CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208159Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: December 8, 2015
From: Todd R. Palmby, PhD
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
To: File for NDA 208159 Vistogard (uridine triacetate)
Re: Approvability for Pharmacology and Toxicology
Indication: for emergency treatment of adult and pediatric patients:

- Following a fluorouracil or capecitabine overdose, or
- Who exhibit early-onset, severe or life-threatening toxicity affecting the cardiac or central nervous system, and/or early-onset, unusually severe adverse reactions (e.g., gastrointestinal toxicity and/or neutropenia) within 96 hours following the end of fluorouracil or capecitabine administration.

On July 7, 2015, Wellstat Therapeutics completed the rolling submission of NDA 208159 for Vistogard (uridine triacetate) [redacted] (b) (4) Nonclinical pharmacology and toxicology literature and original reports for studies to support NDA 208159 for Vistogard (uridine triacetate) for the proposed indications were reviewed by W. David McGuinn, Jr, MS, PhD, DABT. The nonclinical studies conducted with uridine triacetate for which reports were submitted to this NDA included pharmacology (original reports and literature), pharmacokinetics, safety pharmacology, general toxicology (3-month repeat-dose in dogs; 3- and 6-month repeat-dose in rats), genetic toxicology and reproductive and developmental toxicology. Nonclinical safety studies conducted with uridine triacetate that were submitted to NDA 208159 were previously reviewed by Sruthi King, PhD, under NDA 208169 for Xuriden (uridine triacetate), which was approved by the US FDA Division of Gastroenterology and Inborn Errors Products on September 4, 2015, as uridine replacement therapy in pediatric patients with hereditary orotic aciduria. The review of these studies conducted under NDA 208169 was adequate to support NDA 208159; Dr. King's review of NDA 208159 of nonclinical safety studies with uridine triacetate was referenced for these studies. Relevant results of these studies were summarized in Dr. McGuinn's review of NDA 208159. The main focus of the Pharmacology/Toxicology review for NDA 208159 was the original reports and literature for pharmacology studies.

In pharmacology studies, mice were administered sub-lethal doses of fluorouracil. Subsequent administration of oral uridine triacetate reduced, but did not completely prevent, hematological toxicity, assessed by white blood cell count, in a dose-dependent manner. When mice were administered lethal doses of fluorouracil, administration of oral uridine triacetate increased survival rates to 90% when given within 24 hours. Survival rates decreased with increasing time

between fluorouracil and uridine triacetate administrations (e.g., 20% survival in mice given uridine triacetate 96 hours after fluorouracil). This demonstrated that early uridine triacetate administration following fluorouracil should result in a greater mitigation of toxicity. However, no definitive conclusion should be made from these animal studies regarding the prognosis of patients receiving uridine triacetate at various times following fluorouracil. No specific survival rates at various time intervals for these mouse experiments were included in the label for this reason.

There are two primary mechanisms of fluorouracil or capecitabine that are described in published literature. Fluorouracil is a cytotoxic antimetabolite that interferes with nucleic acid metabolism in cells. Fluorouracil is metabolized to the cytotoxic intermediates 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FdUMP inhibits thymidylate synthase, blocking thymidine synthesis. FUTP is incorporated into RNA proportional to systemic fluorouracil exposure. Uridine is converted into uridine triphosphate (UTP), which competes with FUTP for incorporation into RNA. Whether uridine triacetate can lead to inhibition of thymidylate synthase is not completely clear. There are reports in the literature demonstrating that uridine does not reverse thymidylate synthase inhibition. Other reports show that dUMP can compete with FdUMP at thymidylate synthase sites. The potential for uridine triacetate to affect dUTP levels and impact the thymidylate synthase inhibition by fluorouracil in tumor cells in vivo is unclear.

The FDA text phrase in the label for the Established Pharmacologic Class (EPC) of uridine triacetate in the Indications and Usage section of the Highlights is "pyrimidine analogue." This was thought to be the most scientifically accurate and clinically meaningful phrase without being promotional or misleading.

There is a potential that uridine triacetate administration may affect the anti-tumor efficacy of fluorouracil. The relative contributions of RNA toxicity and DNA toxicity of fluorouracil to the anti-tumor activity in various diseases have not been adequately demonstrated in humans. Xenograft mouse models of human tumors treated with fluorouracil followed by administration of uridine triacetate did not indicate an effect on fluorouracil anti-tumor activity. However, these studies were not conclusive. In addition, mouse models are not adequate to demonstrate that uridine triacetate will not impact fluorouracil efficacy in humans. The Applicant did not submit adequate clinical data to demonstrate that uridine triacetate does not affect the efficacy of fluorouracil.

Uridine triacetate resulted in little toxicity even at high daily doses in nonclinical toxicology studies. In repeat-dose toxicology studies, uridine caused no significant adverse effects in dogs or rats. Rats were administered the maximum feasible dose of 2000 mg/kg/day, which was the NOAEL in the 6-month study. Uridine triacetate was not genotoxic in the Ames test, the in vitro mouse lymphoma assay or the in vivo mouse micronucleus test. Rodent carcinogenicity

studies were not conducted with uridine triacetate. There were no findings suggestive of tumorigenic potential in the 6-month repeat-dose toxicity study in rats. The majority of patients indicated to receive the emergency treatment of uridine triacetate are being treated with genotoxic fluorouracil for advanced cancer. In addition, patients receiving uridine triacetate for the emergency treatment of fluorouracil or capecitabine overdose or who exhibit early-onset severe or life-threatening toxicities will receive 20 doses every 6 hours for a total of 5 days of treatment. Therefore, long-term carcinogenicity studies are not warranted to support approval of this NDA for the proposed indications. Oral uridine triacetate did not affect fertility or general reproductive performance in male or female rats, and did not product maternal toxicity or teratogenic effects in an embryo-fetal developmental toxicity study in rats at doses up to 2000 mg/kg/day, which is approximately 50% of the recommended human dose of 40 g per day based on body surface area.

Recommendation: I concur with Dr. McGuinn's conclusion that submitted pharmacology and toxicology data support the approval of NDA 208159 for Vistogard. There are no outstanding non-clinical issues that would preclude the approval of Vistogard for the proposed indications.

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/s/

TODD R PALMBY
12/08/2015

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
PHARMACOLOGY and TOXICOLOGY NDA REVIEW AND EVALUATION

Application Number	NDA 208159
Supporting Documents	3 (eCTD Sequence 0001)
Applicant Letter Date	July 10, 2015
CDER Stamp Date	July 10, 2015
Product	Vistogard™ (uridine triacetate)
Indication	For the emergency treatment of adult and pediatric patients: • following fluorouracil or capecitabine overdose, or • who exhibit early-onset, severe or life-threatening toxicity affecting the cardiac or central nervous system, and/or early-onset, unusually severe adverse reactions (e.g., gastrointestinal toxicity and/or neutropenia) within 96 hours following the end of fluorouracil or capecitabine administration.
Review Division	Division of Hematology Oncology Toxicology (Division of Oncology Products 1)
Applicant	Wellstat Therapeutics Corporation (Wellstat) Gaithersburg, MD
Reviewer	W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T.
Supervisor	Todd Palmby, Ph.D.
Division Director	John Leighton, Ph.D., D.A.BT. (acting, DHOT) (Geoffrey Kim, M.D. (DOP1))
Project Manager	Jeannette O'Donnell
Medical Officer	Gwynn Ison, M.D.
Chemist	Donghau (Robert) Lu, Ph.D.
Clinical Pharmacologist	Runyan Jin, Ph.D.
Statistics	Joyce Cheng, Ph.D.

Disclaimer:

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208159 are owned by Wellstat Therapeutics Corporation or are data for which Wellstat has obtained a written right of reference. Any information or data necessary for approval of NDA 208159 that Wellstat does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208159.

