 (control), 250, 500, 1,000 (divided dose, 6 h between doses)	250 mg/kg/d: no observed effect. 500 mg/kg/d: no observed effect. 1,000 mg/kg/d: One dam delivered 2 pups (with 11 pups still *in utero*) on gd-18 prior to euthanization. No external malformations or developmental variations were observed. Conclusion: FTC was well tolerated during pregnancy in mice at doses up to 1000 mg/kg/day.	2959	IND [redacted] Serial 032 [redacted]

Text Table 5.6.
Summary of Reproductive Toxicity Studies

Study Type, Species/ Strain	No./Sex/ Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
Dose-Range Finding (for Seg II) Rabbit/ New Zealand White, Hra:(NZW)SPF	5 (20) females	PO (gavage) Gestation Days 7-19 C-section on gd 29	0 (control), 250, 500, 1,000 (divided dose, 6 h between doses)	250 mg/kg/d: no observed effect. 500 mg/kg/d: no observed effect 1,000 mg/kg/d: no effect. All does survived until the necropsy with no treatment-related findings. Intrauterine growth and survival were unaffected. No external malformations or developmental variations were observed. Conclusion: No maternal or developmental toxicity was observed at any dose level.	3139	IND [redacted] Serial No. 032 [redacted]
Developmental Toxicity (Seg II) Mouse/ Crl:CD-1®BR	25 (100) females	PO (gavage) Gestation days 6-15 TK samples from 5/gp on gd 17 C-section on gd-18	0 (vehicle), 250, 500, 1,000 (divided dose, 6 h between doses)	250 mg/kg/d: no observed effect. 500 mg/kg/d: no observed effect. 1,000 mg/kg/d = no effect. There were no increased incidences of external, internal or skeletal variations or malformations. TK: Toxicokinetic evaluations demonstrated dose proportional plasma levels of drug with mean values at 0.5 hr post dose of 52 ± 15, 88 ± 14, and 186 ± 14 for the low-, mid, and high-dose groups, respectively. Conclusion: No teratogenic or other embryotoxic effects at 1,000 mg/kg/d. The NOAEL for maternal effects was >1,000 mg/kg/d.	4115	IND [redacted] Serial No. 040 [redacted]

Text Table 5.6.
Summary of Reproductive Toxicity Studies

Study Type, Species/ Strain	No./Sex/ Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
Fetal Toxicokinetic Mouse/ Crl:CD-1®BR	4 control and 8 treated females	PO (gavage) Gestation days 6-15 TK samples from dams (plasma) and fetuses (body homogenate) on gd 15	0 (vehicle), 1000 (divided dose, 6 h between doses)	1000 mg/kg/d: One female was non-gravid. Intrauterine growth and survival were unaffected by treatment. TK: The mean (± standard deviation) maternal plasma concentration of emtricitabine was 137.1 ± 28.0 µg/mL (n=7). The mean concentration of emtricitabine in pooled fetal homogenate was 55.7 ± 10.4 µg/mL. The mean fetal/maternal concentration ratio was 0.41 ± 0.04.	10424	IND [redacted] Serial No. 276 [redacted]
Developmental Toxicity (Seg II) Rabbit/ New Zealand White, Hra:(NZW)SPF	20 (80) females Satellites (TK): 5 (15) females	PO (gavage) Gestation Days 7-19 TK samples on gd 19 (does) and 20 (does + fetuses) C-section on gd-29	0 (control), 100, 300, 1,000 (divided dose, 6 h between doses)	100 mg/kg/d = no observed effect. 300 mg/kg/d = Body weight gain and feed consumption were reduced during gestation intervals of 15-18 and 18-20. 1,000 mg/kg/d = Body weight gain and feed consumption were reduced during gestation intervals of 11-15, 15-18 and 18-20. One doe aborted on gd-23. Intrauterine growth and survival were unaffected by FTC. Conclusion: There were no increased incidences of external, internal or skeletal variations or malformations at any dose. The NOAEL for maternal toxicity was 100 mg/kg/d.	9318	IND [redacted] Serial No. 276 [redacted]
Fetal Toxicokinetic Rabbit/NZW	5 (15) females	PO (gavage) Gestation Days 7-19 TK samples on gd 19 (does) and 20 (does + fetuses)	0 (control), 100, 300, 1,000 (divided dose, 6 h between doses)	At 1,000 mg/kg/day the AUC_{0-24} was 1257.8 hr*µg/mL and the C_{max} was 143.3 µg/mL		IND [redacted] Serial No. 276 [redacted]

