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RESEARCH**

APPLICATION NUMBER:

208169Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on N208169 Xuriden Uridine Triacetate.

From A. Jacobs, AD

Date: 8/7/15

1. I concur that there are no pharm-tox approval issues, and that the nonclinical labeling is appropriate.
2. I have conveyed my other comments to the reviewer and supervisor, and they have addressed my comments, as appropriate.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ABIGAIL C JACOBS
08/07/2015

**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: 208169
Supporting document/s: 01
Applicant's letter date: January 8, 2015
CDER stamp date: January 8, 2015
Product: Uridine Triacetate
Indication: Uridine replacement therapy (b) (4) with hereditary orotic aciduria (HOA)
Applicant: Wellstat Therapeutics Corporation
Gaithersburg, MD
Review Division: Division of Gastroenterology and Inborn Errors
Products
Reviewer: Sruthi Tallapragada King, PhD
Supervisor/Team Leader: Sushanta Chakder, PhD
Division Director: Donna Griebel, MD
Project Manager: Jessica Benjamin, MPH

Disclaimer

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1 Executive Summary

1.1 Introduction

Uridine triacetate (2',3',5'-tri-O-acetyluridine) is an acetylated form of uridine, which is being developed by Wellstat Therapeutics Corporation for the treatment of hereditary orotic aciduria (HOA), a rare congenital autosomal recessive disorder, of which there are approximately 25 known cases worldwide. There are 4 HOA patients identified in the United States, all of whom were recruited into and completed the pivotal 6-week clinical trial and are now receiving uridine triacetate under the treatment extension phase of the protocol. The applicant is seeking approval of uridine triacetate (formulated as oral granules in 2 g and (b) (4) packets) for the treatment of HOA at a starting dose of 60 mg/kg/day. The dose may be increased up to (b) (4) mg/kg/day as clinically indicated.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical safety package for uridine triacetate included safety pharmacology studies, repeat-dose toxicology studies in dogs (3 month) and rats (3 and 6 months), genetic toxicology studies, and reproductive toxicology studies in rats (Segment 1 fertility and early embryonic development study and Segment 2 embryo-fetal development study).

In *in vitro* studies, uridine triacetate inhibited hERG channel current ($IC_{50} = 3137 \mu M$) and affected action potential duration (APD_{90}) at the doses $> 66.3 \mu M$. There was no effect on APD_{60} at $9600 \mu M$, which was the highest feasible concentration tested. Furthermore, because uridine triacetate undergoes complete and rapid deacetylation to uridine in the plasma after oral administration, the observed *in vitro* cardiac effects do not warrant concern. This was confirmed in the repeat-dose toxicology studies with uridine triacetate where no adverse cardiac effects were noted (in rats and dogs).

In animals, uridine triacetate had a generally favorable safety profile. In repeat-dose toxicology studies in rats (3 months and 6 months) and dogs (3 months), there were no significant, treatment-related toxicities or deaths at up to the 2000 mg/kg/day, which was the maximum feasible dose. The no observed adverse effect level (NOAEL) dose from the 6-month repeat-dose toxicity study in rats was 2000 mg/kg/day, which is (b) (4) times the maximum recommended human dose of (b) (4) mg/kg/day, on a body surface area basis. Uridine triacetate was not genotoxic in *in vitro* and *in vivo* assays. Carcinogenicity studies were not conducted with uridine triacetate; however, there were no findings suggestive of tumorigenic potential in the 6-month repeat-dose toxicity study in rats. Uridine triacetate did not affect fertility and reproductive ability in rats of either sex and did not produce maternal toxicity (during gestation) or teratogenic effects in developing fetuses at up to 2000 mg/kg/day, which was the highest dose administered.

(b) (4)

Uridine triacetate is absorbed readily after oral administration and rapidly deacetylated to uridine and free acetate, producing elevated plasma uridine levels, without detectable plasma levels of the acetylated form. At equimolar doses, the bioavailability of uridine triacetate after oral administration is 4-7x greater than uridine, which has poor oral bioavailability (6-10%) and produces dose-limiting diarrhea at high doses. Uridine triacetate provides systemic uridine and therefore, an exogenous source of pyrimidine nucleotides by bypassing the deficient metabolic pathway in HOA patients.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, this product is approvable for indication proposed.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Applicant's submitted version:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

(b) (4)

Recommended Version:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no available data on XURIDEN use in pregnant women to inform any drug-associated risks. When administered orally to pregnant rats during the period of organogenesis, uridine triacetate at doses similar to the maximum recommended

human dose (MRHD) of (b) (4) mg/kg per day was not teratogenic and did not produce adverse effects on embryo-fetal development [see *Data*]. The background risk of major birth defects and miscarriage for the indicated population are unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Data

Animal Data

In an embryo-fetal development study, uridine triacetate was administered orally to pregnant rats during the period of organogenesis at doses up to 2000 mg/kg per day (about (b) (4) times the maximum recommend human dose (MRHD) of (b) (4) mg/kg per day on a body surface area basis). There was no evidence of teratogenicity or harm to the fetus and no effect on maternal body weight and overall health.

Applicant's Submitted Version:

13.1 NONCLINICAL TOXICOLOGY

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of uridine triacetate. (b) (4)

(b) (4)

(b) (4)





Recommended Version:

13.1 NONCLINICAL TOXICOLOGY

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of uridine triacetate.

Uridine triacetate was not genotoxic in the Ames test, the mouse lymphoma assay and the mouse micronucleus test.

Orally administered uridine triacetate did not affect fertility or general reproductive performance, in male and female rats at doses up to 2000 mg/kg per day (about (b) (4) times the maximum recommended human dose (MRHD) of (b) (4) mg/kg per day on a body surface area basis).

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 4105-38-8

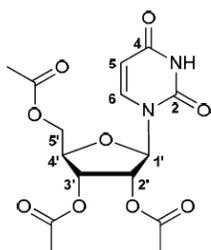
Generic Name: Uridine triacetate

Code Name: PN401, TAU, vistonuridine, UTA

Chemical Name: 2',3',5'-tri-O-acetyluridine

Molecular Formula/Molecular Weight: C₁₅H₁₈N₂O₉ / 370.31

Structure or Biochemical Description:



Pharmacologic Class: Pyrimidine analog

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 118931 Uridine Triacetate

2.3 Drug Formulation

Granules packed in 2g and (b) (4) packets

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern



(b) (4)

(b) (4)

The applicant's table below summarizes the impurity level release from the registration batches of uridine triacetate and a subsequent batch.

Table 3.2.S.4.5-3 Impurity Level Release results from Three Uridine Triacetate Drug Substance Registration Batches and One Subsequent Batch

		Batch Number
Compound	Specification (% HPLC AUC)	(b) (4)
		(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

The applicant is seeking the approval of uridine triacetate for the indication of treatment of hereditary orotic aciduria. The recommended starting dose of oral uridine triacetate is 60 mg/kg/day and can be increased to a maximum dose of (b) (4) mg/kg/day, as clinically indicated. The daily dose of uridine triacetate can be administered as a single dose or (b) (4). Uridine triacetate can be administered (b) (4) or mixed with soft foods such as applesauce, yogurt or pudding. (b) (4) Uridine triacetate will be available as 2 g or (b) (4) packets.

2.7 Regulatory Background

Wellstat Therapeutics Corporation, developed uridine triacetate as a uridine replacement therapy. The current supplier of uridine is discontinuing production and Wellstat was contacted by FDA (OND/DGIEP, Office of Orphan Products Development, Office of Drug Shortages, and the Office of Rare Diseases) to develop a replacement

product for HOA patients. Orphan Drug Designation was granted for uridine triacetate for the treatment HOA on August 9, 2013. Uridine triacetate was also designated as a potential new drug for a rare pediatric disease for the treatment of hereditary orotic aciduria on August 9, 2013 (as defined in section 529(1)(3) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360ff(a)(3), Request #13-401V). On April 30, 2014, Breakthrough Therapy Designation was also granted for uridine triacetate for the treatment of HOA in pediatric patients.

At the pre-IND meeting on August 7, 2013, the Division recommended that a 6-month repeat-dose toxicity study in rats (with no recovery period), a Segment I Fertility and Early Embryonic Development Study in rats, and a Segment II Embryo-fetal development study in one species would be needed to support the NDA. The Division recommended that the proposed clinical trial could be initiated prior to completion of the Segment I study, provided that the Informed Consent Documents clearly indicate the lack of preclinical data and communicate the risks involved. The Division also recommended that the Segment III study pre- and postnatal development (PPND) study could be conducted post-approval.

At the pre-NDA meeting on December 16, 2014, the Division agreed that the nonclinical safety package for uridine triacetate was complete and no additional nonclinical studies would be required to support the NDA.

During the Midcycle communication meeting (via teleconference) held on April 29, 2015, the Division reminded the applicant that a PMR will be issued for the PPND study with orally administered uridine triacetate in rats. However, upon further internal discussion, it was decided that a PMR for the PPND would not be issued. The applicant is to be informed at the late cycle meeting, which had not occurred at the time this review was finalized.

3 Studies Submitted

3.1 Studies Reviewed

Study #	Study Title	GLP	Page
<i>Pharmacology</i>			
120119.XFM	Effect of Uridine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	Yes	15
120120.XFM	Effect of Uridine on Action Potentials in Isolated Rabbit Cardiac Fibers	Yes	15
121130.XFM	Effect of Uridine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	Yes	15
120201.XFM	Effect of Uridine on Action Potentials in Isolated Rabbit Cardiac Fibers	Yes	16
R.401.12.01	Evaluation of PN401 (uridine triacetate) in a recombinant hERG Potassium Ion Channel Membrane Binding Assay	No	16
<i>Pharmacokinetics</i>			
R.401.14.02	Comparative Oral Pharmacokinetics of Uridine and Uridine Triacetate in Mice	No	17
13WELLP1R1, Study	Determination of the P-gp Interaction Potential for the Sponsor's	No	17

II	Test Articles, Uridine and Uridine Triacetate		
13WELLP1R1_Study I	CYP Inhibition by Uridine and Uridine Triacetate	No	17
Toxicology			
68	Acute Oral Toxicity Test in Rats	Yes	18
(b) (4) Study No 552	PN401: A 3-month Oral Dose Toxicity Study	Yes	19
(b) (4) Study No 71	Sub Chronic Toxicology Study in Rats	Yes	23
(b) (4) Study No 551	PN401: A 3-month Oral Dose Toxicity Study in the (b) (4) Rat	Yes	26
2648-100	Subacute Oral Toxicity Study in Dogs	Yes	31
20047236	A 6-month Study of Uridine Triacetate Administered by Oral Gavage (Twice Daily) in Rats	Yes	32
Genetic Toxicology			
9600345	Uridine Triacetate: Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli	Yes	40
16457-0-401	Mutagenicity Test on PN401 In the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)	Yes	43
9600346	Uridine Triacetate: In vitro Mammalian Cell Gene Mutation Test in Mouse Lymphoma L5178Y TK+/- Cells	Yes	47
16457-0-455CO	Genetic Toxicity Evaluation of PN401 In An In Vitro Mouse Micronucleus Oral Limit Dose Assay	Yes	50
Reproductive Toxicology			
20047304	Study of Fertility and Early Embryonic Development to Implantation of Uridine Triacetate Administered by Oral Gavage (Twice Daily) in Rats	Yes	55
20040947	An Embryo-fetal Development Study of Uridine Triacetate by Oral Gavage (Twice Daily) in Rats	Yes	59

3.2 Studies Not Reviewed

(b) (4)

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

No primary pharmacology study reports were submitted.

4.2 Secondary Pharmacology

No secondary pharmacology study reports were submitted.

4.3 Safety Pharmacology

Table 1 below summarizes the findings from the Safety Pharmacology studies submitted by the applicant.

Table 1: Safety pharmacology studies conducted with uridine triacetate and uridine

Study # Study Title	Doses used	GLP Status	Findings
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Study Initiation Date			
<p>120119.XFM</p> <p>Effect of Uridine Triacetate on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells</p> <p>November 1, 2013</p>	<p>1 mM (measured 0.6 mM), 10 mM uridine (measured 9.6 mM) triacetate</p>	<p>GLP</p>	<ul style="list-style-type: none"> • Uridine triacetate inhibited hERG current by $74\% \pm 1.4\%$ at 9.6 mM and $18\% \pm 2.9\%$ at 0.6 mM (mean \pm SD, N=3) • $IC_{50} = 3.1$ mM uridine triacetate • Positive control 60 nM terfenadine inhibited hERG current by $77.1 \pm 1.5\%$ (mean \pm SD, N=3) • Vehicle (HEPES-buffered saline) inhibited hERG by $1.1 \pm 0.4\%$ (mean \pm SD, N=3) <p><i>Conclusion: Uridine triacetate inhibited hERG current by 74% at a maximum concentration of 9.6 mM, as compared to vehicle control.</i></p>
<p>120120.XFM</p> <p>Effect of Uridine Triacetate on Action Potentials in Isolated Rabbit Cardiac Fibers (New Zealand White Rabbits)</p> <p>February 5, 2014</p>	<p>66.3, 774, 8582 μM</p>	<p>GLP</p>	<p>1 sec cycle length:</p> <ul style="list-style-type: none"> • Statistically significant prolongation of action potential duration (APD₉₀) at 774 μM and 8582 μM by $12.8 \pm 3.0\%$ and $26.5 \pm 7.9\%$, respectively, as compared to Vehicle (Rabbit Purkinje fiber Tyrode's solution), $p < 0.05$. • Statistically significant increase in resting membrane potential (RMP) at 8582 μM ($13.1 \pm 3.9\%$) and a decrease in action potential amplitude (APA) ($50.8 \pm 4.2\%$) and maximum rate of depolarization ($85 \pm 2.1\%$), as compared to Vehicle, $p < 0.05$ <p>0.5 sec cycle length:</p> <ul style="list-style-type: none"> • Statistically significant prolongation of action potential duration (APD₉₀) at 774 μM and 8582 μM by $12.1 \pm 1.8\%$ and $38.9 \pm 11.3\%$, respectively, as compared to Vehicle, $p < 0.05$. • Statistically significant increase in resting membrane potential at 8582 μM ($12.6 \pm 2.6\%$) and a decrease in action potential amplitude ($55.7 \pm 4.7\%$) and maximum rate of depolarization ($86.5 \pm 1.7\%$), as compared to Vehicle, $p < 0.05$ <ul style="list-style-type: none"> • 50 μM Sotalol (positive control) produced a 37.4-62.3% prolongation in APD₆₀ and APD₉₀ <p><i>Conclusion: Uridine triacetate produced a statistically significant increase in APD at the highest dose tested (8582 μM), increased RMP, decreased APA and rate of depolarization, as compared to vehicle control.</i></p>
<p>121130.XFM</p> <p>Effect of Uridine on Cloned hERG Potassium Channels Expressed in</p>	<p>10 mM</p>	<p>GLP</p>	<ul style="list-style-type: none"> • Uridine inhibited hERG channel current by $4.2 \pm 1.0\%$ (mean \pm SD, N = 3) • Vehicle control (HEPES-buffered physiological saline) inhibited hERG channel current by $2.5 \pm 0.4\%$ (mean \pm SD,

Human Embryonic Kidney Cells November 26, 2013			<p>N = 3)</p> <ul style="list-style-type: none"> Positive control (60 nM terfenadine) inhibited hERG current by $85.3 \pm 0.0\%$ (mean \pm SD, N = 2) <p><i>Conclusion: Uridine did not produce statistically significant inhibition of hERG current at 10 mM, as compared to vehicle control. IC₅₀ for inhibition of hERG by uridine is greater than 10 mM.</i></p>
120201.XFM Effect of Uridine on Action Potentials in Isolated Rabbit Cardiac Fibers February 7, 2014	0.1, 1, 10 mM	GLP	<ul style="list-style-type: none"> Uridine produced minimal changes in the action potential parameters in vitro. In most cases, treatment-related changes in APD₆₀ and APD₉₀ were negligible (-2.0 – 5.6%), as compared to vehicle (3.3 -12.2%) Statistically significant decrease in APD₉₀ was noted at 0.1 and 1 mM (-2.0 and 0.4%, respectively), as compared to vehicle treatment at 0.5 sec basic cycle length (BCL). There were no significant changes in RMP, APA or dV/dt_{max} related to treatment with uridine. Positive control dl-Sotalol produced 78.6% and 70.9% increase in APD₆₀ and APD₉₀, respectively at 1 sec BCL and 57.5 and 55% increase in APD₆₀ and APD₉₀, respectively at 0.5 sec BCL. <p><i>Conclusion: Uridine at concentrations up to 10 mM did not produce significant changes to action potential parameters in vitro, as compared to vehicle treatment.</i></p>
R.401.12.01 Evaluation of PN401 (uridine triacetate) in a recombinant hERG Potassium Ion Channel Membrane Binding Assay April 4, 2012	10 μ M, 100 μ M, 1 mM	Not GLP	<ul style="list-style-type: none"> Using radioligand binding assays, the IC₅₀ of uridine triacetate for binding hERG was determined. <p><i>Conclusion: Under the experimental conditions, the IC₅₀ for binding hERG was shown to be > 1 mM.</i></p>

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The results from the PK/ADME studies conducted with uridine triacetate and uridine are summarized in **Table 2** below.