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1 Executive Summary

1.1 Introduction

Uridine triacetate (2',3',5'-tri-O-acetyluridine) is a prodrug of uridine. Wellstat Therapeutics Corporation has developed this compound for the treatment of patients who suffer unacceptably high exposure to 5-fluorouracil and consequent toxicosis. After an over exposure to 5-fluorouracil, patients will take a high antidotal dose of uridine triacetate orally for five days. Various deacetylase enzymes in the gastrointestinal tract, liver and blood hydrolyze the compound to uridine. Uridine is sequentially phosphorylated to uridine triphosphate (UTP), an essential component of RNA. 5-Fluorouracil gains a ribose and is phosphorylated through the same enzymatic pathways to form 5-fluorouridine triphosphate. This toxin competes with uridine triphosphate during the biosynthesis of RNA causing serious metabolic errors. These errors accumulate and lead to cellular necrosis or apoptosis. Excess uridine from oral uridine triacetate and its subsequent metabolic products compete with the product of 5-fluorouracil throughout the biosynthetic pathway toward the formation of RNA thus diminishing the formation of metabolically erroneous RNAs and proteins. This antidotal activity preserves cells that would otherwise die. 5-fluorouracil also forms metabolites that bind to thymidylate synthase, thereby disrupting the formation of thymidylate and the subsequent synthesis of DNAs. The effects of excess uridine triacetate treatment on toxicities arising from this pathway have not been determined.

On January 8, 2015, Wellstat submitted an NDA (208169) for uridine triacetate (Xuriden) to Division of Gastroenterology and Inborn Errors Products (CDER/ODEIII/DGIEP) for uridine replacement therapy in children with hereditary orotic aciduria. DGIEP approved Xuriden as an orphan drug September 4, 2015. The toxicology reviewer for DGIEP was Sruthi T. King, Ph.D. The clinical reviewer was Carla Epps, M.D.

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 all these studies, uridine triacetate demonstrated very little toxicity even at high daily doses as one might expect of an acetylated pyrimidine natural product.

The studies of uridine triacetate efficacy reviewed here showed that uridine triacetate and uridine both prevent further damage due to 5-FU over exposure as measured by white cell parameters once they are given, but these treatments do not reverse the damage by day 8. By day 12 white cell parameters remain below historical controls, but show signs of recovery. The antidotal activity of uridine triacetate demonstrates a dose response with a plateau at the highest doses of these experiments. These results suggest that the clinical dose is higher than necessary to achieve the desired clinical response.

When 5-FU is given to mice at a relatively high dose without [redacted] (b) (4) or at a low dose with [redacted] (b) (4), uridine triacetate significantly increases survival. Survival decreases as

the interval between the administration of 5-FU and the administration of uridine triacetate increases. Administration of uridine triacetate more than 96 hours after the 5-FU dose is ineffective.

Once given, uridine triacetate stops the progressive damage caused by overexposure to 5-FU in the intestines of mice. This antidotal effect can be seen qualitatively as improved tissue health in micrographs and quantitatively as increased two dimensional surface areas of the intestinal villi. The area of the intestinal villi after uridine triacetate treatment was statistically equivalent to that of saline controls.

Though these efficacy experiments are poorly designed and in some places poorly controlled and missing data the total body of evidence indicates that uridine triacetate prevents further damage from high exposures to 5-FU once it is administered. It does not appear to significantly hasten recovery. (b) (4) The evidence of efficacy in animals supports the evidence of clinical efficacy for this setting.

Dr. King reviewed the toxicology studies of uridine triacetate under NDA 208169. These studies are adequate to support the safety of uridine triacetate for the indication covered under NDA 208159. There are no significant toxicological concerns with the use of uridine triacetate for the proposed indication.

1.3 Recommendations

1.3.1 Approvability

There are no Toxicological problems that would prevent the approval of Uridine Triacetate.

1.3.2 Additional Non Clinical Recommendations

The data from animal studies supports the clinical efficacy of Uridine Triacetate.

1.3.3 Labeling

The toxicology and pharmacology sections of the label for this NDA are largely the same as those for Xuriden (NDA 208169). The dose comparisons are different because the dose of uridine triacetate for this indication is significantly higher than that of the Xuriden indication. The mechanism section, 12.1, was expanded to read as follows:

"12.1 Mechanism of Action

Uridine triacetate is an acetylated pro-drug of uridine. Following oral administration, uridine triacetate is deacetylated by nonspecific esterases present throughout the body, yielding uridine in the circulation. Uridine competitively inhibits cell damage and cell death caused by fluorouracil.

Fluorouracil is a cytotoxic antimetabolite that interferes with nucleic acid metabolism in normal and cancer cells. Cells anabolize fluorouracil to the cytotoxic intermediates 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FdUMP inhibits thymidylate synthase, blocking thymidine synthesis. Thymidine is required for DNA replication and repair. Uridine is not found in DNA.

The second source of fluorouracil cytotoxicity is the incorporation of its metabolite, FUTP, into RNA. This incorporation of FUTP into RNA is proportional to systemic fluorouracil exposure. Excess circulating uridine derived from VISTOGARD is converted into uridine triphosphate (UTP), which competes with FUTP for incorporation into RNA."

The label for Vistogard also contains a section added as 13.2 in order to briefly describe the efficacy findings in animal model studies. This section reads as follows:

"13.2 Animal Toxicology and/or Pharmacology

In mice given a sub-lethal dose of fluorouracil, the administration of oral uridine triacetate diminished hematological toxicity as a function of increasing dose, but did not completely prevent hematological toxicity. In mice given a lethal dose of fluorouracil, administration of oral uridine triacetate increased survival to 90% when given within 24 hours. Survival diminished with increasing interval between the fluorouracil dose and uridine triacetate treatment demonstrating that earlier administration of uridine triacetate is more beneficial. In similar experiments in mice, uridine triacetate treatment diminished damage to the intestinal mucosa caused by fluorouracil treatment."

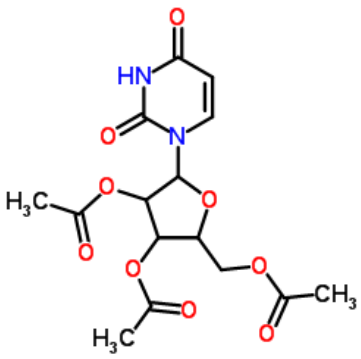
2 Drug Information

2.1 Drug

CAS Registry Number	4105-38-8
Proprietary Name	VISTOGARD (formerly known as [redacted] (b) (4))
Generic Name	Uridine Triacetate Uridine 2',3',5'-triacetate Triacetyluridine 2',3',5'-Triacetyluridine 2',3',5'-Tri-O-acetyluridine

The abbreviation used throughout this review will be UTA (TAU in some graphs).

Code Name	PN401
Chemical Name IUPAC	[(2R,3R,4R,5R)-3,4-Diacetyloxy-5-(2,4-dioxopyrimidin-1-yl)-oxolan-2-yl]methyl acetate
Chemical Name CAS	1-(2',3',5'-tri-*O*-acetyl-ß-D-ribofuranosyl)-2,4(1H,3H)-pyrimidinedione
Molecular Formula	$C_{15}H_{18}N_2O_9$
Molecular Weight	370.31 grams per mole
Chemical Structure	



Pharmacologic Class FDA text for Established Pharmacologic Class (EPC) is “pyrimidine analogue”

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND [redacted] (b) (4)
IND 118931 Commercial PN401, Wellstat
IND 039571 Commercial Triacetyluridine, Pro-Neuron Inc. original IND submission

There are numerous research INDs; most are withdrawn or terminated.

NDA 208169 Xuriden, Uridine Triacetate, Wellstat, CDER/ODEIII/DGIEP
Indication: Uridine replacement therapy in pediatric patients with hereditary orotic aciduria

2.3 Drug Formulation

Wellstat provides Uridine Triacetate as granules (b) (4) for oral ingestion containing 10 grams of the drug product. The granules are comprised of uridine triacetate (95%) with "Opadry® (b) (4) Clear" (b) (4) . The granules also contain Natural Orange Juice Flavor (b) (4) . The contents (b) (4) ingested by the patient or infused via nasograstric tube.

2.4 Comments on Impurities or Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Dosing	
Dose	10 grams ((b) (4) granules)
Schedule	every six hours for a total of 20 doses
Route	Oral
Total dose	200 g

Clinical Protocol

Protocol Number 401.10.001: An Open-Label Protocol for the Use of Uridine Triacetate as an Antidote to Treat Patients at Excess Risk of 5-Fluorouracil Toxicity Due to Over-dosage or Impaired Elimination

Primary Objectives

- To provide uridine triacetate as an antidote to treat adult patients at excess risk of 5-FU toxicity due to overdose (defined as administration of 5-FU at a dose or infusion rate greater than the intended dose or MTD for the patient's intended regimen) or patients presenting with rapid onset of serious toxicity known or suspected to be due to impaired elimination or mutations known to result in increased susceptibility to 5-FU toxicity.
- To evaluate survival for 30 days or until chemotherapy is resumed if within the 30-day observation period, in patients treated with uridine triacetate who are at excess risk of 5-FU toxicity due to over-dosage or presenting with rapid onset of serious toxicity.