Text Table 5.6.
Summary of Reproductive Toxicity Studies

Study Type, Species/ Strain	No./Sex/ Grp. (Total No.)	Route/ Dosing Duration	Dose (mg/kg/d)	Results	TPI Doc. #	IND No., Serial No., Date Submitted
Pre- & Postnatal Development (Seg III) Mouse/ Crl:CD-1®BR	25 (100) females only	PO (gavage) Gestation Day 6 through lactation Day 20	0 (control), 250, 500, 1,000 (divided dose, 6 h between doses)	100 mg/kg/d = no observed effect. 500 mg/kg/d = no observed effect 1000 mg/kg/d = F_1 dams had estrous cycles slightly longer (0.6 days) than controls. Conclusion: The NOAEL for reproductive and postnatal toxicity in the mouse was 1000 mg/kg/d.	5063	IND [redacted] Serial No. 048[redacted]

5.3.5. Genotoxicity (Mutagenicity) Studies

The following table summarizes the genotoxicity (mutagenicity) studies conducted for the emtricitabine program.

Text Table 5.7.
Summary of Genotoxicity (Mutagenicity) Studies

Study Type	Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Reverse Mutation Assay With Confirmatory Assay (*in vitro*)	FTC dissolved in DMSO was tested for mutagenic activity in the Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay. The concentrations tested were 5,000, 3,300, 1,000, 333 and 100 µg/plate in the presence and absence of S9 metabolic activation. The tester strains were *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli strain* WP2*uvr*A.	Positive control substances gave the expected positive results. FTC did not cause an increase in the number of revertants per plate in the tester strains in either the presence or absence of metabolic activation. The confirmatory assay, also gave negative results for mutagenic activity.	460	IND [redacted] Serial No. 000 [redacted]
Reverse Mutation Assay (*in vitro*)	FTC was tested in two Ames *Salmonella/* mammalian-microsome assays: the plate incorporation assay and the preincubation assay. The tester strains used were TA1535, TA1537, TA1538, TA98, and TA100, both in the presence and absence of Arochlor-induced rat liver S9 metabolic action. The range of concentrations of FTC were 100 to 5,000 µg/plate.	There was no detectable mutagenic activity at any concentration of FTC when tested by either Ames assay method.	7147	IND [redacted] Serial No. 000 [redacted]
Mouse Lymphoma Assay (*in vitro*)	FTC dissolved in sterile distilled water was tested in the L5178Y/TK $^{+/-}$ mouse lymphoma mutagenesis assay. Exposure to FTC were in the presence and absence of Arochlor-induced rat liver S9 metabolic activation. Cells at concentrations of 1,000, 2,000, 3,000, 4,000 and 5,000 µg/mL FTC were cloned and evaluated with and without S9.	The colony sizing for the methyl methanesulfonate positive control yielded the expected increase in small colonies, verifying the adequacy of the assay. Consistent with the low order of cytotoxicity established for FTC, no toxicity was observed in the cloned cultures regardless of FTC concentration.	501	IND [redacted] Serial no. 000 [redacted]

Text Table 5.7.
Summary of Genotoxicity (Mutagenicity) Studies

Study Type	Methods	Results/Conclusions	TPI Doc. #	IND No., Serial No., Date Submitted
Mouse Micronucleus Assay (*in vivo*)	FTC dissolved in sterile distilled water was given by gavage to groups of 5 male ICR mice. The doses were 0, 500, 1,000 and 2,000 mg/kg. The positive control was cyclophosphamide (60 mg/kg by gavage). Bone marrow was aspirated from the femur at 24 and 48 hours postdose. The slides of bone marrow were stained with May-Gruenwald-Giemsa stain. Light microscopy was used to count the number of micronuclei per 2000 polychromatic erythrocytes.	The positive control produced a marked and significant increase in micronuclei. No significant increase in micronucleated polychromatic erythrocytes was observed, at either time point or at any dose, in mice given FTC.	502	IND [redacted] Serial No. 000 [redacted]

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