Table 2: PK/ADME studies conducted with uridine triacetate.

Study # Study Title	Doses used	GLP Status	Findings
------------------------	------------	---------------	----------

<p>R.401.14.02 Comparative Oral Pharmacokinetics of uridine and uridine triacetate in mice</p>	<p>Molar equivalent doses of uridine and uridine triacetate used “Low” dose: 300 mg/kg uridine, 460 mg/kg uridine triacetate (N = 30 females/group) “High” dose: 4000 mg/kg uridine and 6066 mg/kg uridine triacetate (N = 24 females/group)</p>	<p>Not GLP</p>	<ul style="list-style-type: none"> When administered by oral gavage at molar equivalent doses to female BALB/c mice, uridine triacetate has higher oral bioavailability, as compared to orally administered uridine. Systemic exposure (AUC and Cmax) to uracil also higher after oral administration of uridine triacetate, as compared to uracil. PK results from comparative oral studies are shown in the table below: <table border="1" data-bbox="816 506 1421 982"> <thead> <tr> <th colspan="6">PK parameters for uridine</th> </tr> <tr> <th>Dose (mg/kg)</th> <th></th> <th>AUC_(0-t) (µM·h)</th> <th>C_{max} (µM)</th> <th>T_{max} (h)</th> <th>t_{1/2} (h)</th> </tr> </thead> <tbody> <tr> <td>300</td> <td>Uridine</td> <td>14.12</td> <td>7.02</td> <td>0.25</td> <td>0.77</td> </tr> <tr> <td>460</td> <td>Uridine triacetate</td> <td>121.60</td> <td>197.80</td> <td>0.25</td> <td>0.69</td> </tr> <tr> <td></td> <td>Fold increase</td> <td>8.6</td> <td>28.2</td> <td></td> <td></td> </tr> <tr> <td>4000</td> <td>Uridine</td> <td>206.8</td> <td>60.8</td> <td>1.0</td> <td>1.19</td> </tr> <tr> <td>6066</td> <td>Uridine triacetate</td> <td>2627.3</td> <td>864.0</td> <td>2.0</td> <td>1.20</td> </tr> <tr> <td></td> <td>Fold increase</td> <td>12.7</td> <td>14.4</td> <td></td> <td></td> </tr> <tr> <th colspan="6">PK parameters for uracil</th> </tr> <tr> <td>300</td> <td>Uridine</td> <td>26.92</td> <td>14.32</td> <td>0.25</td> <td>1</td> </tr> <tr> <td>460</td> <td>Uridine triacetate</td> <td>258.17</td> <td>312.40</td> <td>0.5</td> <td>0.38</td> </tr> <tr> <td></td> <td>Fold increase</td> <td>9.6</td> <td>21.8</td> <td></td> <td></td> </tr> </tbody> </table>	PK parameters for uridine						Dose (mg/kg)		AUC _(0-t) (µM·h)	C _{max} (µM)	T _{max} (h)	t _{1/2} (h)	300	Uridine	14.12	7.02	0.25	0.77	460	Uridine triacetate	121.60	197.80	0.25	0.69		Fold increase	8.6	28.2			4000	Uridine	206.8	60.8	1.0	1.19	6066	Uridine triacetate	2627.3	864.0	2.0	1.20		Fold increase	12.7	14.4			PK parameters for uracil						300	Uridine	26.92	14.32	0.25	1	460	Uridine triacetate	258.17	312.40	0.5	0.38		Fold increase	9.6	21.8		
PK parameters for uridine																																																																											
Dose (mg/kg)		AUC _(0-t) (µM·h)	C _{max} (µM)	T _{max} (h)	t _{1/2} (h)																																																																						
300	Uridine	14.12	7.02	0.25	0.77																																																																						
460	Uridine triacetate	121.60	197.80	0.25	0.69																																																																						
	Fold increase	8.6	28.2																																																																								
4000	Uridine	206.8	60.8	1.0	1.19																																																																						
6066	Uridine triacetate	2627.3	864.0	2.0	1.20																																																																						
	Fold increase	12.7	14.4																																																																								
PK parameters for uracil																																																																											
300	Uridine	26.92	14.32	0.25	1																																																																						
460	Uridine triacetate	258.17	312.40	0.5	0.38																																																																						
	Fold increase	9.6	21.8																																																																								
<p>13WELLP1R1, Study II Determination of P-gp Interaction potential for the sponsor’s test articles, uridine and uridine triacetate</p>	<p>Nonspecific binding experiment: 0.5 µM uridine triacetate or uridine Caco-2 tolerability study: 1000, 5000, 15000 µM uridine or uridine triacetate P-gp interaction study: 0.5, 5, 50 mM uridine triacetate or uridine</p>	<p>Non GLP</p>	<ul style="list-style-type: none"> Uridine triacetate showed little to no nonspecific binding (recovery > 80% from experimental device) Because of the unusually low recovery of uridine from experimental device (< 10%), additional experiments (P-gp assessment) were not done. Caco-2 cell monolayer can tolerate up to 15000 µM of uridine triacetate or uridine Uridine triacetate inhibited P-gp with an IC₅₀ of 344 µM. Uridine was not a significant inhibitor of P-gp at up to 15000 µM. Uridine triacetate may be a P-gp substrate at 5 and 50 µM 																																																																								
<p>13WELLP1R1_Study 1 CYP inhibition and induction by uridine and uridine triacetate</p>	<p>0, 0.0137, CYP inhibition studies: 0.0412, 0.123, 0.370, 1.11, 3.33, 10.0 mM uridine triacetate or uridine CYP induction studies: 50 and</p>	<p>Non GLP</p>	<ul style="list-style-type: none"> Uridine inhibited CYP1A2, 2A6, 2B6, 2C9, 2D6, 2E1, and 3A with an IC₅₀ > 10 mM. Uridine inhibited CYP2C19 and 3A (midazolam) with an IC₅₀ of 5.1 and 2.0 mM, respectively, in human liver microsomes. Uridine triacetate inhibited CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, and 3A (testosterone) with an IC₅₀ > 10 mM. Uridine triacetate inhibited 2C19 and 3A (midazolam) with an IC₅₀ of 6.6 and 8.3 mM, respectively, in human liver microsomes. 																																																																								

	500 μ M uridine triacetate and 50, 500, 5000 μ M uridine		<ul style="list-style-type: none">• Uridine triacetate at 5 mM produced cytotoxicity (33% cell viability), as measured by the MTS assay, under the conditions of the study.• In fresh human liver hepatocytes, no induction of CYP1A2, 2B6, or 3A4 mRNA was observed at up to 500 μM uridine triacetate and 5000 μM uridine
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6 General Toxicology

6.1 Single-Dose Toxicity

Study title: (b) (4) Study No. 68: Acute Oral Toxicity Test in Rats

When administered as a single oral dose of 5000 mg/kg, uridine triacetate was not toxic and did not produce any treatment-related adverse effects on clinical signs or body weight at up to 14 days post-dose.

6.2 Repeat-Dose Toxicity:**Study title: 3-month oral dose toxicity study in the beagle dog**

Study no.: (b) (4) Study 552
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: September 30, 1994
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PN401, Lot # 1911-A-5P-Load 2, 99.13%
 Vehicle: Gelatin capsules, #11 Torpac (8000#), Batch # 296

Key Study Findings

When administered orally to beagle dogs (N = 4/sex/group), uridine triacetate was well tolerated at doses up to 1500 mg/kg/day (administered in 2 equal doses, 6 h apart). The NOAEL dose was 1500 mg/kg/day. Peak plasma uridine and uracil levels were noted at 2 h post dose and returned to baseline by 6 h post dose, suggesting that there was no accumulation of either uridine or uracil with twice daily dosing of uridine triacetate.

Methods

Doses: 0, 375, 750, 1500 mg/kg/day
 Frequency of dosing: Twice daily, doses administered 6 h apart
 Route of administration: Oral
 Dose volume: N/A
 Formulation/Vehicle: Test article was loosely packed into capsules for oral administration. Control animals received the same number of empty capsules as the high dose animals.
 Species/Strain: Dog/Beagle
 Number/Sex/Group: 4/sex/group
 Age: 5 months of age
 Weight: 5.9-9.1 kg (males), 5.2-7.1 kg (females)
 Satellite groups: None
 Unique study design: No
 Deviation from study protocol: None which affected the outcome of this study

Observations and Results**Mortality**

Animals were observed twice daily for signs of mortality and morbidity, with detailed individual examination once a week during the course of the study. There were no unscheduled treatment-related deaths during the course of the study. One male assigned to the mid-dose (MD) group was euthanized on Day 1 prior to dose

administration due to oral papillomatosis. The applicant noted that it was likely other animals were infected with the virus, despite precautionary measures taken to minimize infection.

Clinical Signs

Animals were observed twice daily for signs of mortality and morbidity, with detailed individual examination once a week during the course of the study.

There were no treatment-related changes in body temperature, heart rate or respirations in either sex. Four males (1 control, 1 MD, and 2 high dose (HD)) also developed papillomas between Study Days 26-31; however, the presence of the papillomas did not interfere with the animals' wellbeing, eating or drinking behaviors. Therefore, because of the absence of any adverse physiological effects, the affected animals were allowed to remain in the study and did not affect overall study results.

Body Weights

Body weights were recorded at pretest, prior to randomization, twice a week during the week before dosing, on Day 1 of dosing, weekly thereafter, and at necropsy.

There were no treatment-related changes in body weight during the course of the study in either sex.

Feed Consumption

Individual food consumption was measured twice before dosing initiation and weekly thereafter.

There were no treatment-related changes in food consumption during the course of the study in either sex.

Ophthalmoscopy

Animals were examined before initiation of treatment and during Week 12.

There were no treatment-related ophthalmoscopic findings in either sex.

ECG

ECGs were conducted before treatment initiation and during Weeks 6 and 12.

There were no treatment-related changes to ECG in either sex.

Hematology

Sampling for hematology analysis was conducted at Week -2 and Week -1 before

initiation of dosing, and during Weeks 6 and 12 of the study.

There was a statistically significant decrease of 8.3% and 13.8% in prothrombin time in MD and HD males, respectively, during Week 12, as compared to controls ($p \leq 0.05$). There was a slight decrease in WBC in MD and HD females during Week 12, but these changes were not statistically significant. There was a statistically significant decrease (~13%) in prothrombin time in HD females during Week 12, as compared to controls ($p \leq 0.01$). There were no other treatment-related changes in hematological parameters during the course of the study.

Clinical Chemistry

Sampling for clinical chemistry analysis was conducted at Week -2 and Week -1 before initiation of dosing, and during Weeks 6 and 12 of the study.

There were occasional treatment-related changes in clinical chemistry parameters, but a dose response was not apparent. There was small but statistically significant increase (1.8%) in CI level in LD males and a decrease (9.5%) in Phos level in MD males during Week 12, as compared to controls ($p \leq 0.01$ and $p \leq 0.05$, respectively). There were no toxicologically significant treatment-related changes in clinical chemistry parameters.

Gross Pathology

Full necropsy was conducted on Day 86 of the study.

The observed gross lesions are shown in the applicant's table 2 below.

TABLE 2
SUMMARY OF
GROSS LESIONS

TISSUE	LESION**	Group 1		Group 2		Group 3		Group 4	
		Male*	Female*	Male*	Female*	Male*	Female*	Male*	Female*
Liver	Adhesion	1 (101)							
Kidney	Enlarged					1 (354)		1 (401)	
Kidney	Absent					1 (354)		1 (401)	
Spleen capsule	Lesion, white		1 (154)						1 (453)
Heart valve	Hematocyst					1 (351)			
Gingiva	Papilloma(s)			2 (202, 204)		1 (303)			
Tongue	Papilloma			1 (204)					
Testes	Small					1 (304)			

*=Figures are total affected within the group followed by, in parentheses, the animal numbers of the affected animals.

**=Lesion description is abbreviated, in some cases, from the total description on the necropsy records.

An enlarged kidney was noted in a MD female and a HD male. This was thought to be a compensatory physiological adaptation to the congenital absence of the other kidney. Oral papillomas were noted in several LD and MD males. However, the presence of the papillomas did not affect the wellbeing of the affected animals. Therefore, this finding was not considered to be toxicologically significant. One MD male had small testes.

Organ Weights

The following organs were harvested and weighed: adrenals, heart, kidneys, liver, spleen, and testes (with epididymides).

There were no statistically significant treatment-related changes in organ weights in any dose group.

Histopathology

Adequate Battery: Yes (Histopathology studies were done for Group 1 and Group 4 animals only). The harvested tissues were: animal identification, adrenals, aorta (thoracic), bone/marrow (sternum), brain (three levels), cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, and vagina.

Peer Review: No

Histological Findings: Chronic inflammation was noted in the kidney of 1 control male and vasculitis was noted in the testes of one HD male. Vasculitis and mineralization were noted in the stomach and chronic inflammation was noted in the liver of 1 HD male. Chronic meningeal inflammation in the brain was noted in 2 control females. C-cell hyperplasia in the thyroid lobes were noted in 1 control female and 2 HD females. Chronic inflammation of the tongue was noted in 3 HD females. Lymphocytic inflammation in the stomach was noted in 2 control females and 1 HD female. In the spleen, capsular fibrosis was noted in a control and HD female, while capsular mineralization was noted in 1 control female. Thickened meninges of the optic nerves were noted in 1 HD female.