Secondary Objectives

- To assess the occurrence, severity, and duration of hematological, GI, skin, neurological, and cardiovascular toxicities in patients at excess risk of 5-FU toxicity due to overdose or presenting with rapid onset of serious toxicity
- To assess the occurrence, severity, and duration of mucositis, diarrhea, and skin and neurological toxicities, commonly associated with 5-FU dosing, in patients at excess risk of 5-FU toxicity due to over-dosage or impaired elimination
- To assess systemic levels of uridine and uracil in treated patients
- To assess the safety and tolerability of uridine triacetate in treated patients

Clinical Trial Design

The applicant provided the results of an open-label trial designed to provide expanded access to uridine triacetate (b) (4) for patients at excess risk of 5-FU toxicity due to overdose or patients exhibiting rapid onset of serious toxicity following 5-FU administration. When an investigator associated with the trial considered a patient at excess risk of 5-FU toxicity or exhibited early onset of serious toxicity following 5-FU administration they contacted Wellstat. Patients with an overdose (e.g., due to infusion pump malfunction or incorrect programming) were often identified quickly and prior to the patient presenting with symptoms of toxicity. Patients with rapid onset of serious toxicity were identified based on presentation of serious symptoms and toxicities associated with 5-FU. The investigator then provided the following information to Wellstat for determination of eligibility of the patient for treatment under the expanded access Protocol 401.10.001: demographics, disease information, prior disease-directed treatment, including 5-FU therapy, details of the over dosage such as dose, cause, and times of infusions, and symptoms associated with the over dosage, as well as other chemotherapies included in the regimen. The patient was eligible for emergency treatment if the circumstance met all inclusion and exclusion criteria. If the patient was eligible for treatment, the Wellstat provided the trial-related materials to the Investigator, which included the study protocol, ICF template, treatment regimen, and dosing log. Wellstat then immediately shipped uridine triacetate to the Investigator. Patients were to begin treatment with uridine triacetate as soon as possible, and no later than 96 hours after completion of 5-FU dosing. In addition to uridine triacetate, patients could also receive supportive care at the discretion of the treating physician. The patient's clinical course and outcome, including survival, were to be assessed for 30 days following the 5-FU overdose unless the patient died or resumed chemotherapy within the 30-day period

The primary efficacy endpoint was survival after a 30 day following the 5-FU overdose; secondary endpoints included assessments of the occurrence, severity, and duration of neutropenia, thrombocytopenia, leukopenia, mucositis, diarrhea, skin, neurological and cardiovascular toxicities, and systemic levels of uridine and uracil. Safety and tolerability of uridine triacetate was evaluated by assessments of vital signs, laboratory values and AEs.

2.7 Regulatory Background

- Pro-Neuron Inc. submitted the original IND (039571) for uridine triacetate on May 5, 1992.
- In June of 2002 Pro-Neuron Inc. changed their corporate name to Wellstat Therapeutics Corporation.
- On July 6, 2010 Wellstat met with the FDA to discuss End of Phase 2 development.
- On August 15, 2013 Wellstat again met with the FDA to discuss End of Phase 2 development.
- On August 27, 2014, Wellstat met with the FDA (OHOP) in a Type A meeting to discuss the filing of an NDA. We advised Wellstat that approval might be achieved through application of the animal rule in conjunction with existing clinical data. We discussed the design of a new and statistically well powered GLP study in rodents to demonstrate the efficacy of uridine triacetate. Subsequently, we advised Wellstat that their existing animal studies would be sufficient.
- Wellstat submitted this NDA on July 10, 2015. The clinical review team subsequently determined the clinical data to be sufficient to support a full review of this application without the need for reliance on nonclinical efficacy data.

3 Studies Submitted

Dr. King reviewed the following studies. These were all the studies Wellstat submitted to NDA 208169 except a dose range-finding embryo-fatal development study . Wellstat submitted these studies to NDA 208159 in addition to the three efficacy studies reviewed here (*v.i.*). Refer to Dr. King's review of the nonclinical safety studies under NDA 208169 dated 6/18/2015.

Study Number	**Study Title**	GLP
Pharmacology		
120119.XFM	Effect of Uridine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	Yes
120120.XFM	Effect of Uridine on Action Potentials in Isolated Rabbit Cardiac Fibers	Yes
121130.XFM	Effect of Uridine on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	Yes
120201.XFM	Effect of Uridine on Action Potentials in Isolated Rabbit Cardiac Fibers	Yes
R.401.12.01	Evaluation of PN401 (uridine triacetate) in a recombinant hERG Potassium Ion Channel Membrane Binding Assay	No

Pharmacokinetics		
R.401.14.02	Comparative Oral Pharmacokinetics of Uridine and Uridine Triacetate in Mice	No
13WELLP1R1, Study 1	Determination of the P-gp Interaction Potential for the Sponsor's Test Articles, Uridine and Uridine Triacetate	No
13WELLP1R1 Study 2	CYP Inhibition by Uridine and Uridine Triacetate	No
Toxicology		
68	Acute Oral Toxicity Test in Rats	Yes
FRC Study No 552	PN401: A 3-month Oral Dose Toxicity Study	Yes
Biocon Study No 71	Sub Chronic Toxicology Study in Rats	Yes
FRC Study No 551	PN401: A 3-month Oral Dose Toxicity Study in the CD® Rat	Yes
2648-100	Subacute Oral Toxicity Study in Dogs	Yes
20047236	A 6-month Study of Uridine Triacetate Administered by Oral Gavage (Twice Daily) in Rats	Yes
Genetic Toxicology		
9600345	Uridine Triacetate: Bacterial Reverse Mutation Test in Salmonella typhimurium and Escherichia coli	Yes
16457-0-401	Mutagenicity Test on PN401 In the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)	Yes
9600346	Uridine Triacetate: In vitro Mammalian Cell Gene Mutation Test in Mouse Lymphoma L5178Y TK+/- Cells	Yes
16457-0-455CO	Genetic Toxicity Evaluation of PN401 In An In Vitro Mouse Micronucleus Oral Limit Dose Assay	Yes
Reproductive Toxicology		
20047304	Study of Fertility and Early Embryonic Development to Implantation of Uridine Triacetate Administered by Oral Gavage (Twice Daily) in Rats	Yes
20040947	An Embryo-fetal Development Study of Uridine Triacetate by Oral Gavage (Twice Daily) in Rats	Yes

3.1 Studies Reviewed

Study Number	Study Title	GLP
R.401.14.01	Effects of Uridine Triacetate on 5-Fluorouracil-Induced	No

	Hematologic Toxicities in Mice	
R.401.14.03	Effects of Uridine Triacetate [PN401] in Two Models of 5-Fluorouracil (5-FU) Overexposure in Mice: (b) (4)	No
R.401.15.01	Anti-Tumor Efficacy of 5-Fluorouracil with and without Uridine or Uridine Triacetate in the CD8F1 Murine Mammary Carcinoma System	No

3.2 Studies Not Reviewed

Study Number	Study Title	GLP
20040946	A dose range-finding embryo-fatal development study	No

3.3 Previous Reviews Referenced

Dr. Will Coulter reviewed some of these studies for the original submission to IND 039571 in 1991. Dr. King and I have reviewed all those studies again.

Abbreviations

EC	Enzyme Catalog number (http://enzyme.expasy.org)
5-FU	5-Fluorouracil
COA	Certificate of Analysis
DPD	Dihydropyrimidine Dehydrogenase
5-EU	Ethynyluracil
HPMC	Hydroxypropylmethylcellulose
SMZ	Sulfamethoxazole
TMP	Trimethoprim
UTA	Uridine triacetate (in some graphs as TAU)

REVIEW

4 Pharmacology

4.1 Primary Pharmacology

1) Effects of Uridine Triacetate on 5-Fluorouracil-Induced Hematologic Toxicities in Mice

Study Number	R.401.14.01
Filename	r4011401-report-body.pdf, Module 4.2.1
Laboratory	Wellstat Therapeutics Corp., Gaithersburg, MD 20878
Study Date	June 1990
GLP	No
Audited	No
Drug	Uridine Triacetate, 1911-A-2 [redacted] (b) (4) Purity 99.19 % by HPLC (September 23, 1991)

Experiment 1: The effects of uridine triacetate on hematological toxicities caused by 5-FU in mice after orally of parenterally administered uridine.

Method

Dose: Table 1 below shows the dose groups in Experiment 1. Notably the experiment does not include an untreated control group, that is a group not treated with 5-FU. Nor does it include a control group treated with vehicle only.

Table 1: Doses for Study 1, Experiment 1

Group	5-FU mg/kg IP	Antidote	Route	Treatment dose mg/kg	Treatment dose mmol/kg
1	150	Vehicle Control	PO	0	0
2	150	Uridine	PO	400	1.64
3	150	Uridine	PO	800	3.28
4	150	Uridine	IP	400	1.64
5	150	Uridine Triacetate	PO	500	1.35

Schedule: Starting two hours after the administration of 5-FU, animals received eight control doses of vehicle or eight antidote treatment doses. All mice received 5-FU (150 mg/kg, IP) at 12:00 PM on Day 1. Treatments (control (vehicle), uridine or uridine triacetate) as shown above were administered 3 times on Day 1 (2:00 PM, 4:00 PM & 6:00 PM), and 5 times on Day 2 (9:00 AM, 11:00 AM, 1:00 PM, 3:00 PM & 5:00 PM).

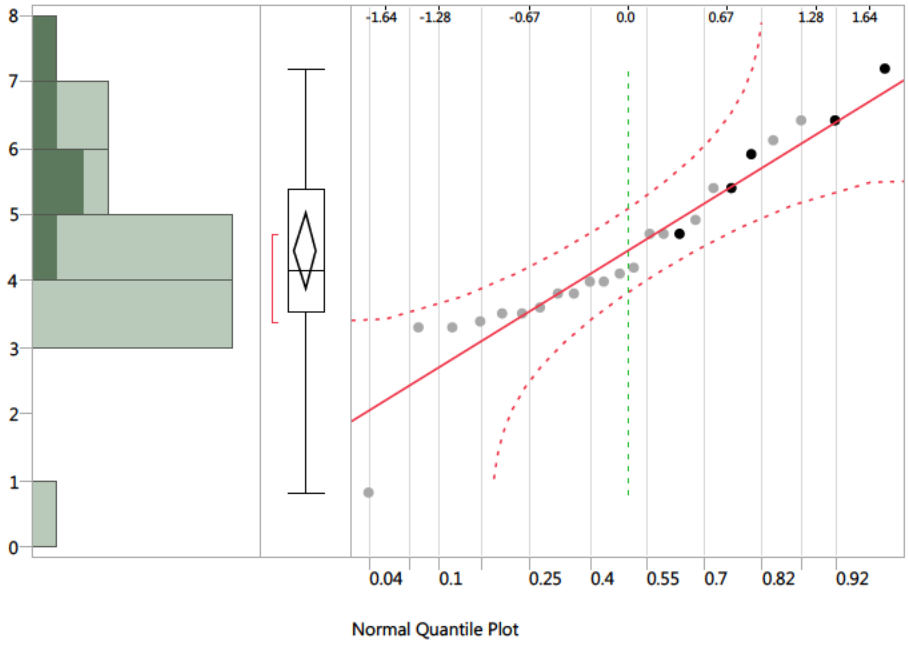
Route: See table above

Dose Volume	0.2 to 0.4 mL
Formulation	Deionized water for oral administration Normal saline for IP administration
Species	Female BALB/C mice
Number	Nine per dose group
Age	Not specified
Weight	About 20 grams
Design	The investigators necropsied five animals per group Day 8 and four animals per group on Day 12 and collected blood samples at both times. They counted cells in the marrow (day 8 only) and weighed the spleens to determine hematopoietic recovery.
Analysis	The investigators did an analysis of variance and used an unspecified parametric post hoc test on each data pair. They did not present results of comparisons between treatment groups. The report did not originally include the individual data values. The Applicant submitted the individual animal data in response to an information request.
Parameters	Marrow count, Spleen weight, WBC, neutrophils, lymphocytes, platelets, RBC

Results

I analyzed all the individual data using JMP software. The following graph shows the distribution of the white blood cell (WBC) count data. I chose WBC for this analysis because among the analytical parameters it shows the clearest response. The values for the uridine triacetate PO 500 mg group (Group 5, main treatment group) are displayed in a darker shade. Significant divergence from the red line in the normal quadrille plot indicates a lack of normality. The plot on the left shows the number animals within each the range of each ordinal value between the extreme values of 0 and 8. If the data set were normal the plot should show a Gaussian distribution.

Figure 1: Test of the WBC data for Normality, Distributions of WBCs



Summary Statistics for Figure 1

Mean	4.5
Std Dev	1.4
Std Err Mean	0.28
Upper 95% Mean	5.0
Lower 95% Mean	3.8
N	24

The outlier at the low end of the plot is mouse O4. The applicant reports the following.

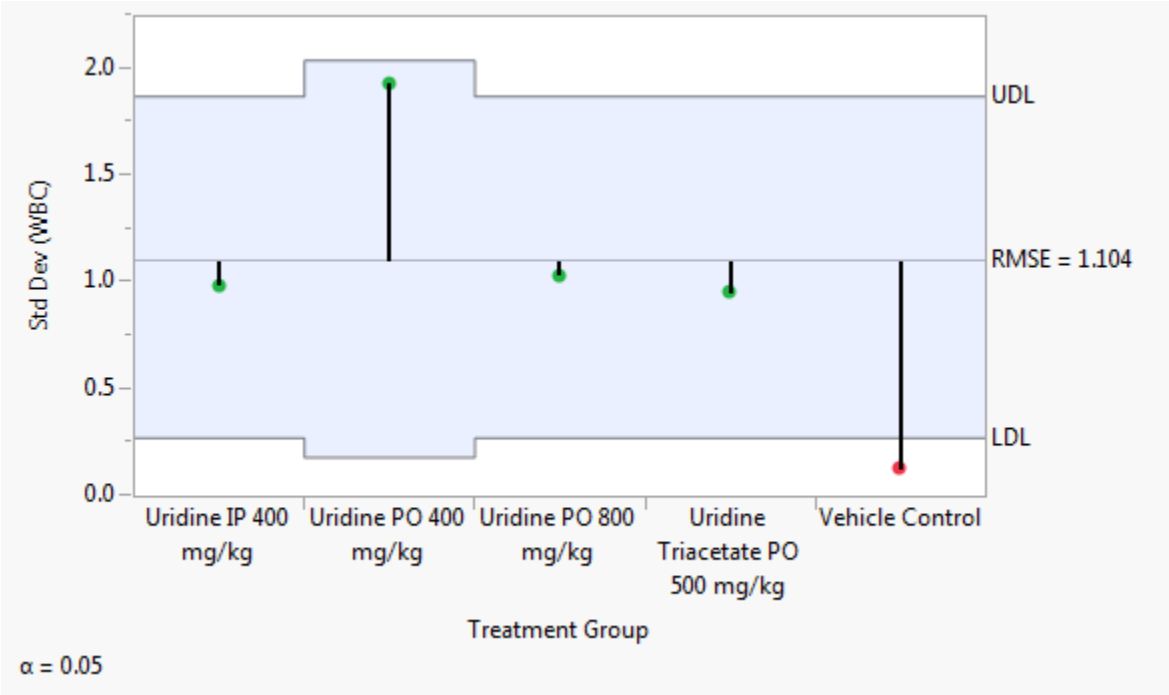
"* CBC data from mouse ID# O4 in the group receiving 400 mg/kg uridine p.o. were deemed to be unreliable, likely due to a sporadic infection and/or undocumented gavage accident, based on WBC counts of 0.8 K/µL, whereas all other mice in all other groups had WBC counts ≥3.5 K/µL. Marrow cell counts were also >3× higher than for any other mice in the group."