Special Evaluation

None

Toxicokinetics

TK parameters were assessed on samples collected on Day 1 and Day 80. Oral administration of uridine triacetate produced systemic exposure to uridine and its metabolite uracil. Exposure to both uridine and uracil increased with dose. Peak plasma concentration of uridine was observed at 2 h post-dose and plasma levels returned to baseline by 6 h, suggesting that there was no accumulation of uridine with twice daily dosing. For uracil, T_{max} was at 2 h for animals dosed with ≤ 350 mg/kg/day (BID) and at 4 h for animals dosed with 750 mg/kg/day (BID); however, plasma levels returned to baseline by 6 h. The TK results are summarized in **Table 3** below.

Table 3: TK parameters for uridine and uracil in beagle dogs orally dosed with uridine triacetate for 3 months

Daily Dose (mg/kg)	0 (Control)		187.5 mg/kg bid (375 mg/kg/day)		375 mg/kg bid (750 mg/kg/day)		750 mg/kg bid (1500 mg/kg/day)	
	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics: Day 80								
Plasma Uridine: C _{max} (µM) ^a	NA	NA	66.98	50.27	212.83	200.71	678.55	447.20
Plasma Uracil: C _{max} (µM) ^a	NA	NA	147.28	107.01	320.08	373.78	586.05	479.48
Plasma Uridine: AUC _{0-t} (µM•h) ^a	NA	NA	124.80	78.64	412.43	398.00	1402.45	956.74
Plasma Uracil: AUC _{0-t} (µM•h) ^a	NA	NA	268.62	203.85	757.58	908.65	2216.34	1589.75

Dosing Solution Analysis

Study samples were analyzed at the beginning and end of the study. Test article was shown to be stable during the course of the study.

Study title: Sub-chronic toxicology study in rats

Study no.: 71
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: November 7, 1991
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: 2',3',5'-Tri-o-acetyluridine, 1911-B-1, 99.49%

Key Study Findings

An emulsion of uridine triacetate in corn oil/water/Tween-80 was administered at up to 5 g/kg/day by oral gavage to F344 rats for 6 weeks. There were several deaths of females in all dose groups early in the study which were attributed to dosing-related errors. No overt treatment-related toxicities or target organs of toxicity were identified. However, there was irreversible mineralization in the kidneys in females in all dose groups, including controls, and in 1 HD male. A NOAEL dose was not identified.

Methods

Doses: 0, 1.25, 2.5, 5 g/kg/day (administered in 2 equal doses)
 Frequency of dosing: Twice daily, five times per week for 6 weeks
 Route of administration: Oral gavage
 Dose volume: 1 mL/100 g body weight
 Formulation/Vehicle: Emulsion in corn oil/water/Tween-80
 Vehicle: Corn oil and water (1:1) + 2.5% Tween-80 (polysorbate 80, polyoxyethylenesorbitan)

mono-oleate)
Species/Strain: Rat/F344
Number/Sex/Group: 12/sex/group
Age: 10 weeks old (male), 11 weeks old (female)
Weight: Not specified
Satellite groups: None
Unique study design: 75% of treated animals were sacrificed at the end of 6 weeks and remaining animals were maintained for a 2-week, treatment-free period before sacrifice.
Deviation from study protocol: None which affected the results of the study

Observations and Results

Mortality

There were several unscheduled deaths in females early on in the dosing period (before Day 12): 4 controls, 3 LD, 2 MD, and 1 HD. The deaths of these animals occurred within the first 12 days of dosing. Necropsy on these animals revealed test article in lungs, chest cavity and/or abdominal cavity, suggesting dosing-related injury as the cause of death. The gavage needle was subsequently changed from a 16-gauge, 3 inch feeding needle with a ball end to 18-gauge, 1.5 inch feeding needle of the same type. No additional deaths attributed to dosing-related injury were recorded. One HD male animal died later in the study (sacrificed on Day 25) due to aspiration of test article into lungs.

Clinical Signs

One control female was observed to have lost weight, exhibiting hunched posture and a rough coat. Upon examining this animal, the veterinarian recommended withholding the next 4 doses to allow the animal to recover, as the observed clinical signs were consistent with either damage to lungs an internal organs or irritated esophagus. The animal was returned to regular dosing once symptoms improved. This animal regained weight and was on par with other animals in the same dose group. Necropsy on this animal at the end of dosing revealed white-yellow exudate in the thoracic cavity, severe pyrogranulomatous pleuritic and pneumonitis, and mononuclear cell infiltrate in the lungs.

The rats which died (or were sacrificed) early on in the dosing period exhibited lethargy, rough coats and hunched posture. These animals had white material (probably test article) in lungs, chest cavity, and/or abdominal cavity; these were likely related injuries while dosing. One HD male developed head tilt, which lasted throughout the dosing period and recovery period; however, histopathology of brain and ear of this animal did not reveal any abnormalities.

Other clinical signs include alopecia and swollen eye (unilateral) in a few animals; however, affected animals healed without any intervention.

Body Weights

All animals gained weight over the course of the study. There were no treatment-related effects on body weight.

Feed Consumption

Not reported

Clinical Chemistry and Hematology

There were occasional statistically significant changes in a few clinical chemistry and hematology parameters; however, dose-dependence was unclear.

Gross Pathology

The major finding in both sexes across all dose groups was white or red foci in the lungs and thymus, with fewer foci in recovery animals. Enlarged lymph node was found in 4/8 HD males, although there was no correlating histopathology finding. Red foci or red coloration of the thymus was noted in both sexes, with the incidence of this finding higher in recovery animals.

Organ Weights

Not reported

Histopathology

Adequate Battery: Yes

Peer Review: Not indicated

Histological Findings: Mineralization of the kidney was observed in females in all dose groups (3 of 6 control, 5 of 6 LD, 6 of 7 MD, and 1 of 8 HD) and in 1 HD male (N = 8/dose group). Mineralization was observed in 2, 3, 3, and 3 recovery females in the control, LD, MD, and HD groups, respectively (N=3/dose) and 1/3 HD males. Mononuclear cell infiltrate in the lungs was observed in females in all dose groups (3/6 control, 5/6 LD, 6/7 MD, 4/8 HD) and in males in all dose groups (6/8 control, 6/8 LD, 6/8 MD, 5/8 HD). Granulomatous pneumonitis was observed in 2/6 control, 1/7 MD, and 2/8 HD females and in 2/8 control, 3/8 LD, 2/8 MD, and 1/8 HD males. Pyrogranulomatous pneumonitis was observed on 1/6 control females. These and other findings, such as histiocytosis and congestion were observed in a few animals in both sexes across all dose groups, suggesting aspiration of test article. Pneumonitis recovered after the 2-week treatment-free period in females but mononuclear cell infiltration in 1/3 HD females, 1 Control, 3 LD, and 1 MD males did not (N=4 recovery animals/dose group). Two females each in the control, MD, and HD had hemorrhage in the thymus, as did 2/8, 3/8, and 5/8 males in the Control, MD, and HD groups, respectively. Other observed lesions occurred sporadically in a few animals without apparent dose response, suggesting they are likely not treatment-

related.

Special Evaluation

None

Toxicokinetics

Not reported

Dosing Solution Analysis

Not reported

Study title: A 3-month oral dose toxicity study in the (b) (4) Rat

Study no.: (b) (4) 551
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: October 5, 1994
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: PN401, Lot #s 1911-A-4 and 1911-A-5P,
 99.13% purity
 Carboxymethylcellulose (CMC), Lot #
 64H1176

Key Study Findings

(b) (4) Rats tolerated oral doses of uridine triacetate at up to 1682 mg/kg/day (administered as 2 even doses, given 6 h apart) without significant treatment-related toxicities. There were 2 unscheduled deaths which were ruled incidental and not treatment-related. All animals showed systemic exposure to uridine and uracil after oral administration of uridine triacetate; however, exposure was not dose-proportional.

Methods

Doses: 0, 500, 1000, 2000 mg/kg/day (estimated doses received 408, 828, 1682 mg/kg/day)
 Frequency of dosing: Twice daily in even doses, 6 h apart
 Route of administration: Oral gavage
 Dose volume: 10 mL/kg
 Formulation/Vehicle: Suspension in 5% CMC
 Species/Strain: Ra (b) (4)
 Number/Sex/Group: 10/sex/group (main study), 4/sex/PN401 dose group (TK study)
 Age: 7 weeks old
 Weight: 237.8-286.7 g (males), 174.3-220.1 g (females)

Satellite groups: Yes, for TK
Unique study design: None
Deviation from study protocol: None which affected the results of the study

Observations and Results

Mortality

Animals were examined twice daily for signs of mortality/morbidity and 1-2 hours post-dose.

There were 2 unscheduled deaths: MD female on Study Day 46 and HD male on Study Day 80. The MD female had blood in the peritoneal cavity and dark red coloration of the median liver lobe. The HD male had lungs filled with blood, with death caused by asphyxiation, secondary to aspiration of blood. The applicant did not attribute death of either animal to test article, as these deaths were isolated and all other animals survived until scheduled sacrifice.

Clinical Signs

Animals were examined twice daily for clinical signs and 1-2 hours post-dose.

Observed clinical signs included sores, alopecia, occasional reflux and/or chromodacryorrhea in control and test article-treated animals. One MD female had mild-moderate swelling of the right shoulder, which correlated with an axillary abscess. The MD female which died on Day 46 had languid behavior prior to and after dose administration. All other clinical signs were incidental and were thought to be unrelated to treatment with test article, as they were observed in only a few animals (1 or 2) and a dose response was not apparent.

Body Weights

Body weights were recorded at arrival, at randomization, one day before dosing was initiated, and weekly thereafter during the study.

There were no treatment-related changes in body weight in either sex over the course of the study. Occasional fluctuations in body weight (slight increases and decreases) were noted in all groups.

Feed Consumption

Food consumption was measured for each animal before dosing was started and weekly thereafter.

Overall, there were no treatment-related changes in food consumption. Statistically significant increases in food consumption (<10%) were observed during Weeks 2 and 3 in LD and MD males, as compared to controls ($p \leq 0.05$). There was a statistically significant

increase in food consumption in HD females (21%) during Week 9, as compared to controls ($p \leq 0.01$).

Ophthalmoscopy

Ophthalmoscopic exams were conducted before treatment was started and during Week 12 of the study.

Ocular lesions (panophthalmitis), consistent with injury from orbital bleeding procedure, were noted in 8 animals (1 MD male, 2 HD males, 1 LD female, 1 MD female, 3 HD females). These findings were considered to be related to experimental procedures and not treatment with test article.

ECG

Not conducted

Hematology

Sampling for hematology analysis was conducted during Week 6 and 12 of treatment.

There were small, but statistically significant changes (2-3%, as compared to controls, $p \leq 0.05$): decrease in MCHC (Week 6) and MCV in LD females (Week 12) and an increase in MCHC in MD and HD females during Week 12. Because of the small changes and lack of apparent dose response, the toxicological significance of these changes is not clear. There were no other significant treatment-related effects on hematology parameters.

Clinical Chemistry

Sampling for clinical chemistry analysis was conducted during Week 6 and 12 of treatment.

There was a statistically significant increase in calcium (Ca) level in HD males during Week 6 (10.7% as compared to controls, $p \leq 0.01$); although Ca levels in males in all uridine triacetate treatment groups increased from controls slightly during Week 12, they did not reach statistical significance. There was a 24% decrease in alkaline phosphatase in LD males ($p \leq 0.05$, as compared to controls) during Week 6 and a 55.7% increase ($p \leq 0.05$, as compared to controls) in the same group during Week 12. There was also a 2.5% decrease ($p \leq 0.01$, as compared to controls) in sorbitol dehydrogenase in HD males during Week 12. In females, there was a 7.3% increase in Ca at the HD during Week 6 ($p \leq 0.01$, as compared to controls), a 7.5% increase in K and a 1.04% increase in K at the HD and MD, respectively in Week 12 ($p \leq 0.05$, as compared to controls). The toxicological significance of these observations is not clear.

Urinalysis

Not conducted

Gross Pathology

Animals in the Main Study were sacrificed on Study Days 86 and 87 and full necropsies were performed on Control and HD animals only. Animals in the satellite group were sacrificed on Day 42 and discarded without necropsy.

There were no toxicologically significant changes in gross pathology noted at necropsy.

Organ Weights

The following organs were harvested and weighed: adrenals, heart, kidneys, liver, spleen, testes (with epididymides).

Overall, there were no significant treatment-related changes in organ weights. There was a slight increase (9.1%) in heart-to-body-weight ratio in HD males, as compared to control ($p \leq 0.05$). This was likely due to a decrease in body weight in this group. There was also ~32% increase in adrenals-to-body weight ratio in MD females ($p \leq 0.05$, as compared to controls) only.

Histopathology

Adequate Battery: Yes

The following tissues from the Control and HD groups were harvested for histopathology evaluation: animal identification, adrenals, aorta (thoracic), bone/marrow (sternum), brain (three levels), cecum, colon, duodenum, epididymides, esophagus, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, optic nerves, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, and vagina.

Peer Review: No

There were no microscopic changes which correlated with macroscopic findings in the 2 animals which died while on study. A treatment-related cause of death was not apparent. Of the remaining animals, there were no histopathological changes which could be considered to be related to treatment with uridine triacetate. The findings are summarized in the table (**Table 4**) below.

Table 4: Histopathology findings in ^{(b) (4)} rats treated with uridine triacetate by oral gavage for 3 months

Organ/Tissue	Male		Female	
	Control (N = 10)	HD (N = 10)	Control (N = 10)	HD (N = 10)
Pancreas, Acinar Atrophy	2	0	1	0
Chronic inflammation	0	0	0	1
Heart, Fibrosis in epicardium	1	0	0	0

Cardiomyopathy	5	0	1	2
Liver,				
Chronic inflammation	6	4	5	5
Bile duct hyperplasia	1	0	0	0
Capsular fibrosis	0	0	1	0
Prostate, Chronic inflammation	2	1	0	0
Thyroid lobes, ultimobranchial cyst	0	0	1	0
Stomach,				
Neutrophilic inflammation	0	0	0	1
Squamous epithelium vacuolation	0	0	0	1
Skin, Chronic inflammation	0	0	0	1
Mesenteric lymph node,				
Neutrophilic inflammation	0	0	1	0
Spleen,				
Chronic mesenteric inflammation	0	0	1	0
Eyes,				
Chronic inflammation	0	0	0	1
Hemorrhage	0	0	0	1
Cataract	0	0	0	1
Retinal degeneration	0	0	0	1

Special Evaluation

None

Toxicokinetics (TK)

TK sampling was conducted on Day 1 and Day 39 (Week 6).

All animals showed systemic exposure to uridine and uracil after oral administration of uridine triacetate; however, exposure was not dose-proportional. Because of the high level of uridine phosphorylase in rats, the concentration of uracil was highest at 0.75 h after first dose of uridine triacetate was administered. The TK results from the 6-week study are shown in the applicant's table (**Table 5**) below.