The study report does not further elaborate on the "sporadic infection", a condition difficult to document in mice. The very low WBC would argue against an infection. Neutrophils, lymphocytes, platelets and RBCs were also lower than normal in this animal. This suggests that the animal was either inadvertently overdosed or that the dose entered the viscera directly due to gavage error resulting in a higher systemic exposure, the latter being most likely. All further analysis excludes this mouse unless otherwise noted, as it adds significantly to the variance and likely does not give a true indication of the treatment effect. The data set also contains one missing value. The sample from mouse O1 clotted and CBC

values were unobtainable and appear as missing values. Thus the Uridine PO 400 mg/kg after 5-FU group has only three mice in most of the analyses.

The following chart shows an analysis of the WBC means for each group for variance. This analysis is a parametric test for homoscedasticity. The test compares group standard deviations to the root mean square error. This method assumes that the data is approximately normally distributed. The method requires that each group must have at least four observations, so this analysis includes mouse O4 of the Uridine PO 400 mg after 5-FU group.

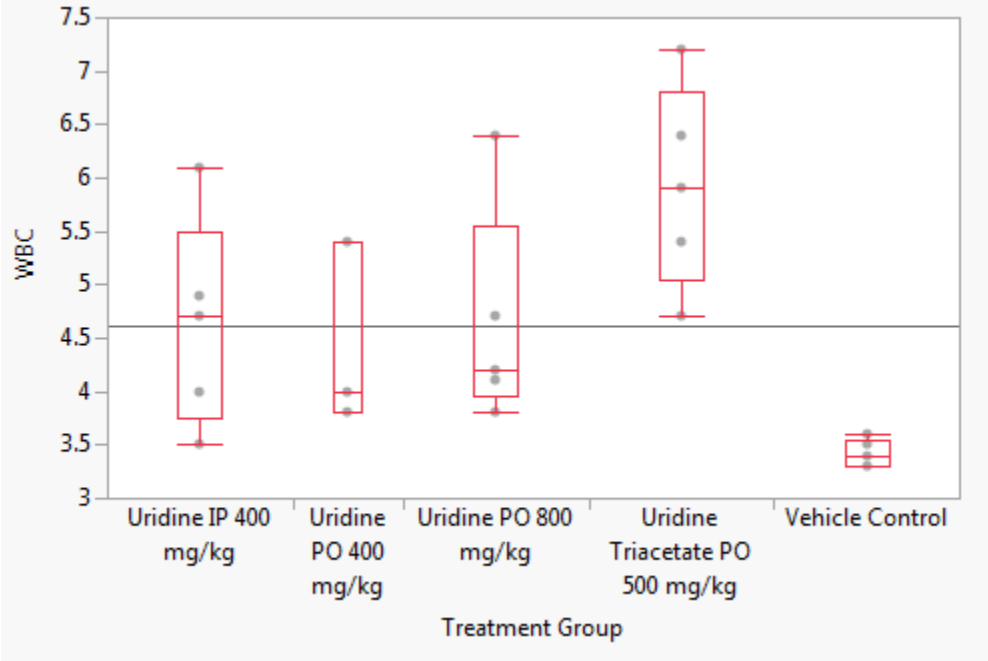
Figure 2: WBC Analysis of Means for Variance



The chart shows a center line indicating the overall root mean square error and an upper and lower decision limit (UDL and LDL). If a group standard deviation falls outside of the decision limits, then that standard deviation is significantly different from the root mean square error. The value for the treatment control group (5-FU alone) is outside the lower decision limit (LDL). This further indicates that the data is heteroscedastic.

As the sample size is very small and the data appears heteroscedastic, parametric analysis will not yield reliable comparisons. Thus, all of the following comparisons are made with Kruskal-Wallis Tests (Rank Sums) followed by non-parametric post-hoc Wilcoxon pairwise comparison. I have included the results of the entire analysis for WBC; all the other parameters are presented as plots with summaries. The reader can find the full analytical reports for each group in Appendix 1.

Figure 3: Oneway Analysis of WBC by Treatment Group Day 8



Mouse 01 missing, Mouse 04 excluded (v.s.)

The chart above shows the data points and the quantiles about the median as red boxes. The variability in the quantiles again demonstrates the differences in the variances between the treated groups and the controls.

Table 2: Quantiles and Medians for WBCs

Level	Minimum	10%	25%	Median	75%	90%	Maximum
Uridine IP 400 mg/kg	3.5	3.5	3.75	4.7	5.5	6.1	6.1
Uridine PO 400 mg/kg	3.8	3.8	3.8	4	5.4	5.4	5.4
Uridine PO 800 mg/kg	3.8	3.8	3.95	4.2	5.55	6.4	6.4
Uridine Triacetate PO 500 mg/kg	4.7	4.7	5.05	5.9	6.8	7.2	7.2
Vehicle Control	3.3	3.3	3.3	3.4	3.55	3.6	3.6

Table 3: Wilcoxon/Kruskal-Wallis Tests (Rank Sums) for WBCs on Day 8

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0) /Std0
Uridine IP 400 mg/kg	5	64.0	60.0	12.80	0.262
Uridine PO 400 mg/kg	3	34.5	36.0	11.50	-0.092
Uridine PO 800 mg/kg	5	66.0	60.0	13.20	0.411
Uridine Triacetate PO 500 mg/kg	5	95.0	60.0	19.00	2.578
Vehicle Control	5	16.5	60.0	3.30	-3.213

Chi Square is 13.8, DF = 4, Probability > Chi Square is 0.0077, ANOVA is significant
The non-parametric analysis of variance is significant.

Table 4: Nonparametric Comparisons for Each Pair Using Wilcoxon Method for WBCs

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	3.20	1.90	1.68	0.09	1.30	-1.00	3.10
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	3.00	1.91	1.57	0.12	1.20	-0.70	3.20
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	2.93	1.78	1.65	0.10	1.60	.	.
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	0.80	1.78	0.45	0.65	0.20	.	.
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	0.00	1.91	0.00	1.00	0.00	-2.00	2.40
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-0.27	1.78	-0.15	0.88	-0.20	.	.
Vehicle Control	Uridine PO 400 mg/kg	-3.73	1.78	-2.10	**0.036***	-0.60	.	.
Vehicle Control	Uridine IP 400 mg/kg	-4.20	1.90	-2.21	**0.027***	-1.30	-2.80	0.00
Vehicle Control	Uridine PO 800 mg/kg	-4.80	1.91	-2.51	**0.012***	-0.80	-3.10	-0.30
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-4.80	1.91	-2.51	**0.012***	-2.50	-3.90	-1.20

The analysis presented above shows that the Vehicle Control values are different from all the treatment groups and that all the treatment groups are equivalent. In this experiment, treatment with uridine triacetate was statistically indistinguishable from treatment with all of the uridine regimens. The p-values suggest the possibility of such a difference, but the experiment is does not have the power to demonstrate it.

Marrow Count – Day 8

Figure 4: Oneway Analysis of Marrow Count by Treatment Group Day 8

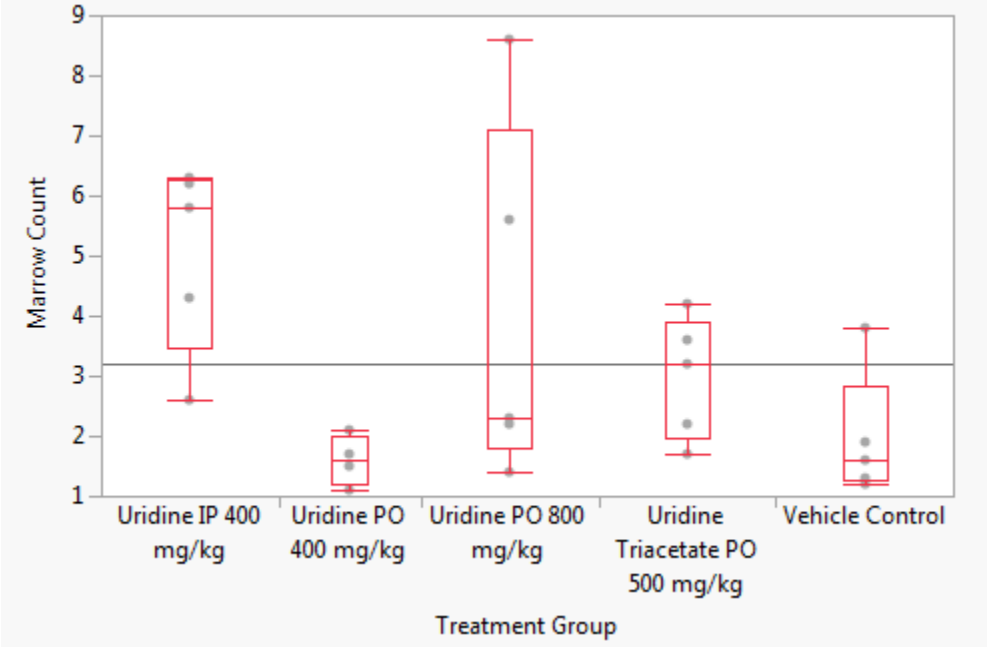


Table 5: Nonparametric Comparisons for Each Pair Using Wilcoxon Method for Marrow Count Day 8