Table 5: TK parameters for uridine and uracil in (b) (4) rats treated with uridine triacetate by oral gavage

Daily Dose (mg/kg)	0 (Control)		204 mg/kg bid (408 mg/kg/day)		414 mg/kg bid (828 mg/kg/day)		841 mg/kg bid (1682 mg/kg/day)	
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10
Toxicokinetics: Day 39								
Plasma Uridine: C _{0.75} (µM) ^a	NA	NA	32.39	24.60	59.42	55.50	194.0	321.0
Plasma Uracil: C _{0.75} (µM) ^a	NA	NA	409.3	470.8	676.8	887.0	930.9	1048

Dosing Solution Analysis

Dosing solution analysis was conducted pre-study and during Weeks 1, 3, 6, 10, and 12.

Dosing solution analysis revealed that the concentrations of the dosing preparation were much lower than expected, possibly due to analytical error. The average percent difference from target values was 18.5%, 17.2% and 15.8% lower than expected for the LD, MD, and HD, respectively. The estimated concentration of test article administered to each dosing group was 408, 828, and 1682 mg/kg/day.

Study title: Subacute oral toxicity study in dogs

Study no.: 2648-100
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: November 21, 1991
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: TAU, 1911-B-1, 99.49%

Methods

Doses: 0, 0.75, 1.5 g/kg/day for 5 days
 Frequency of dosing: Twice daily (8 h apart)
 Route of administration: Oral gavage
 Dose volume: 3 mL/kg/day
 Formulation/Vehicle: Emulsion in corn oil/water/Tween-80
 Vehicle: Corn oil and water (1:1) + 2.5% Tween-80 (polysorbate 80, polyoxyethylenesorbitan mono-oleate)
 Species/Strain: Dog/Beagle
 Number/Sex/Group: 2/sex/dose
 Age: 37-43 weeks of age
 Weight: 12.3-15.1 kg (males), 8.5-10.3 kg (females)
 Satellite groups: None
 Unique study design: None
 Deviation from study protocol: None which affected study results

Key Study Findings

Uridine triacetate (denoted as TAU) was administered orally to Beagle dogs at up to 1.5 g/kg/day for 5 days. There were no deaths or overt treatment-related toxicities. A few treatment-related changes in gross pathology (mottled lung, pale area of lung in one HD male and 1 control female (pale color only) and enlarged ovaries (1 HD female)); however these changes are likely incidental and not directly related to treatment. The highest dose administered (1.5 g/kg/day) was generally well tolerated.

Study title: A 6-month study of uridine triacetate administered by oral gavage (twice daily) in rats

Study no.:	(b) (4) 20047236
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 20, 2013 (Sponsor signature)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Uridine triacetate, Q000001326W, 100.5% 0.75% (w/v) hydroxypropyl methylcellulose (HPMC) in reverse osmosis deionized (RODI) water, 2BK0258, 2CD0422

Key Study Findings

Uridine triacetate was well tolerated in (b) (4) rats at oral doses of up to 2000 mg/kg/day (administered as two even doses, 6h apart). The NOAEL dose was 2000 mg/kg/day, based on the absence of significant treatment-related toxicities. Systemic exposure to uridine and uracil after administration of uridine triacetate increased in a greater than dose proportional manner. There were no histopathological findings suggestive of tumorigenic potential of uridine triacetate at doses up to 2000 mg/kg/day.

Methods

Doses: 0, 500, 2000 mg/kg/day (0, 250, 1000 mg/kg/dose)
Frequency of dosing: Twice daily, approximately 6 hours apart
Route of administration: Oral gavage
Dose volume: 10 mL/kg
Formulation/Vehicle: Suspension in 0.75% HPMC in in reverse osmosis deionized water
Vehicle: 0.75% (w/v) hydroxypropyl methylcellulose (HPMC) in reverse osmosis deionized water
Species/Strain: Rat/ (b) (4)
Number/Sex/Group: 15/sex/group (Main Study)
Age: 8 weeks old
Weight: 176-289 g at initiation of dosing
Satellite groups: Yes (for toxicokinetics (TK)): 3/sex/ (b) (4) group; 9/sex/treatment group
Unique study design: None
Deviation from study protocol: None which affected the study results

Observations and Results

Mortality

Study animals were observed twice daily for general health/mortality and moribundity throughout the study period.

There were 2 unscheduled deaths: 1 control male (dead on Day 164) and one 500 mg/kg/day male (dead on Day 98). Discolored lungs and thymus were observed in the 500 mg/kg/day male at necropsy, although microscopic observation did not reveal any findings. No cause of death was identified for either animal, as no treatment-related clinical observations were noted prior to deaths. All other animals survived to scheduled necropsy.

Clinical Signs

Cage side observations were made once daily beginning one week before dosing and through the dosing period (1-3 h after the first daily dose). Detailed clinical observations on each animal were performed at least once a week, beginning one week before dosing.

No significant treatment-related clinical observations were noted during the dosing period.

Body Weights

Body weights were measured once weekly beginning one week before dosing and continued until scheduled euthanasia.

There were a few statistically significant, treatment-related increases in mean body weight, particularly at the HD in males and females, as compared to control animals, throughout the dosing period; however, these changes were sporadic and were less than 10%, as compared to controls, and therefore, not considered to be toxicologically significant.

Feed Consumption

Feed consumption was measured once a week beginning 2 days prior to initiation of dosing and continued through the dosing period.

There were sporadic changes in treatment-related changes (increases or decreases) in food consumption; however, in the absence of a clear dose response, these minor changes (less than 10%, as compared to control) were not considered to be toxicologically significant.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted during the week before dosing (Day -7) was initiated and during the last week of treatment (Day 178).

There were no treatment-related adverse ophthalmoscopic findings in main study animals.

Hematology

Samples were collected from Main Study (b) (4) and high dose (HD) animals on Study Day 183/184 and analyzed for the parameters listed in the applicant's table 5 below:

Text Table 5
Hematology Parameters

Red blood cell count	White blood cell count
Hemoglobin concentration	Neutrophil count (absolute)
Hematocrit	Lymphocyte count (absolute)
Mean corpuscular volume	Monocyte count (absolute)
Red blood cell distribution width	Eosinophil count (absolute)
Mean corpuscular hemoglobin concentration	Basophil count (absolute)
Mean corpuscular hemoglobin	Large unstained cells
Reticulocyte count (absolute)	Other cells (as appropriate)
Platelet count	

Activated partial thromboplastin time, fibrinogen, and prothrombin time were also evaluated.

There were sporadic increases and decreases in hematology parameters in both sexes without corresponding dose response during the dosing period. Because of high variability (standard deviation), these changes were not statistically significant. A

statistically significant decrease (5.9%) in partial thromboplastin time was observed in HD males ($p \leq 0.01$, as compared to controls). There was a treatment-related increase in platelet count in females, with a statistically significant increase of 12% at the HD ($p \leq 0.05$, as compared to controls). There was also a dose-dependent, statistically significant increase of 14.4% and 20.6% in activated partial thromboplastin time (APTT) in females treated with 500 and 2000 mg/kg/day uridine triacetate, respectively, ($p \leq 0.05$ and $p \leq 0.001$, respectively, as compared to controls).

Clinical Chemistry

Samples were collected from Main Study ^{(b) (4)} and high dose (HD) animals on Study Day 183/184 and analyzed for the parameters listed in the applicant's table 7 below:

Text Table 7
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin (calculated)
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine kinase	Glucose
Total bilirubin ^a	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride

^a When total bilirubin was > 0.5 mg/dL, direct bilirubin was measured and indirect bilirubin was calculated.

Overall, there were no treatment-related, toxicologically significant changes in clinical chemistry parameters in animals treated with uridine triacetate, as compared to controls. There was a statistically significant increase (1%) in chloride level in HD females ($p \leq 0.05$, as compared to control).

Urinalysis

Samples were collected from Main Study ^{(b) (4)} and high dose (HD) animals on Study Day 183/184 and analyzed for the following parameters: color, appearance/clarity, specific gravity, volume, pH, protein, glucose, bilirubin, ketones, and blood.

There were no treatment-related, toxicologically significant findings in urinalysis parameters.

Gross Pathology

On Study Day 183/184, Main Study animals underwent scheduled euthanasia. Necropsy was conducted, tissues were harvested and preserved for histopathology, and organ weights were noted. Animals which died while on study underwent necropsy and tissues were saved for further investigation when possible. Necropsy included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity, external surface of brain, thoracic, abdominal, and pelvic cavities and associated organs.

The 500 mg/kg/day male which died on study material accumulation on the skin and mottled discoloration/dark foci on the lung and thymus, while the control male had no such findings. There were no corresponding histopathology findings in either the control or 500 mg/kg/day male. Furthermore, no treatment-related clinical signs were noted in either animal and therefore, the cause of death of these two animals was considered to be incidental and not treatment-related.

There were incidental findings in animals of both sexes across all treatment groups; however, there were no dose-dependent, treatment-related findings in uridine triacetate-treated animals, as compared to controls.

Organ Weights

The following organ weights were determined: brain, epididymis, adrenal gland, pituitary gland, prostate gland, thyroid (including parathyroid) gland, heart, kidney, liver, lung, ovary, spleen, testis, thymus, and uterus.

There were incidental changes (increases or decreases in organ weights) in uridine triacetate-treated animals (both sexes). Although some of these changes reached statistical significance, as compared to controls, an apparent dose-dependent relationship was not apparent. There were no correlating changes in histopathology. Therefore, any changes that were observed were not considered to be of toxicological significance.

Histopathology

Adequate Battery: Yes (see applicant's table 13 below)

Text Table 13
Tissue Collection and Preservation

Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Body cavity, nasal	Larynx
Bone marrow smear	Liver
Bone marrow	Lung
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Muscle, skeletal
Cervix	Nerve, optic ^a
Epididymis	Nerve, sciatic
Esophagus	Ovary
Eye ^a	Pancreas
Gland, adrenal	Skin
Gland, harderian	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary	Stomach
Gland, seminal vesicle	Testis ^b
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Urinary bladder
Kidney	Uterus
Large intestine, cecum	Vagina

^a Preserved in (b) (4) fixative.
^b Preserved in Modified (b) (4) fixative.

Peer Review: Yes

Histological Findings: There were occasional histopathological findings in test article-treated animals; however, in most cases, they were also observed in vehicle-treated animals. The severity of most findings ranged from minimal to mild, except for 2 HD females which had moderate mandibular lymph node plasmocytosis. The following observations were noted only in HD animals: pelvic dilatation and mononuclear infiltration in 1 of 15 females, hemorrhage in lungs (1 of 15 males), tension lipidus in liver of 1 of 15 females, lobule atrophy and fibrosis in islet of Langerhans (1 of 15 males), lymphoid depletion in thymus of 1 of 15 females, and vaginal inclusion cyst in 1 of 15 females. There were no findings suggestive of tumorigenic potential of uridine triacetate. **Table 6** below summarizes the histopathology findings from this study.

Table 6: Histopathology findings in (b) (4) rats treated with uridine triacetate for 6 months via oral gavage

Organ/Finding	Males			Females		
	0 mg/kg/day (N=14)	500 mg/kg/day (N=14)	2000 mg/kg/day (N=15)	0 mg/kg/day (N=15)	500 mg/kg/day (N=15)	2000 mg/kg/day (N=15)
Adrenal Gland, Hypertrophy (mild)	0	0	0	1	0	0
Thrombosis	0	0	0	0	0	1

(mild)						
Mammary gland, Adenocarcinoma	---	---	---	0	1	0
Lactation, minimal	---	---	---	2	0	0
Lactation, mild	---	---	---	1	1	2
Galactocele	---	---	---	1	1	2
Pituitary gland, Adenoma, pars distal is	1 (minimal)	0	1 (mild)	0	0	0
Cyst	1 (minimal)	0	0	0	0	0
Kidney, Fibrosis	0	0	2 (mild)	0	0	0
Pelvic Dilatation	0	0	2 (mild)	0	0	1 (minimal) 1 (mild)
Mononuclear infiltration in pelvis	0	0	0	0	0	1 (minimal)
Liver, Hepatocellular vacuolation	0	0	2 (minimal)	0	0	0
Tension Lepidus's	1	0	2	1	0	1
Lungs, Hemorrhage	0	0	1 (minimal)	0	0	0
Lymph node (mandibular), plasmocytosis	2 (mild)	0	3 (mild)	1 (mild)	0	2 (mild) 2(moderate)
Lymph node (mesenteric): hemorrhage	1 (mild)	0	1 (mild)	0	0	0
Pancreas, Lobule atrophy	0	0	1 (mild)	0	0	0
Fibrosis (islet of Langerhans)	0	0	1 (mild) 1 (minimal)	0	0	0
Stomach, cyst	0	0	1 (mild)	0	0	0
Thymus, Hemorrhage	2 (minimal)	0	1 (mild)	0	0	0
Lymphoid depletion	2 (minimal) 1 (mild)	0	1 (mild)	0	0	2 (mild)
Vagina, epidermal inclusion cyst	---	---	---	0	0	1

Toxicokinetics

Sampling for TK analysis from satellite animals (all groups) was conducted at timepoint 0 (predose) and at 15 min, 45 min, 2 h, 4 h, and 6 h postdose on Study Days 1, 90, and 182. The 6 h sample was collected just prior to the afternoon dose.

Endogenous uridine and uracil were detected in animals from the placebo group. There were no gender differences in exposure to uridine or uracil. Maximum plasma concentrations of uridine were noted at 0.25 h post-dose on for 250 mg/kg/day on all sampling days and for 1000 mg/kg/day on Day 182, while T_{max} on Days 1 and 90 for 1000 mg/kg/day was 0.75 h. The exposure to uridine (C_{max} and AUC) generally increased in a greater than dose proportional manner on all sampling days. There was a slight increase in exposure to uridine from Day 1 to Day 90 (2.2-4.6-fold), and exposure was generally similar between Days 90 and 182.

Maximum exposure to uracil was noted at 0.75 to 2 h after dosing with uridine triacetate. Exposure (AUC) increased in a greater than dose proportional manner in females on Days 1 and 182 and on Day 1 in males. C_{max} increased in a dose-proportional manner on Day 1 and in a less than dose-proportional manner on Days 90 and 182 in both sexes. Exposure to uracil was generally similar on all sampling days. TK parameters for uridine and uracil were evaluated and are summarized in **Table 7** below.

Table 7: TK parameters for uridine (A) and uracil (B) in (b) (4) rats treated with uridine triacetate via oral gavage

Table 7A:

TK parameters of Uridine	Females			
	Dose (mg/kg)	T_{max} (h)	C_{max} (h)	$AUC_{(0-t)}$ ($\mu\text{mol}\cdot\text{h/L}$)
Day 1	250	0.25	16.3 \pm 3.11	14.3 \pm 1.39
	1000	0.75	143 \pm 31.3	178 \pm 28.9
Day 90	250	0.25	145 \pm 13.7	65.2 \pm 5.24
	1000	0.75	388 \pm 38.1	472 \pm 34.6
Day 182	250	0.25	45.0 \pm 4.15	26.9 \pm 1.67
	1000	0.25	217 \pm 17.6	316 \pm 94.3
	Males			
Day 1	250	0.25	11.3 \pm 1.5	13.9 \pm 0.782
	1000	0.75	150 \pm 26.0	172 \pm 23.0
Day 90	250	0.25	35.2 \pm 2.27	30.0 \pm 1.16
	1000	0.75	329 \pm 60.8	414 \pm 53.3
Day 182	250	0.25	28.9 \pm 1.92	26.9 \pm 1.67
	1000	0.25	205 \pm 34.4	262 \pm 23.1

Table 7B:

TK parameters of Uracil	Females			
	Dose (mg/kg)	T_{max} (h)	C_{max} (h)	$AUC_{(0-t)}$ ($\mu\text{mol}\cdot\text{h/L}$)
Day 1	250	0.75	229 \pm 21.9	271 \pm 21.1
	1000	0.75	977 \pm 41.6	2770 \pm 21.1
Day 90	250	0.75	432 \pm 23.6	602 \pm 49.4
	1000	0.75	925 \pm 35.8	2960 \pm 238
Day 182	250	0.75	31 \pm 13.5	422 \pm 19.8
	1000	2.00	839 \pm 59.3	2830 \pm 491
	Males			
Day 1	250	0.75	273 \pm 24.5	464 \pm 22.6

	1000	0.75	899 ± 43.1	3220 ± 177
Day 90	250	0.75	470 ± 19.3	815 ± 57.1
	1000	2.00	925 ± 35.8	2960 ± 238
Day 182	250	0.75	318 ± 4.62	425 ± 57.0
	1000	0.75	643 ± 15.6	1980 ± 169

Dosing Solution Analysis

Homogeneity of test article formulation was assessed during Study Weeks 1, 13, and 26. Concentration of dose formulations were analyzed during Weeks 1, 7, 13, 20, and 26. Stability over a 14-day period was assessed for 25 and 100 mg/mL concentration of test article in 0.75% HPMC. Test article homogeneity and stability were within prespecified limits and considered acceptable. The concentration of test article for Study Week 7 (100 mg/mL sample) and Weeks 13/26 (20 mg/mL sample) were 128% and 80.5% of the target concentration, respectively. Therefore, study animals received a slightly higher dose during Week 7 and a slightly lower dose during Week 13/26.

7 Genetic Toxicology

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

Study title: Uridine Triacetate: Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Escherichia coli*

Study no.: 9600345
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: July 8, 2013
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: Uridine Triacetate, Q000001243, 99.8%
 Dimethyl sulfoxide (DMSO), RNBC5920

Key Study Findings

Uridine triacetate was not mutagenic in the *in vitro* reverse mutation assay in bacterial cells, under the conditions tested.

Methods

Strains: *S. typhimurium* TA1535 *hisG46 rfa ΔuvrB*
S. typhimurium TA1537 *hisC3076 rfa ΔuvrB*
S. typhimurium TA98 *hisD3052 rfa ΔuvrB*
 pKM101
S. typhimurium TA100 *hisG46 rfa ΔuvrB*
 pKM101
E. coli WP2 *trp uvrA*

Concentrations in definitive study: 1.58, 5.0, 15.8, 50, 158, 500, 1581, 5000
 µg/plate using the plate incorporation
 version of the bacterial reverse mutation
 test

Basis of concentration selection: Based on limit dose of up to 5000 µg/plate
 since no toxicity was detected at
 concentrations < 5000 µg/plate

Negative control: Dimethyl sulfoxide (DMSO)

Positive control:

	Dose and strain	S9
Sodium azide (NaZ)	0.5 µg/plate for TA1535 and TA100	-
9-aminoacridine hemihydrate (9AC)	50 µg/plate for TA1537	-
2-nitrofluorine (2NF)	1 µg/plate for T98	-
4-Nitroquinoline N-oxide (NQO)	0.5 µg/plate for WPA <i>uvrA</i>	-
2-Aminoanthracene (2AA)	5 µg/plate for TA1535 20 µg/plate for WP2 <i>uvrA</i>	+
Benzo[a]pyrene (BaP)	5 µg/plate for TA1537, TA98, TA100	+

Formulation/Vehicle: Test article was prepared in DMSO
 Incubation & sampling time: Plates (containing test article ± S9 mix)
 were incubated at 37 °C for 64 h and 26
 minutes prior to evaluation. Plates were
 evaluated for background lawn (a measure
 of test article toxicity) and number of
 revertant colonies.

Study Validity

The study is valid and acceptable, based on the following:

1. Selection of bacterial strains was adequate.

- The mean revertant colonies in the vehicle treatment group were within historical control ranges and the positive controls for each strain produced the appropriate increase in mean revertant colonies to be considered as a valid study.
- Dose selection for the plate incorporation assay was appropriate, based on the limit dose, since test article was nontoxic at concentrations < 5000 µg/plate.
- The S9 fraction was within acceptable limits (10% w/v).

Results

The test article was not toxic and did not precipitate out of solution. Uridine triacetate did not increase the number of revertant colonies at up to 5000 mg/plate. The results are shown in the applicant's tables (**Table 8**) below.

Table 8: Results from *in vitro* mutagenicity assay with uridine triacetate using the plate incorporation method in the absence (**A**) and presence (**B**) of metabolic activation (Shown as Mean revertants/plate at each dose for each strain, with corresponding positive and negative controls)

Table 8A:

Metabolic Activation	Test Item	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			<u>TA1535</u>	<u>TA1537</u>	<u>TA98</u>	<u>TA100</u>	<u>WP2 <i>uvrA</i></u>
Without Activation	DMSO	-	16 ± 5	19 ± 2	21 ± 7	123 ± 10	39 ± 1
	Uridine Triacetate	50	18 ± 4	11 ± 7	30 ± 8	106 ± 12	37 ± 11
		158	16 ± 5	15 ± 3	32 ± 4	124 ± 9	40 ± 5
		500	15 ± 2	11 ± 3	31 ± 4	124 ± 4	44 ± 5
		1581	17 ± 2	13 ± 6	34 ± 5	123 ± 3	33 ± 5
		5000	17 ± 4	10 ± 3	27 ± 4	119 ± 13	44 ± 5
	NaAz	0.5	234 ± 5			427 ± 20	
	9AC	50		513 ± 60			
	2NF	1			157 ± 19		
	NQO	0.5					169 ± 17

Concentrations expressed in the terms of material as supplied.

N/A = Not applicable, DMSO = dimethyl sulfoxide, NaAz = Sodium azide, 9AC = 9-Aminoacridine hemihydrate, 2NF = 2-Nitrofluorene, NQO = 4-Nitroquinoline N-oxide, SD = Standard deviation, Water = Sterile Water for Irrigation, USP

Table 8B:

Metabolic Activation	Test Item	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2 <i>uvrA</i>
With Activation	DMSO	-	15 ± 2	14 ± 2	32 ± 4	127 ± 6	51 ± 8
	Uridine	50	17 ± 6	15 ± 5	33 ± 6	144 ± 15	49 ± 4
	Triacetate	158	19 ± 2	13 ± 4	38 ± 8	144 ± 17	46 ± 2
		500	19 ± 6	13 ± 2	41 ± 3	136 ± 10	47 ± 5
		1581	21 ± 2	15 ± 5	38 ± 2	140 ± 2	43 ± 10
		5000	21 ± 1	17 ± 4	41 ± 16	137 ± 5	38 ± 12
	2AA	5	355 ± 24				
	2AA	20					129 ± 12
	BaP	5		54 ± 9	248 ± 14	821 ± 18	

Concentrations expressed in the terms of material as supplied.

N/A = Not applicable, DMSO = dimethyl sulfoxide, 2AA = 2-Aminoanthracene, BaP = Benzo[a]pyrene, SD = Standard deviation, Water = Sterile Water for Irrigation, USP

Study title: Mutagenicity Test on PN401 in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)

Study no.: 16457-0-401

Study report location: EDR

Conducting laboratory and location: (b) (4)

Date of study initiation: November 11, 1994

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: PN401, 1911-A-SP

Key Study Findings

Uridine triacetate (PN401) was not mutagenic in the *in vitro* reverse mutation assay in bacterial cells, under the conditions tested.

Methods

Strains: TA98, TA100, TA1535, TA1537, TA1538

Concentrations in definitive study: For the definitive plate incorporation assay, the doses tested were 5000, 3330, 1000, 667, and 333 µg/plate (± S9 mix).

Basis of concentration selection: A preliminary dose range finding assay (10 doses) was conducted using TA100 in the presence and absence of S9 mix at the following concentrations of test article: 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3330, 5000 µg/plate. Cytotoxicity was assessed at a maximal dose of 5 mg/plate of test article. If no cytotoxicity was observed, then the high dose for the mutagenicity assay was set at 5 mg/plate.

Negative control: DMSO

Positive control:

	<i>Dose and strain</i>	S9
2AA	2.5 µg/plate for TA98, TA100, TA1535, TA1537, TA1538	+
2NF	1.0 µg/plate for TA98	-
NaZ	2.0 µg/plate for TA100, TA1535	-
ICR-191	2.0 µg/plate for TA1537	-
2NF	1.0 µg/plate for TA1538	-

Formulation/Vehicle: DMSO

Incubation & sampling time: The plate incorporation method was used to expose tester strains to the test article (± S9). Plates were incubated at 37 °C for 48 ± 8 h and evaluated for background lawn (a measure of cytotoxicity), presence of test article precipitation, and the number of revertant colonies.

Study Validity

The study is valid and acceptable, based on the following:

1. The selection of bacterial strains was adequate.
2. The mean revertant colonies in the vehicle treatment group were within historical control ranges for all strains except for TA1538. Therefore, mutagenicity of uridine triacetate was not assessed in this tester strain. A separate experiment (Experiment 16457-B2) was conducted with TA1538 (± S9 mix). The results of Experiment 16457-B2 met all the criteria for a valid and acceptable study.
3. The positive controls for each strain produced the appropriate increase in mean revertant colonies.
4. Dose selection for the plate incorporation assay was based on the limit dose, since test article was nontoxic at concentrations < 5000 µg/plate.

5. The S9 fraction was within acceptable limits (10% w/v).

Results

There was no cytotoxicity observed (no background lawn present) in the dose range-finding study with TA 100 (\pm S9) at up to 5000 μ g/plate of test article, as compared to vehicle treatment. Since the mean vehicle control value for TA1538 was not within historical control limits, the assay was redone. In the subsequent study (Experiment 16457-B2), results were acceptable. Uridine triacetate at up to 5000 μ g/plate did not produce any increase in TA1538 revertants, in the presence and absence of S9 mix. Under the conditions of the study, uridine triacetate was not mutagenic at up to 5000 mg/plate. The results are summarized in the applicant's tables (**Tables 9, 10**) below.

Table 9: Results from *in vitro* mutagenicity assay with uridine triacetate using the plate incorporation method in the absence (**A**) and presence (**B**) of metabolic activation (Shown as Mean revertants/plate at each dose for each strain, with corresponding positive and negative controls)

Table 9A

Metabolic Activation	Test Item	Dose Level (μ g/plate)	Revertants/plate (Mean \pm SD)				
			TA1535	TA1537	TA98	TA100	TA1538
Without Activation	DMSO	-	9 \pm 4	5 \pm 3	14 \pm 3	101 \pm 16	71 § \pm 9
	Uridine Triacetate	333	9 \pm 4	5 \pm 0	22 \pm 12	100 \pm 1	NC \pm -
		667	10 \pm 3	5 \pm 1	16 \pm 2	110 \pm 5	NC \pm -
		1000	11 \pm 2	7 \pm 2	18 \pm 7	93 \pm 11	NC \pm -
		3300	12 \pm 1	9 \pm 6	12 \pm 4	96 \pm 8	NC \pm -
		5000	10 \pm 1	6 \pm 3	18 \pm 3	102 \pm 14	NC \pm -
	NaAz	2.5	518 \pm 36			548 \pm 71	NC \pm -
	2NF	1			200 \pm 21		NC \pm -
	ICR-191	0.5		403 \pm 81			

§ NC = Not counted due to unacceptable mean vehicle control value.

DMSO = dimethyl sulfoxide, NaAz = sodium azide, 2NF = 2-nitrofluorene; ICR-91 = acridine half-mustard mutagen; SD = Standard deviation

Table 9B:

Metabolic Activation	Test Item	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	TA1538
With Activation	DMSO	-	12 ± 3	14 ± 0	25 ± 8	125 ± 7	90 § ± 4
	Uridine	333	15 ± 4	11 ± 5	28 ± 3	129 ± 16	NC ± -
	Triacetate	667	14 ± 4	13 ± 3	24 ± 8	109 ± 4	NC ± -
		1000	10 ± 5	11 ± 1	24 ± 14	123 ± 18	NC ± -
		3300	13 ± 2	10 ± 4	25 ± 8	128 ± 4	NC ± -
		5000	19 ± 3	9 ± 4	34 ± 7	120 ± 9	NC ± -
	2AA	2.5	152 ± 22	132 ± 23	893 ± 30	884 ± 36	NC ± -

§ NC = Not counted due to unacceptable mean vehicle control value.

DMSO = dimethyl sulfoxide, 2AA = 2-Aminoanthracene, SD = Standard deviation

Table 10: Results from Experiment 16457-B2 with uridine triacetate using the plate incorporation method in the absence (A) and presence (B) of metabolic activation (Shown as Mean revertants/plate at each dose for each strain, with corresponding positive and negative controls)

Table 10A:

Metabolic Activation	Test Item	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	TA1538
Without Activation (repeat TA1538)	DMSO	-	±	±	±	±	8 ± 1
	Uridine Triacetate	333	±	±	±	±	5 ± 1
		667	±	±	±	±	10 ± 4
		1000	±	±	±	±	11 ± 3
		3300	±	±	±	±	12 ± 1
		5000	±	±	±	±	9 ± 5
	2NF	1			±	288 ± 32	

DMSO = dimethyl sulfoxide, 2NF = 2-nitrofluorene; SD = Standard deviation

Table 10B:

Metabolic Activation	Test Item	Dose Level (µg/plate)	Revertants/plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	TA1538
With Activation (repeat TA1538)	DMSO	-	±	±	±	±	16 ± 4
	Uridine Triacetate	333	±	±	±	±	16 ± 6
		667	±	±	±	±	21 ± 3
		1000	±	±	±	±	15 ± 3
		3300	±	±	±	±	23 ± 6
		5000	±	±	±	±	22 ± 2
	2AA	2.5	±	±	±	±	1266 100

DMSO = dimethyl sulfoxide, 2AA = 2-Aminoanthracene, SD = Standard deviation

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Uridine Triacetate: *In Vitro* Mammalian Cell Gene Mutation Test in Mouse Lymphoma L5178Y TK+/- Cells

Study no.: 9600346
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: July 4, 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Uridine Triacetate, Q000001243, 99.8%
DMSO, RNBC5920

Key Study Findings

Uridine triacetate was not mutagenic in the mouse lymphoma assay both in the presence and absence of metabolic activation, under the conditions tested.