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	3.60	1.83	1.97	0.049*	1.5	-0.4	3.1
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	2.93	1.84	1.59	0.111	0.95	-0.7	7.5
Vehicle Control	Uridine PO 400 mg/kg	0.23	1.84	0.12	0.903	0.1	-0.9	2.7
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	-0.20	1.91	-0.10	0.917	-0.1	-6.4	2.2
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	-2.00	1.91	-1.04	0.296	-1.2	-4.8	4.3
Vehicle Control	Uridine PO 800 mg/kg	-2.80	1.91	-1.46	0.144	-1	-7.3	1.6
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-2.80	1.91	-1.46	0.144	-1	-2.9	1.6
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	-3.60	1.91	-1.88	0.060	-2.2	-4.5	1
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-4.28	1.84	-2.33	0.020*	-4.1	-5.2	-0.5
Vehicle Control	Uridine IP 400 mg/kg	-4.40	1.91	-2.30	0.022*	-3.1	-5	-0.5

These results are equivocal and difficult to interpret because of the small sample size and variability, but again it suggests little difference between uridine triacetate treatment and uridine treatment.

Spleen Weight

Spleen weight showed no significant variation by treatment group (p = 0.52). All groups were statistically equivalent (not shown).

Neutrophils

Figure 5: Oneway Analysis of Neutrophils Count by Treatment Group Day 8

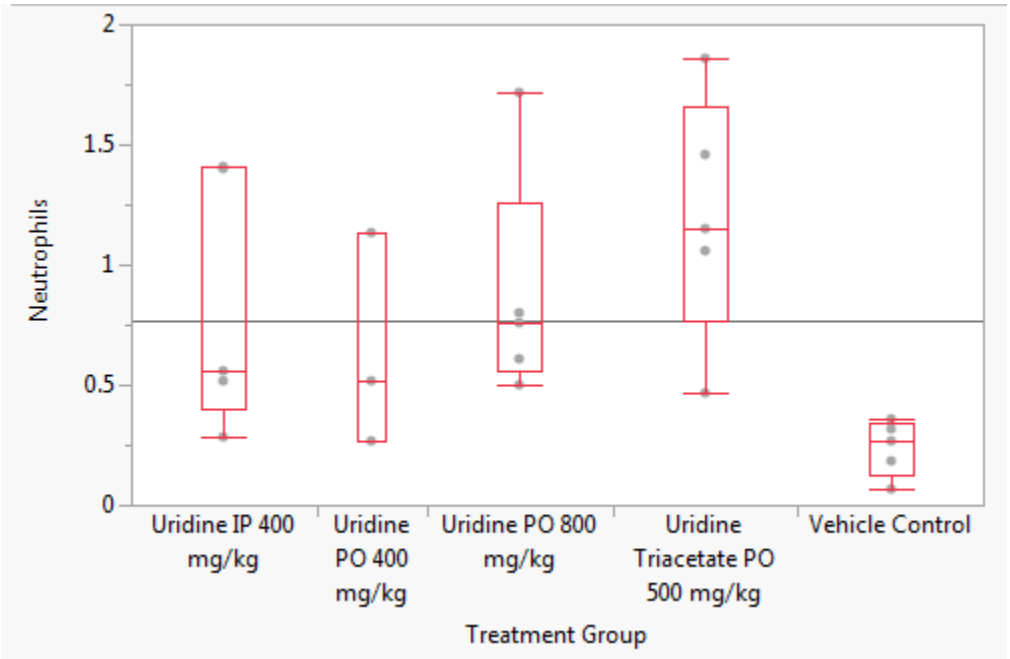


Table 6: Nonparametric Comparison of Each Pair Using Wilcoxon Method for Neutrophils Day 8

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	2.13	1.79	1.19	0.23	0.63	.	.
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	1.60	1.91	0.84	0.40	0.46	-0.93	1.34
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	1.60	1.91	0.84	0.40	0 39	-0.66	1.25
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	1.07	1.79	0.60	0.55	0 24	.	.
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	0.80	1.91	0.42	0.68	0.2	-0 9	1.2
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-1.33	1.78	-0.75	0.45	-0.25	.	.
Vehicle Control	Uridine PO 400 mg/kg	-2.40	1.78	-1.35	0.18	-0.25	.	.
Vehicle Control	Uridine IP 400 mg/kg	-4.00	1.91	-2.09	**0.037***	-0.38	-1.33	0.04
Vehicle Control	Uridine PO 800 mg/kg	-4.80	1.91	-2.51	**0.012***	-0.48	-1.54	-0.18
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-4.80	1.91	-2.51	**0.012***	-0.97	-1.68	-0.15

Lymphocytes

Figure 6: Oneway Analysis of Lymphocyte Count by Treatment Group Day 8

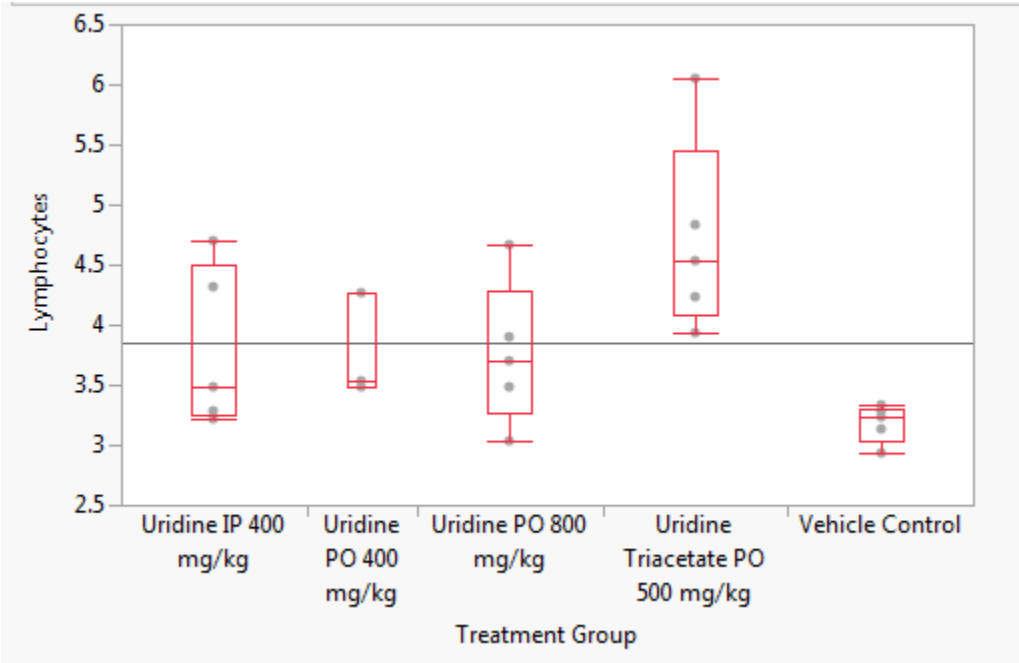


Table 7: Nonparametric Comparison of Each Pair Using Wilcoxon Method for Lymphocytes Day 8

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	3.60	1.91	1.88	0.06	0.9	-0.44	2.56
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	2.80	1.91	1.46	0.14	0.94	-0.47	2.76
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	2.67	1.79	1.49	0.14	0.75	.	.
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	0.27	1.78	0.15	0.88	0.05	.	.
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	0.00	1.91	0.00	1.00	0.01	-1.27	1.38
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	0.00	1.79	0.00	1.00	0.01	.	.
Vehicle Control	Uridine IP 400 mg/kg	-3.20	1.91	-1.67	0.09	-0.35	-1.57	0.06
Vehicle Control	Uridine PO 800 mg/kg	-3.20	1.91	-1.67	0.09	-0.55	-1.54	0.24
Vehicle Control	Uridine PO 400 mg/kg	-3.73	1.79	-2.09	**0.037***	-0.4	.	.
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-4.80	1.91	-2.51	**0.012***	-1.31	-2.92	-0.66

Platelets

Figure 7: Oneway Analysis of Platelets by Treatment Group Day 8

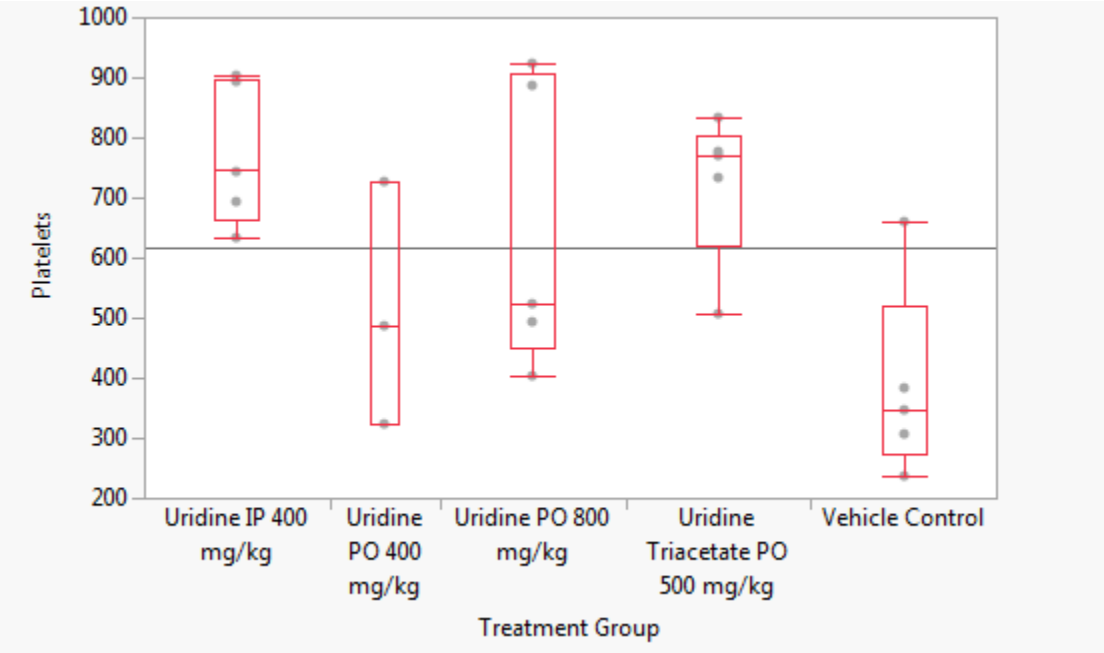


Table 8: Nonparametric Comparisons for Each Pair Using Wilcoxon Method for Platelets Day 8

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	3 20	1.79	1.79	0 07	246.00	.	.
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	1 60	1.79	0 89	0 37	159.00	.	.
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	0.40	1 91	0 21	0 83	104.00	-380	373
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	-0.40	1 91	-0 21	0 83	-59.00	-385	143
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	-1 60	1 91	-0 84	0.40	-170.00	-489	254
Vehicle Control	Uridine PO 400 mg/kg	-1 60	1.79	-0 89	0 37	-105.00	.	.
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-2 67	1.79	-1.49	0.14	-257.00	.	.
Vehicle Control	Uridine PO 800 mg/kg	-3 60	1 91	-1 88	0 06	-215.00	-649	164
Vehicle Control	Uridine IP 400 mg/kg	-4.40	1 91	-2 30	0.022*	-395.00	-654	-34
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-4.40	1 91	-2 30	0.022*	-393.00	-538	-75

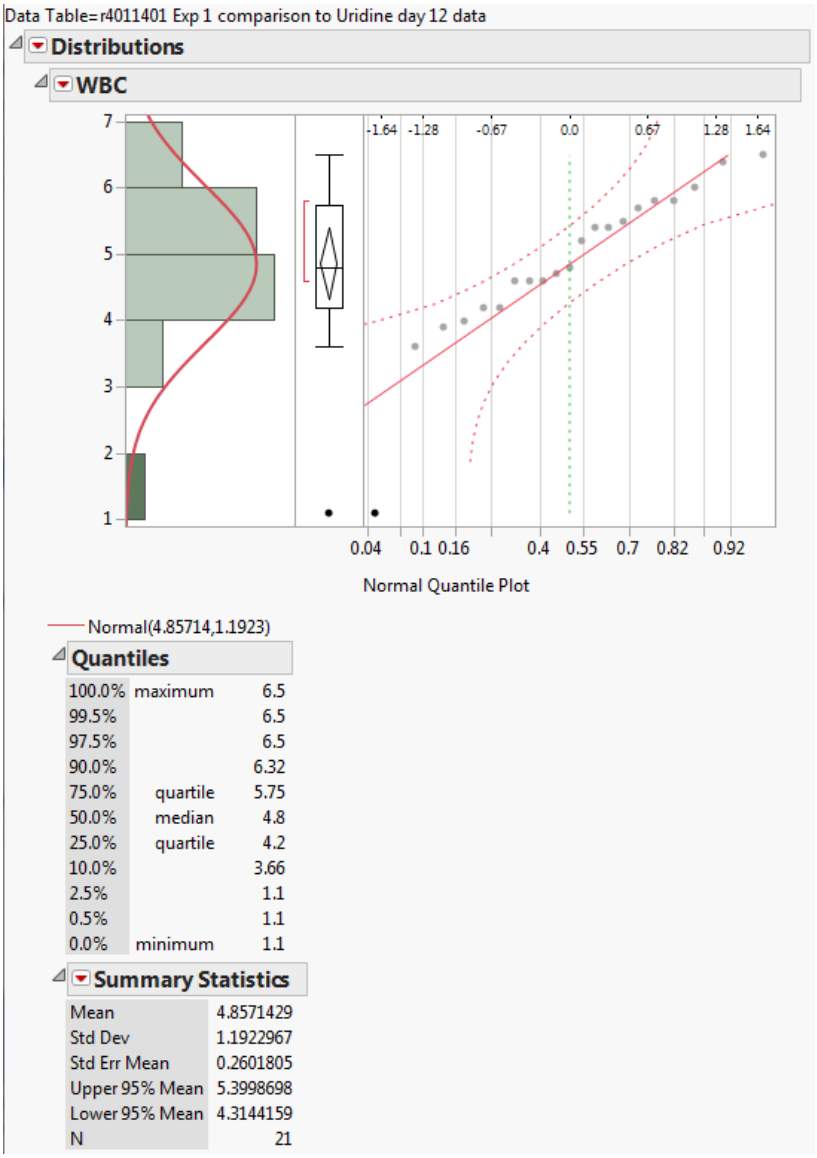
RBCs

RBCs showed no significant variation by treatment group (p = 0.52). All groups were statistically equivalent (not shown).

Day 12 results

The following plot shows that the WBC data is roughly normally distributed across all the treatment groups with the exception of one outlier (black).

Figure 8: Analysis of the Distribution of WBC Values on Day 12.