Methods

Assay Version: Microwell
Cell line: Mouse lymphoma L5178Y TK+/- (clone 3.7.2C)
Concentrations in definitive study: 23.1, 46.3, 92.5, 185, 370 µg/mL
Basis of concentration selection: A preliminary toxicity test was conducted with uridine triacetate at 11.7, 20.8, 37.0, 65.8, 117, 208, 370, 658, 1170, 2081, 3700, 6580, 11700, 20807, and 37000 µg/mL (limit dose of 1 mM).
Negative control: DMSO
Positive control: 4-nitroquinoline N-oxide (3 h and 24 h without S9 activation)
Benzo[a]pyrene (3 h with S9 activation)
Formulation/Vehicle: DMSO
Incubation & sampling time: Cultures were incubated for 3 h and 24 h without S9 activation and 3 h with S9 activation. After treatment, cells were washed and counted and density was adjusted after 24 h, if necessary. Cells were cloned in medium with and without trifluorothymidine (TFT), the selective agent. The plates were incubated for 7-12 days before evaluation relative total growth (RTG) and mutation frequency (MF) were calculated.

Study Validity

The study is valid and acceptable based on the following:

1. The plating efficiency (PE) viability for vehicle control cultures was 65-120% and the mean spontaneous mutant frequency exceeded ~50 mutants per 10^6 cells and was within historical control range of the testing laboratory.
2. The positive controls produced an increase of 300 mutants per 10^6 viable cells with $\geq 40\%$ increase reflected in small colony mutant frequency, as compared to vehicle treatment.

Results

1. The results of the dose range-finding experiment are shown in the applicant's table below (**Table 11**) and revealed that the test article was non-toxic up to the limit dose of 1 mM.

Table 11: *In Vitro* Mammalian Cell Gene Mutation Test in Mouse Lymphoma L5178Y TK+/- Cells: Results from dose range-finding experiment

Material	Final conc. ($\mu\text{g/mL}$)	% Plating efficiency (PE) †			% Relative total growth (RTG) ‡		
		3 Hours (0S9)	3 Hours (+S9)	24 Hours (0S9)	3 Hours (0S9)	3 Hours (+S9)	24 Hours (0S9)
DMSO	-	91	76	96	100	100	100
Uridine Triacetate	0.117	92	92	101	95	129	117
	0.208	75	112	89	89	166	107
	0.370	98	87	89	121	115	102
	0.658	116	101	112	151	150	129
	1.17	87	89	101	107	136	108
	2.08	116	87	87	136	148	103
	3.70	89	73	92	106	117	99
	6.58	79	73	77	95	113	100
	11.7	87	98	77	103	141	99
	20.8	92	101	92	109	137	119
	37.0	98	73	92	119	95	115
	65.8	87	98	79	117	139	94
	117	82	71	92	104	107	108
	208	82	82	98	109	122	119
370	92	105	84	105	143	93	

† Absolute Day 2 plating efficiency (%)

‡ Day 2 plating efficiency compared with the vehicle control and corrected for growth during treatment and expression (i.e. RG0, RG1 and RG2)

DMSO Dimethyl sulfoxide

2. Treatment with uridine triacetate at up to 1 mM did not produce an increase in the mutant frequency, as compared to treatment with vehicle (results are shown in the applicant's table (**Table 12**) below).

Table 12: *In Vitro* Mammalian Cell Gene Mutation Test in Mouse Lymphoma L5178Y TK+/- Cells: Results from definitive study

Treatment	Final conc. ($\mu\text{g/mL}$)	% PE Viability	% RTG	Mutant Frequency			Dose Response
				SC	LC	Total	
<i>3 hour treatment in the absence of S9</i>							
DMSO	-	89	100	34	59	94	} NR
Test Item	23.1	83	88	35	62	97	
	46.3	93	104	28	44	72	
	92.5	97	108	32	66	98	
	185	100	115	28	39	67	
	370	78	88	31	49	80	
NQO	0.40	66	51	210	236	446	
<i>3 hour treatment in the presence of S9</i>							
DMSO	-	100	100	19	34	53	} NR
Test Item	23.1	77	80	13	37	50	
	46.3	85	91	26	37	63	
	92.5	90	92	24	75	99	
	185	84	81	26	62	88	
	370	95	95	14	34	47	
BaP	1.4	67	51	201	180	382	
<i>24 hour treatment in the absence of S9</i>							
DMSO	-	97	100	11	51	63	} NR
Test Item	23.1	93	97	15	62	77	
	46.3	97	85	4	56	60	
	92.5	96	96	14	71	85	
	185	94	80	15	48	63	
	370	103	92	4	56	61	
NQO	0.14	74	18	177	323	500	
NR	Negative response, <i>i.e.</i> no substantial increase (equal to or higher than 126 mutants per 10^6 viable cells) in the mutation frequency over the concurrent vehicle control						
+	Positive response, <i>i.e.</i> substantial increase in the mutation frequency over the concurrent vehicle control						
PE	Plating efficiency						
RTG	Relative total growth						
SC	Small colony						
LC	Large colony						
DMSO	Dimethyl sulfoxide						
NQO	4-Nitroquinoline N-oxide						
BaP	Benzo[a]pyrene						

- Dosing solution analysis revealed that all preparations were within $\pm 10\%$ and $\pm 15\%$ of the nominal concentration for stock solutions and lower level solutions, respectively.

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

Study title: Genetic Toxicity Evaluation of PN401 In an *In Vivo* Mouse Micronucleus Oral Limit Dose Assay

Study no: 16457-0-455CO
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: November 11, 1994
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: PN401, 1911-A-4
0.75% aqueous hydroxyl propyl methyl cellulose (HPMC)

Key Study Findings

Under the conditions tested, uridine triacetate did not produce an increase in micronuclei in bone marrow polychromatic erythrocytes and was therefore negative in the mouse micronucleus assay.

Methods

Doses in definitive study: 2000 mg/kg
Frequency of dosing: Single dose
Route of administration: Oral gavage
Dose volume: 20 mL/kg; positive control dosed with 10 mL/kg
Formulation/Vehicle: Suspension/0.75% HPMC
Species/Strain: Mice/ (b) (4)
Number/Sex/Group: 5/sex/group (3 groups: 24 h, 48 h, 72 h)
Satellite groups: No
Basis of dose selection: Limit dose of 2000 mg/kg body weight
Negative control: 0.75% HPMC (20 mL/kg)
Positive control: Cyclophosphamide(CP) 80 mg/kg (treatment for 24 h only)

Study Validity

A finding was considered positive when there was a statistically significant, dose-dependent increase in micronucleated polychromatic erythrocytes (PCEs) or the statistically significant response is reproducible for at least one dose level. A finding was considered negative if there was neither a statistically significant and reproducible increase in PCEs at one dose level nor a statistically significant dose response.

Based on the criteria set forth, the study is valid and acceptable.

Results

Uridine triacetate did not produce an increase in PCEs when administered as a single dose to (b) (4) mice at an oral limit dose of 2000 mg/kg. Vehicle treatment and CP treatment produced results which were within historical data of the testing laboratory. Based on the criteria for a positive (and negative) response, under the conditions of this assay, uridine triacetate was negative in the *in vivo* mouse micronucleus test. The study results are shown in the applicant's table below.

Table 13: Micronucleus data summary table from the *in vivo* mouse micronucleus assay (NCE: normochromic erythrocyte, PCE: polychromic erythrocyte)

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E.			RATIO PCE:NCE MEAN ± S.E.	
			MALES	FEMALES	TOTAL	MALES	FEMALES
VEHICLE CONTROL 0.75% Aqueous HPMC	20 ml/kg	24	0.08 ± 0.04	0.10 ± 0.05	0.09 ± 0.03	0.77 ± 0.08	0.79 ± 0.10
		48	0.04 ± 0.04	0.04 ± 0.02	0.04 ± 0.02	0.40 ± 0.10	0.59 ± 0.10
		72	0.12 ± 0.07	0.08 ± 0.02	0.10 ± 0.04	0.76 ± 0.15	0.62 ± 0.12
POSITIVE CONTROL CP	80 mg/kg	24	3.68 ± 0.45*	1.86 ± 0.33*	2.77 ± 0.40	0.53 ± 0.04	0.90 ± 0.19
TEST ARTICLE	2000 mg/kg	24	0.08 ± 0.06	0.02 ± 0.02	0.05 ± 0.03	0.49 ± 0.15	0.69 ± 0.12
		48	0.04 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.58 ± 0.14	0.64 ± 0.12
		72	0.08 ± 0.04	0.02 ± 0.02	0.05 ± 0.02	0.64 ± 0.07	0.58 ± 0.05

* Significantly greater than the corresponding vehicle control, p<0.05.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

The sponsor submitted a justification for a waiver of the requirement to conduct a 2-year rodent carcinogenicity study with uridine triacetate, which is being developed for HOA. The sponsor has submitted a Carcinogenicity Risk Assessment document in support of this waiver request. In this document, the sponsor proposed that uridine triacetate is not likely to be tumorigenic in both rats and humans and a 2-year rat study is not needed. The supporting data is summarized below.

Pharmacology, Pharmacokinetics, and Biochemical Properties:

Uridine triacetate is an acetylated form of uridine. The rationale for using uridine triacetate comes from previous clinical experience with oral uridine supplementation in

HOA patients. Exogenous uridine has been used for various indications, including diabetic nephropathy, mitochondrial and neurometabolic disorders, and 5-fluorouracil toxicity, with minimal safety concerns. In humans, uridine is the primary form of circulating pyrimidine and is transferred to tissues such as the brain, heart and other tissues, from the liver, the primary site of de novo uridine synthesis. Intracellular uridine concentration is regulated by the activity of uridine kinase, which converts uridine to UMP, which enters cellular metabolic pathways for conversion into uridine di- and tri-phosphate, di- and tri-phospho sugars and cytosine nucleotides and metabolites. Excess uridine is excreted or catabolized into intermediates in the canonic pyrimidine pathway, and extracellular uridine has not been shown to activate cell surface receptors which contribute to cytokine stimulation and/or neoplastic growth.

Uridine triacetate undergoes rapid and complete conversion to uridine upon absorption after oral administration. Pharmacokinetic studies in animals (rats and dogs) and humans have shown that no detectable levels of uridine triacetate in the circulation after oral administration. *In vitro* studies with uridine triacetate in human plasma have shown rapid de-acetylation by plasma esterases. Preliminary human PK data from clinical trial (Protocol 401.13.001) show that a single oral dose of 60 mg/kg/day uridine triacetate produced an increase in plasma uridine within 1-2 h post dose, plasma concentrations of uridine were maintained for up to 8 h post-dose.

The proposed starting dose of 60 mg/kg/day of uridine triacetate for HOA patients will provide a maximum dose of 30 mg/kg/day of acetate in the systemic circulation. Acetate is the byproduct of fatty acid metabolism. The amount of acetate in the proposed dose of uridine triacetate is far exceeded by the amount of acetate resulting from the breakdown of the fat consumed in a day (2-3 g/kg/day) by children.

Carcinogenic potential of uridine triacetate:

There are no long term safety studies with uridine triacetate. Uridine has been used at doses of 150-200 mg/kg/day with no reports of neoplasia. There have been no reports of neoplasia or neoplastic disease in patients receiving uridine triacetate at up to 300 mg/kg/day (or 2 g/m² TID) for up to 19 years for the treatment of mitochondrial disease or for the treatment of neurometabolic syndrome associated with excessive 5'-nucleotidase activity.

In animals, chronic toxicity of uridine triacetate has been tested in the R6/2 and N171-82Qrodent models of Huntington's Disease and in the Tg2576 and Tg2576XP301L (TAPP) Alzheimer's Disease models. Dietary studies with uridine triacetate in these models at doses of 4-8% of the feed (approximately 8000-16,000 mg/kg/day) for up to one year failed to produce any neoplastic findings. In the Alzheimer's disease model, mice were fed up to 12 g/kg/day of uridine triacetate for 1 year and failed to produce any treatment-related morbidity or tumors. The doses studied in the Alzheimer's animal

models far exceed the proposed daily dose of uridine triacetate for HOA². Although these studies are not considered carcinogenicity studies, they demonstrate long term exposure of animals to high doses of uridine triacetate.

In the 6-month chronic toxicity study in rats, there were no observations of nonspecific pharmacologic effects at up to 2000 mg/kg/day (maximum feasible dose). Likewise, there were no such observations in the 3-month toxicity studies in rats and dogs or in the reproductive toxicology studies conducted with uridine triacetate. In the 6-month chronic toxicity study in rats and in the 3-month study in rats and dogs, there were no treatment-related effects suggestive of hormonal imbalance. No such effects were observed in the clinical trials with uridine triacetate. Uridine (endogenous or exogenous) and uridine triacetate have not been shown to induce hormonal perturbations in either sex, which could contribute to tumorigenesis. No effects suggestive of treatment-related immunosuppression were observed in any of the species studied.

There were no changes in histopathology in tissues of rats treated for 3 months or 6 months with uridine triacetate at doses up to 2000 mg/kg/day which were suggestive of carcinogenic potential (hypertrophy, diffuse or focal cellular hyperplasia, chronic inflammation, tumors or preneoplastic changes). There was one incidence of mammary gland adenocarcinoma in a female treated with 500 mg/kg/day for 6 months. All other histopathological findings in the 3- and 6-month rat studies were considered to be incidental and not related to treatment with uridine triacetate.

Uridine triacetate was shown to be non-mutagenic at up to 5000 µg/plate in two Ames assays and negative in an *in vitro* mouse lymphoma assay at up to 370 µg/mL (1 mM). In an *in vivo* mouse micronucleus test, uridine triacetate was tested up to a limit dose of 2000 mg/kg and failed to demonstrate genotoxic effects.

Toxicokinetics:

Comparison of TK parameters across species indicates that plasma levels of uridine are higher in animals after oral administration of uridine triacetate, as compared to HOA patients. Systemic exposure (AUC) to uridine in rats treated with 2000 mg/kg/day of uridine triacetate for 6 months was similar to exposure in HOA patients on Day 28 (shown in **Table 14** below). The NOAEL dose for each of the animal studies was the highest dose of uridine triacetate administered.

² Using allometric scaling, the dietary dose of uridine triacetate used in the Alzheimer's mice (12 g/kg/day) is equivalent to a human dose of approximately 1 g/kg or 1000 mg/kg. The recommended starting dose of uridine triacetate for HOA is 60 mg/kg/day. The dose used in the Alzheimer's mice was approximately 16.7x higher than the proposed human dose for HOA.

Table 14: Comparison of uridine plasma concentration after oral administration of uridine triacetate in humans and animals

Study	Uridine Triacetate (mg/kg/day)	Mean C _{max} (µM)	Mean AUC _{0-t} (µM·h)
Pediatric patients with HOA*	60	Day 1: 91.3 Day 28: 88.7	Day 1: 326 Day 28: 289
12-week rat	1680	258 [§]	
12-week dog	1500	563 [§]	1180 [§]
6-month rat [£]	2000	211 [§]	289 [§]

* Protocol # 401.13.001

£ TK sampling analysis from Day 182 of dosing

§ Average of C_{max} (males and females pooled)

TK analysis for uracil revealed levels below the limit of quantitation in patient samples (shown in the **Table 15** below). Systemic exposure to uracil (C_{max} and AUC) in rats was higher as expected, as rats have high endogenous uridine phosphorylase activity.

Table 15: Comparison of uracil plasma concentration after oral administration of uridine triacetate in humans and animals

Study	Uridine Triacetate (mg/kg/day)	Mean C _{max} (µM)	Mean AUC _{0-t} (µM·h)
Pediatric patients with HOA*	60	BLQ [€]	BLQ [€]
12-week rat	1680	989.5	
12-week dog	1500	532.8	1903
6-month rat [£]	2000	741	2405

* Protocol # 401.13.001

£ TK sampling analysis from Day 182 of dosing

§ Average of C_{max} (males and females pooled)

€ Below the limit of quantitation

In summary, the applicant has submitted a carcinogenicity risk assessment document for uridine triacetate and is requesting a waiver of the 2-year rodent carcinogenicity study. Uridine triacetate is being developed for the treatment of HOA in pediatric and adult patients. The applicant's rationale is supported by the following:

1. Uridine triacetate is rapidly and completely converted to uridine. Uridine triacetate is undetectable in plasma after oral administration.
2. Oral administration of uridine triacetate for up to 6 months at doses up to 2000 mg/kg/day was not associated with hormonal perturbations in rats or dogs.
3. Repeat-dose toxicology studies in rats (up to 6 months) and dogs (3 months) at doses up to 2000 mg/kg/day did not reveal any treatment-related changes in histopathology which were suggestive of carcinogenic potential.
4. Uridine triacetate was non-genotoxic *in vitro* and *in vivo*.
5. Long term studies in rodent models of Alzheimer's Disease and Huntington's Disease, repeated oral daily administration of uridine triacetate at up to 12 g/kg/day for one year, which is ~16x greater than the proposed clinical dose of 60 mg/kg/day, failed to produce any morbidity related to tumor formation.
6. Exposure comparison indicated that plasma levels of uridine at the NOAEL doses were higher in animals, as compared to humans.

Based on the information provided in the carcinogenicity risk assessment document, the applicant's request to waive the 2-year rodent carcinogenicity study appears to be reasonable. Therefore, from a nonclinical standpoint, the 2-year carcinogenicity study may be waived and need not be conducted to support marketing approval for uridine triacetate for the treatment of HOA.

The CDER/Executive Carcinogenicity Assessment Committee (ECAC) agreed with the Division's recommendation to grant the waiver. The applicant was informed of this recommendation via teleconference on April 29, 2015 (Please see Meeting Minutes from the Midcycle Communication Meeting).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Study of Fertility and Early Embryonic Development to Implantation of Uridine Triacetate Administered by Oral gavage (Twice Daily) in Rats

Study no.:	20047304
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	October 21, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Uridine Triacetate, Q000001326W, 100.5% w/w 0.75% (w/v) hydroxypropyl methylcellulose (HPMC) in reverse osmosis membrane-processed deionized water, 2CD0422, 1BC0738

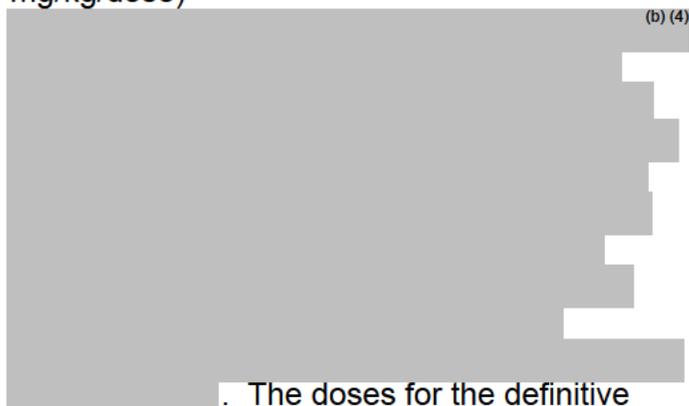
Key Study Findings

Uridine triacetate at oral doses up to 2000 mg/kg/day did not affect male or female fertility parameters. There were no treatment-related deaths, adverse effects on clinical signs, body weights, or food consumption. There were no gross lesions or changes in reproductive organ weights (males). There was no effect on sperm count, motility and density in male rats or on estrous cycle, ovarian and uterine examination in female rats at up to 2000 mg/kg/day of uridine triacetate. The NOAEL dose was 2000 mg/kg/day in males and females.

Methods

Doses: 0, 500, 2000 mg/kg/day (0, 250, 1000 mg/kg/dose)

(b) (4)



. The doses for the definitive embryofetal toxicity study ((b) (4) Study # 20040947) were 500 and 2000 mg/kg/day, and the same doses were selected for this study.

Frequency of dosing: Twice daily (6 h apart)
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: Suspension in 0.75% HPMC
Species/Strain: Rat/ (b) (4)
Number/Sex/Group: 20/sex/dose
Satellite groups: None
Study design: Males were dosed with either control or test article beginning 28 days before cohabitation, during cohabitation and continuing through to the day before scheduled euthanasia (total of 49-52 days of twice daily dosing). Females were dosed with either control or test article beginning 14 days before cohabitation, during cohabitation, and continuing through gestation day (GD) 7. Females which did not have evidence of mating were dosed until 7 days after completion of the cohabitation period.

Deviation from study protocol: None which affected the integrity of the data or study outcome

Observations and Results

Mortality

Animals were checked twice daily for signs of morbidity and mortality during the study.

All male and female rats in the study survived until scheduled sacrifice.

Clinical Signs

Animals were observed at least twice daily during the acclimatization period, before each dose, and daily during the post-dose period (0.5 – 1 h post dose).

Three of 20 males in the 500 mg/kg/day group had localized alopecia on limbs, which was statistically significant ($p \leq 0.01$), as compared to the control group. There was also 1 of 20 males which had chromodacryorrhea. These findings were not considered to be related to treatment with uridine triacetate due to the absence of a dose response and their low incidence (number of animals affected). There were no other treatment-related clinical signs in males.

In females, excess salivation (moderate/slight) was observed in 2 animals in the high dose (HD) group. Other observed clinical signs included dark red/yellow right eye, sparse hair on limb, enlarged right eye, rales, corneal opacity, microphthalmia, and/or head tilt in 1 of 20 animals in the HD group. There were no other treatment-related clinical signs in females.

Body Weight

Body weights were recorded at least twice daily during the acclimatization period, daily during the dosing and post-dose periods, and at terminal necropsy.

There were no treatment-related effects on body weight in males and females (during cohabitation and gestation) at up to 2000 mg/kg/day uridine triacetate, as compared to controls. Body weights of males and females at terminal necropsy were similar between control and uridine triacetate treatment groups.

Feed Consumption

Food consumption was recorded weekly during the dose period for male rats and weekly during the dose period and on GD 0, 7, 10, 12, and 13 for females.

There were no treatment-related effects on food consumption in males and females (during cohabitation and gestation) at up to 2000 mg/kg/day uridine triacetate, as compared to controls.

Toxicokinetics

Not conducted

Dosing Solution Analysis

Samples were analyzed from the 1st, 5th, and last dose formulations. All samples were within or equal to $\pm 15\%$ of the nominal concentrations.

Necropsy

Males were euthanized on Study Day 50, 52, or 53 and necropsy was conducted. Tissues were collected and organs were weighed. Sperm analysis was conducted on male mice. For females, scheduled euthanasia was on GD 13. At necropsy, tissues were collected, organs were weighed, and ovarian/uterine examination was conducted. The applicant's table below summarizes the tissue collection protocol:

Text Table 6
Tissue Collection and Preservation

Tissue	Weighed	Collected	Comment
Cervix	-	X	Collected with uterus.
Epididymides	X	X	Individual weight and examination. The remaining portion of the left epididymis (corpus and caput) and right epididymis were fixed in 10% neutral buffered formalin.
Epididymis, left cauda	X	X	-
Gland, prostate	X	X	-
Gland, seminal vesicles	X	X	All male rats. Weighed with and without fluid.
Gross lesions	-	X	-
Ovaries	-	X	-
Testes	X	X	Individual weight and examination; Preserved in Modified (b) (4) fixative then rinsed as per Testing Facility Standard Operating Procedures.
Uterus	-	X	Collected with cervix.

X = Procedure conducted; - = Not applicable.

There were 2 (of 20) control males which had small and flaccid testes and 1 HD male with slight, bilateral pelvic dilatation. No other significant findings were noted at necropsy in males in all dose groups. There were no gross lesions identified at necropsy in females in all dose groups.

Organ weights:

There were no treatment-related effects on organ weights in males, as compared to controls.

Sperm Analysis:

Sperm motility and sperm concentration were evaluated to assess the effects of uridine triacetate on the male reproductive system and function.

Overall, the following parameters were similar between control and uridine triacetate treatment at up to 2000 mg/kg/day: number of motile and nonmotile sperm and total sperm count in the vas deferens and sperm count and density in the cauda epididymis.

Fertility Parameters:

There were no treatment-related effects on fertility in male rats, as compared to controls. The following mating and fertility parameters in males were not affected by treatment with uridine triacetate: number of days in cohabitation, number of rats that mated, fertility index, number of rats with confirmed mating dates, number of pregnancies per number of rats in cohabitation. Overall, mating performance and fertility index were similar between males treated with control and uridine triacetate.

There were no treatment-related differences in estrous cycling between control- and uridine triacetate-treated females. The number of estrous stages during the prehabitation period was similar between females in all dose groups. There was one HD female exhibiting persistent diestrus. The number of females that mated, the fertility index, the number of females with confirmed mating dates, number of females mated by the first male, and the number of pregnant females were similar between control- and uridine triacetate treatment groups.

Ovarian and Uterine evaluation:

In females, the reproductive tract was dissected and the uterus and ovaries were evaluated for the number and distribution of: corpora lutea, implantation sites, placentae (size, color or shape), and the number of live and dead embryos. Uteri of non-pregnant females were also examined to confirm the absence of implantation sites.

Examination of ovaries and uterine contents did not reveal significant differences between control and uridine triacetate treatment. The following parameters were generally similar between control and uridine triacetate-treated females: number of corpora lutea, number of implantations, % preimplantation, number of viable and nonviable embryos, % postimplantation loss, number of dams with viable and nonviable embryos, and number of normal placentae.

9.2 Embryonic Fetal Development

Study title: An Embryo-fetal Development Study of Uridine Triacetate by Oral Gavage (Twice Daily) in Rats

Study no.:	20040947
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 18, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Uridine Triacetate, Q000001243, 99.80% 0.75% (w/v) hydroxyl propyl methyl cellulose (HPMC), 1BC0738

Key Study Findings

Uridine triacetate was not teratogenic in rats when administered to pregnant dams from GD 6 through GD 17. The NOAEL for maternal and fetal developmental toxicity was 2000 mg/kg/day, the highest dose administered.

Methods

Doses: 0, 500, 2000 mg/kg/day (0, 250, 1000 mg/kg/dose)

Frequency of dosing: Twice daily, administered 6 h apart
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: Suspension/HPMC
Species/Strain: Rat/ (b) (4)
Number/Sex/Group: 25 females/group
Satellite groups: Yes, for TK study (5 females/group)
Study design: Doses were based on results from the 3-month oral toxicity study in rats at 500, 1000, and 2000 mg/kg/day (given as two equal doses, 6 h apart). In the definitive study, females were cohabited with breeder males for 5 days. Pregnant females (identified by presence of copulatory plug or observation of sperm on vaginal smear) were treated with twice daily oral doses of uridine triacetate at 1000 and 2000 mg/kg/day from gestation day (GD) 6 through GD 17.

Deviation from study protocol: None which affected the integrity of the data or study outcome

Observations and Results

Mortality

Animals were checked twice daily for signs of mortality and morbidity.

There were 2 unscheduled deaths in the HD group in the Main Study cohort. One of these HD females (#1454) delivered 2 live pups on GD 21 and was found to have 9 live fetuses in utero at necropsy. This animal was observed with a swollen snout on GD 12. Body weight and food consumption observations in this animal were comparable to other animals in the dose group. Gross examination of all offspring revealed no external, soft tissue or skeletal abnormalities; all pups/fetuses were normal for their developmental age. The second HD female (#1471) which was sacrificed was also observed delivering on GD 21. This female exhibited several adverse clinical signs including excess salivation (GD 16), rales on GD 16-18, moderate dehydration on GD 17 and mild dehydration through GD 21, and reduced fecal output on GD 18-19. There was a decrease in body weight on GD 16-17 of 17 g and food consumption was lower on GD 15-18, as compared to other animals in this dose group. Sixteen live pups were delivered with one dead pup. Fetal weights were slightly lower compared to other litters in this group.

Clinical Signs

Animals were observed weekly during acclimatization period, on GD 0, daily before dose administration and during post-dose period.

Observed clinical signs occurred sporadically and did not show a dose response; therefore, they were considered to be unrelated to treatment with uridine triacetate. These included 3 HD females with a swollen snout, which was observed transiently in the affected animals on separate occasions (GD 12, 14, and 16). Other clinical signs included mild/moderate dehydration, slight excess salivation, reduced fecal output, and red urine which were observed in 1 HD female and brown perivaginal substance in 1 LD female.

Body Weight

Animals were weighed once weekly during acclimatization period, on GD 0, daily during dose and post-dose period, and at scheduled sacrifice.

There were no treatment-related effects on body weight. Animals in all dose groups gained weight during the dosing period.

Feed Consumption

Food consumption was measured on GD 0, 6, 9, 12, 15, 18, and 21.

There were no treatment-related effects on food consumption.

Toxicokinetics

Samples for TK analysis were collected on GD 6 and 17 at 0, 15 and 45 min, and 2 h, 4 h, and 6 h after dosing. TK parameters for uridine and uracil were evaluated.

Endogenous uridine and uracil were detected in the plasma of control animals. Systemic exposure (C_{max} and AUC) to uridine and uracil (C_{max}) increased in a greater than dose-proportional manner. There was a greater than dose proportional increase in AUC of uracil. Exposure to uridine and uracil was generally similar on GD 6 and GD 17. Exposure to uracil was higher, as compared to uridine. TK data are summarized in **Table 16** below.

Table 16: TK parameters for uridine and uracil after oral administration of uridine triacetate to pregnant rats

GD	Dose (mg/kg/day)	Uridine			Uracil		
		Tmax (h)	Cmax ($\mu\text{mol/L}$)	AUC ($\mu\text{mol}\cdot\text{h/L}$)	Tmax (h)	Cmax ($\mu\text{mol/L}$)	AUC ($\mu\text{mol}\cdot\text{h/L}$)
6	0	0	2.28 \pm 0.193	6.79 \pm 0.354	6	3.38 \pm 1.66	17.4 \pm 1.07
	250	0.25	19.1 \pm 5.62	27.2 \pm 1.87	0.75	305 \pm 5.69	547 \pm 10.4
	1000	0.75	248 \pm 36.1	391 \pm 56.4	2.0	1130 \pm 31.8	3420 \pm 70.2
17	0	0	1.46 \pm 0.0784	5.88 \pm 0.286	6	3.80 \pm 0.166	20.7 \pm 0.847
	250	0.25	33.2 \pm 5.67	30.4 \pm 2.28	0.75	351 \pm 10.9	623 \pm 19.7
	1000	0.75	170 \pm 56.8	271 \pm 73.4	2.00	917 \pm 216	2800 \pm 544

Dosing Solution Analysis

Dose formulation analysis was conducted on the first and last preparation for each dose group and all study samples had mean concentrations \pm 15% the theoretical concentrations.

Necropsy

TK animals were sacrificed on GD 18 and examined for pregnancy status only. Main Study animals underwent Caesarean-section and ovarian and uterine examinations were conducted, in addition to necropsy (examination of thoracic, abdominal, and pelvic viscera). The applicant's Text Table 7 below lists the tissues which were collected and preserved from all Main Study animals at necropsy and from those which died prior to scheduled sacrifice.

Text Table 7
Tissue Collection and Preservation

Tissue	Collected	Comment
Cervix	X	Collected with uterus. Nonpregnant rats.
Esophagus	X	Infused with 10% neutral buffered formalin. Rats euthanized before scheduled termination.
Heart	X	Rats euthanized before scheduled termination.
Kidney	X	Rats euthanized before scheduled termination.
Liver	X	Rats euthanized before scheduled termination.
Lung	X	Infused with 10% neutral buffered formalin. Rats euthanized before scheduled termination.
Ovaries	X	Nonpregnant rats.
Spleen	X	Rats euthanized before scheduled termination.
Stomach	X	Rats euthanized before scheduled termination.
Trachea	X	Infused with 10% neutral buffered formalin. Rats euthanized before scheduled termination.
Uterus	X	Collected with cervix. Nonpregnant rats.

X = Procedure conducted

No gross lesions were observed at necropsy.

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

The reproductive tract was dissected and examined. The ovaries and uterus were opened and contents were examined for the following: distribution of corpora lutea, implantation sites, placentae (size, color, shape), live and dead fetuses, and early and late resorptions.

There were no treatment-related effects on the number of pregnant dams, corpora lutea, implantations, % pre- and postimplantation loss, litter sizes, number of live and dead fetuses, or the number of resorptions (early, late). There were no dams with all conceptus resorbed in any dose group and no abnormal placentae were noted. There were no treatment-related effects on the ratio of male and female live fetuses. There was a slight decrease (~4%) in live fetal body weight (g/litter) in the uridine-treated dose groups ($p \leq 0.05$, as compared to control). There was a statistically significant decrease

in the number of male and female pups born to uridine triacetate-treated dams, as compared to control (~2.6-4.1%, $p \leq 0.05$).

Offspring (Malformations, Variations, etc.)

External, visceral and skeletal abnormalities in fetuses were recorded and classified as malformations or variations. Fetal examinations included determination of sex, external abnormalities, and body weight.

There was a statistically significant decrease in the number of alterations/litter in the 500 mg/kg/day uridine triacetate: 7 litters, 1 litter, and 10 litters with observed alterations in the control, 500 mg/kg/day and 2000 mg/kg/day uridine triacetate dose groups, respectively. One fetus (litter size = 14) born to a HD female had a thread like tail and no anal opening. There was one litter in the HD group with a thread like tail (in 1 fetus) and no anal opening (1 fetus). There were no other gross alterations noted in any of the litters born to dams in all dose groups. There were no soft tissue fetal alterations noted in any of the dose groups. A few skeletal alterations were observed and are summarized in the table below. There were no malformations observed in the study in any of the dosing groups. These findings are summarized in **Table 17** below.

Table 17: Observed skeletal alterations in pups born to female rats treated with uridine triacetate by oral gavage from GD 6-GD 17

	Group	Control	500 mg/kg/day	2000 mg/kg/day
		# litters evaluated	25	24
	# litters with live fetuses	25	24	22
Finding	#fetuses evaluated	176	168	149
Incompletely ossified zygomatic arch in skull	Litter incidence	2	0	0
	Fetal Incidence	2	0	0
Incompletely ossified squamosal bone in skull	Litter incidence	2	0	0
	Fetal Incidence	2	0	0
Cervical rib present at 7 th cervical vertebra	Litter incidence	0	1	3
	Fetal Incidence	0	1	3
Thoracic vertebra, Centrum Bifid	Litter incidence	3	0	2
	Fetal Incidence	3	0	2
Short ribs	Litter incidence	2	0	2
	Fetal Incidence	2	0	2
Incompletely ossified ribs	Litter incidence	0	0	1
	Fetal Incidence	0	0	1
Incompletely ossified sternal centra	Litter incidence	0	0	3**
	Fetal Incidence	0	0	3**

** $p \leq 0.01$

9.3 Prenatal and Postnatal Development

No study reports from pre- and postnatal development studies were submitted. The sponsor previously agreed to conduct a pre- and postnatal development study post-approval.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Hereditary orotic aciduria (HOA) is a rare congenital disorder (25 known cases worldwide) which is caused by mutations which reduce the activity of uridine 5'-monophosphate synthase (UMPS), the enzyme which converts orotic acid to uridine monophosphate (UMP). UMP is the precursor of pyrimidine nucleotides necessary for RNA and DNA synthesis and is incorporated into other nucleotide cofactors. HOA patients therefore have impaired de novo pyrimidine synthesis. Because of the deficiency in UMP, patients with HOA have a build-up of orotic acid, which is normally regulated by feedback inhibition by intracellular nucleotides. Accumulated orotic acid is excreted in the urine and can cause crystalluria and lead to obstructive uropathy. HOA is characterized by orotic aciduria, megaloblastic anemia or other hematological abnormalities such as impaired lymphocytic function or neutropenia, in addition to physical and developmental delays. There are, however, documented cases of HOA without anemia in the literature.

There are currently no approved treatments for HOA. Uridine replacement in HOA can replenish endogenous concentrations of uridine, restore normal pyrimidine biosynthesis and function, and decrease orotic acid build up and normalize its concentration in the urine. Oral uridine replacement therapy (ranging between 50-300 mg/kg/day), if initiated early at adequate doses, has been shown to be clinically beneficial for patients with HOA, allowing them to lead an essentially normal life. HOA patients would require lifelong uridine replacement therapy. Uridine, however, has poor oral bioavailability and can produce dose-limiting osmotic diarrhea. Uridine triacetate has high oral bioavailability and delivers 4-7 times more uridine into the systemic circulation, as compared to equimolar doses of uridine.

In this NDA, Wellstat Therapeutics Corporation is seeking marketing approval for uridine triacetate, which is the acetylated form for uridine, for the treatment of HOA. Uridine triacetate is formulated as granules in 2 g and (b) (4) packets for oral administration. The proposed starting dose of uridine triacetate is 60 mg/kg/day and can be increased to the maximum recommended human dose of (b) (4) mg/kg/day.

In support of this NDA, the applicant, in agreement with the review division's recommendations, submitted safety pharmacology studies, pharmacokinetics (PK) studies, repeat-dose toxicology studies in dogs (3 months) and rats (3 months and 6 months), genetic toxicology studies, and a fertility and early embryonic development

study in rats and an embryo-fetal development study in rats. A pre- and postnatal study in rats is to be conducted post-approval. At the time of submission, a carcinogenicity study was not conducted. The findings from the nonclinical safety studies are summarized briefly below.

Cardiac safety pharmacology of uridine triacetate included *in vitro* studies only. Uridine triacetate inhibited hERG channel current with an $IC_{50} = 3137 \mu\text{M}$ at a maximum concentration of $9600 \mu\text{M}$, when compared to uridine which did not produce significant hERG inhibition ($IC_{50} > 10,000 \mu\text{M}$). Uridine triacetate produced an increase in action potential duration and resting membrane potential and decreased action potential amplitude and rate of depolarization in isolated rabbit cardiac fibers at the highest dose tested ($8582 \mu\text{M}$), unlike uridine, which did not produce similar effects at up to $10000 \mu\text{M}$. Uridine triacetate undergoes rapid and complete deacetylation after oral administration. Uridine triacetate levels were below the limit of detection in the animal and in human plasma (or whole blood) due to high esterase activity in these compartments. First pass metabolism of uridine triacetate converts all circulating uridine triacetate to uridine. Therefore, the observed *in vitro* cardiac effects are not predictive of adverse cardiac effects *in vivo* (ex: QT prolongation). In the animal toxicology studies, uridine triacetate did not produce any overt systemic toxicity or specific organ toxicity. No treatment-related effects on the central nervous system or cardiovascular and respiratory systems were reported in dogs (3-month repeat-dose study) and in rats (3 month and 6 month repeat-dose study). Furthermore, there have been no adverse cardiac effects or other organ system toxicities reported with clinical use of uridine triacetate in children and adults for various indications.

Pharmacokinetics of uridine triacetate in animals was qualitatively similar between animals and humans. *In vitro* studies demonstrated that uridine triacetate did not inhibit or induce CYP enzyme activity and weakly inhibited P-gp. Toxicokinetics were incorporated into repeat-dose toxicity studies and reproductive toxicity studies in rats. In all studies, study animals were systemically exposed to uridine triacetate and uridine and uracil were the major analytes in the plasma. Plasma uracil levels were higher in rats, dogs, and mice after oral administration of uridine triacetate or uridine, mainly due to higher levels of uridine phosphorylase in animals, as compared to humans. At clinical human dose of 60 mg/kg/day , the plasma level of uridine was lower than what was observed in animals at the NOAEL doses in repeat-dose toxicology studies (Refer to Table 14 page 54 of the review).

Repeat-dose toxicity studies in rats and dogs demonstrated that uridine triacetate has a favorable safety profile. There were no significant, treatment-related toxicities or deaths and the NOAEL doses in both species were the highest doses administered or the maximum feasible dose (1500 mg/kg/day in dogs in the 3-month study and 2000 mg/kg/day in the 6-month rat study). Uridine triacetate was not genotoxic at the limit dose of $5000 \mu\text{g/plate}$ and 1 mM ($370 \mu\text{g/mL}$) in the bacterial mutagenicity test (2 separate assays) and in the L5178Y mouse lymphoma assay, respectively. Uridine triacetate was also negative in the *in vivo* mouse micronucleus assay when administered as a single dose of 2000 mg/kg .

Carcinogenicity studies were not conducted with uridine triacetate. The applicant submitted a Carcinogenicity Risk Assessment Document with the NDA to support their request for a waiver on the requirement to conduct a 2-year rodent carcinogenicity study. The applicant's rationale was supported by: (1) absence of histopathological findings suggestive of tumorigenic potential and no evidence of hormonal perturbations with uridine triacetate in repeat dose toxicity studies at doses up to 2000 mg/kg/day, (2) rapid deacetylation and undetectable levels of uridine triacetate in the plasma after oral administration, (3) no evidence of genotoxicity with uridine triacetate *in vitro* and *in vivo*, (4) absence of mortality or morbidity due to tumor formation in long term studies in rodent models of Alzheimer's Disease and Huntington's Disease with repeated oral doses of uridine triacetate at up to 12 g/kg/day for one year (which is ~16x greater than the proposed clinical dose of 60 mg/kg/day), (5) higher plasma levels of uridine at NOAEL doses in animals, when compared to humans receiving the clinical dose of 60 mg/kg/day. Uridine triacetate would be providing exogenous uridine to HOA patients who are not capable of de novo uridine synthesis, thereby restoring uridine levels to normal. Therefore, based on our review of the submission and consultation with the CDER ECAC, we recommend that the applicant be granted the waiver.

In conclusion, uridine triacetate, which is formulated as granules for oral administration, is an acetylated form for uridine and has high oral bioavailability (4-7x greater than uridine). Uridine triacetate is rapidly metabolized in the plasma and delivers exogenous uridine for incorporation into pyrimidine nucleotides. Uridine along has poor oral bioavailability and produces dose-limiting diarrhea. In HOA patients who have a deficiency in de novo pyrimidine synthesis, uridine triacetate normalizes uridine levels and prevents orotic acid build up and associated hematological effects. HOA patients can live a fairly normal life with uridine replacement therapy, which would be taken lifelong.

The applicant has adequately characterized the safety of uridine triacetate in animals and has provided clinical safety data from the use of uridine or uridine triacetate for various indications. Uridine triacetate has been shown to be well tolerated in adults and children at doses up to 40 g/day, with minimal safety concerns for various indications, including diabetic nephropathy, mitochondrial and neurometabolic disorders, and 5-fluorouracil toxicity. In repeat-dose studies in animals, there were no overt signs of toxicity or morbidities. Orally administered uridine triacetate did not produce significant treatment-related toxicities when administered for 6 months at up to 2000 mg/kg/day in rats. The NOAEL dose from this 6-month study (2000 mg/kg/day) is ~^{(b) (4)} times the maximum recommended human dose of ^{(b) (4)} mg/kg/day. The applicant's table 2.4.4-1 below shows the exposure comparison between humans and animals.

Table 2.4.4-1 Exposure to Plasma Uridine and Uracil Following Oral Uridine Triacetate and Calculated Safety Factors in Rats, Dogs and in Patients with Hereditary Orotic Aciduria

Study	Uridine Triacetate Dosage	Pharmacokinetic Parameter (average)							
		Uridine				Uracil			
		C _{max} (µM)	AUC _{0-t} (µM•h)	T _{max} (h)	t _{1/2} (h)	C _{max} (µM)	AUC _{0-t} (µM•h)	T _{max} (h)	t _{1/2} (h)
6-Month Rat NOAEL^b	2000 mg/kg/day ^a (1000 mg/kg bid)	M: 205 F: 217	M: 262 F: 316	M: 0.25 F: 0.25	NC	M: 643 F: 839	M: 1980 F: 2830	M: 0.75 F: 2.00	NC
Segment 2 Rat NOAEL^c	2000 mg/kg/day ^a (1000 mg/kg bid)	F: 170	F: 271	F: 0.75	NC	F: 917	F: 2800	F: 2.00	NC
3-Month Dog NOAEL^d	1500 mg/kg/day ^a (750 mg/kg bid)	M: 679 F: 447	M: 1402 F: 957	M: 2.0 F: 2.25	M: 0.50 F: 0.49	M: 586 F: 479	M: 2216 F: 1590	M: 3.5 F: 3.0	M: 1.66 F: 1.07
Patients with Hereditary Orotic Aciduria^e (N = 4)	60 mg/kg/day	90	295	Day 1: 1.84 Day 28: 1.51	Day 1: 1.65 Day 28: 2.29	BLQ	BLQ	NA	NA
Safety Factor (based on 6-mo rat):		2.2 to 2.4	0.8 to 1.0			NC	NC		
Safety Factor (based on Seg 2 rat):		1.8	0.9						
Safety Factor (based on 3-mo dog):		4.9 to 7.5	3.2 to 4.7			NC	NC		

All values shown are averages.

BLQ: most values for plasma uracil in patients with HOA were below the limit of quantitation.

NA: not available

NC = not able to be calculated

^a NOAEL in the study (the highest feasible dose).

^b Data shown are from samples taken up to 6 hours after the first (morning) dose on Day 182 (steady-state) in Study 20047236.

^c Data shown are from samples taken up to 6 hours after the first (morning) dose on Gestation Day (GD) 17 (steady-state) in Study 20040947.

^d Data shown are from samples taken up to 6 hours after the first (morning) dose on Day 80 (steady-state) in Study (b) (4) 552.

^e Data shown are from samples taken up to 8 hours after the first (morning) dose (average of Days 1 and 28) in Protocol 401.13.001.

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Taken together, from a nonclinical standpoint, uridine triacetate should be approved for the treatment of HOA at the doses proposed.

12 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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06/18/2015

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06/18/2015