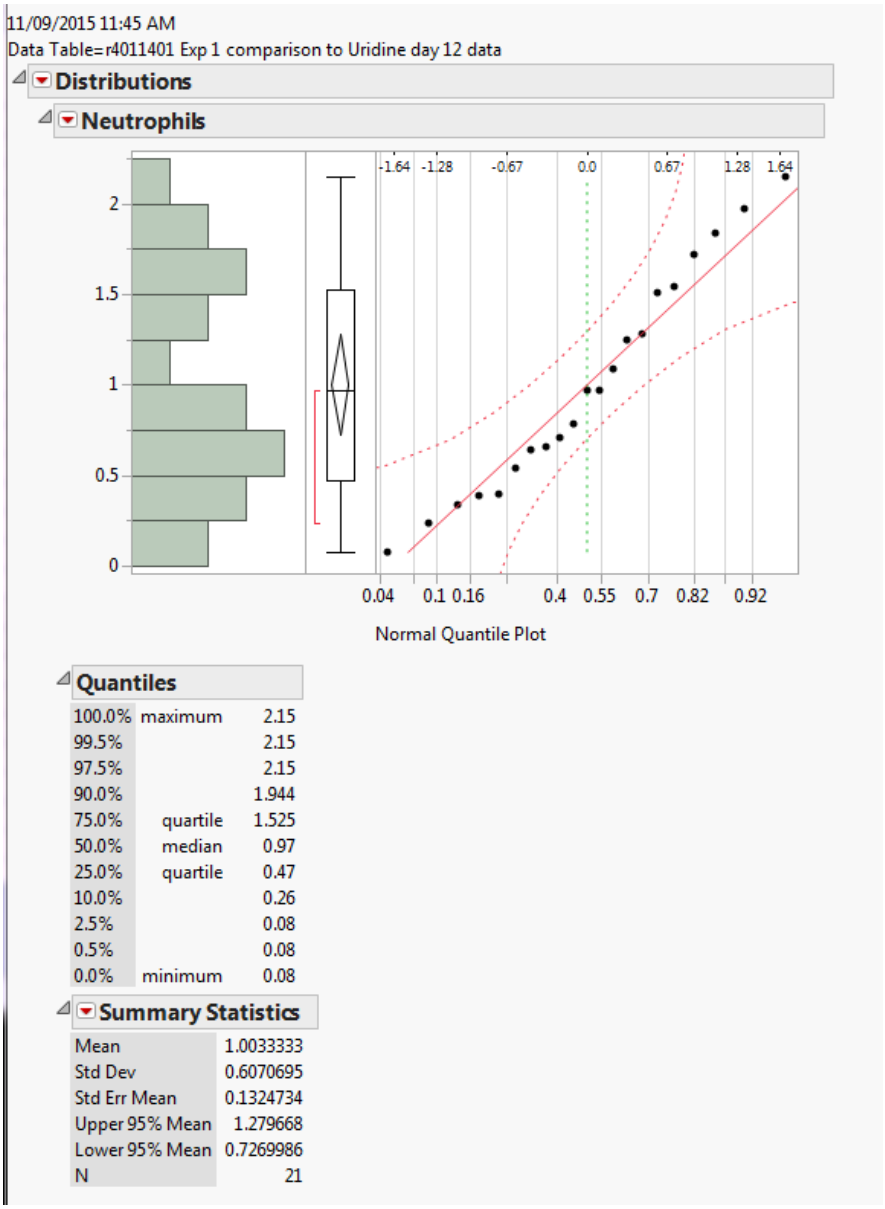
The study report says of this mouse:

"CBC data from Mouse ID# S5 in the group receiving vehicle p.o. were deemed to be unreliable, likely due to a sporadic infection and/or undocumented gavage accident, based on WBC counts of 1.1 K/µL, whereas all other mice in all other groups had WBC counts ≥ 3.6 K/µL. In Table 14-2, mean CBC counts for the Vehicle group are therefore presented both with and without counts from this animal."

As with the case in the 8 day group, this mouse will be excluded from analysis unless otherwise noted.

The following graph shows the shows that at day 12, neutrophils had two distinct distributions. The uridine triacetate group is in black.

Figure 9: Analysis of the Distribution of WBC Values on Day 12.

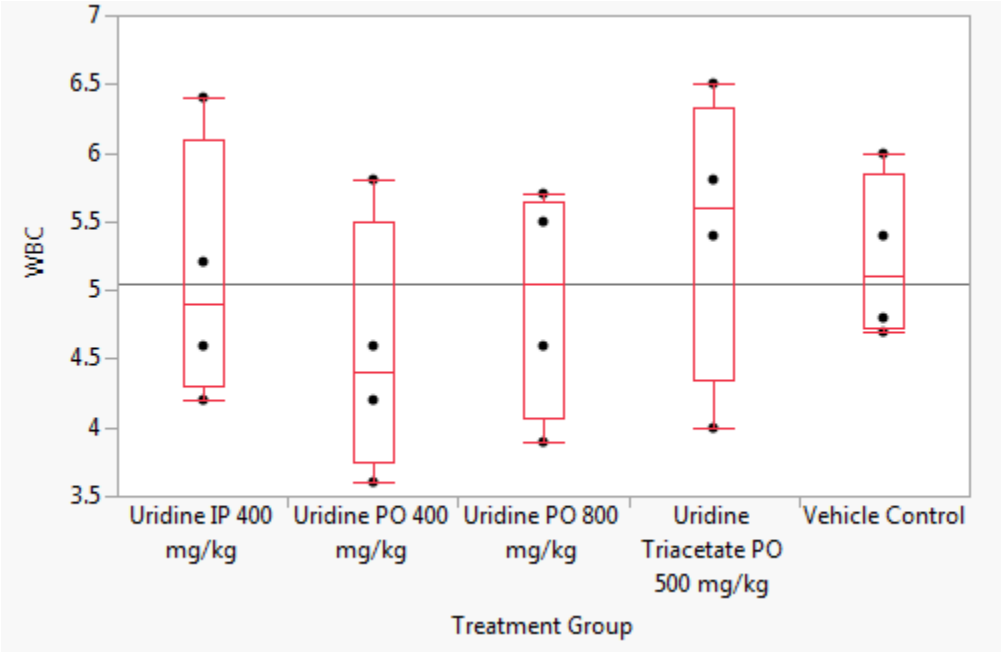


Likewise, lymphocytes, platelets and RBCs were not normally distributed (not shown), so again for the reasons detailed above, I used non-parametric techniques to analyze the 12 day data.

WBC

The following graph shows that there were no statistical differences in the median values among the different dose groups for WBC. The p value for the Kruskal-Wallis analysis was 0.65. Thus all groups, including the 5-FU treated Vehicle Control, had recovered to the same degree by day 12.

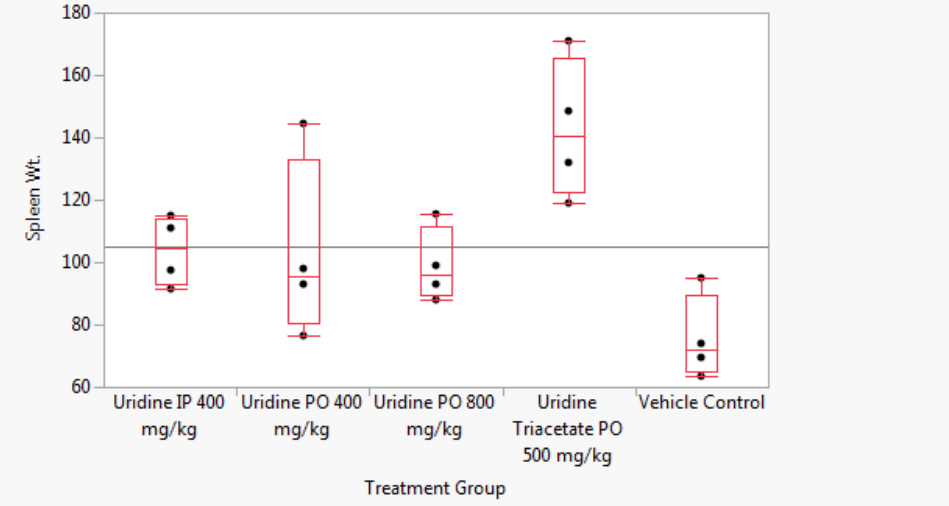
Figure 10: Oneway Analysis of WBCs Count by Treatment Group on Day 12



Spleen Weight

Spleen weight did demonstrate a statistical difference among the dose groups as the following graph shows. The p value for the Kruskal-Wallis analysis was 0.020.

Figure 11: Oneway Analysis of Spleen Wt. by Treatment Group on Day 12



Excluded Rows 1

Quantiles

Level	Minimum	10%	25%	Median	75%	90%	Maximum
Uridine IP 400 mg/kg	91.6	91.6	93.1	104.25	113.825	114.8	114.8
Uridine PO 400 mg/kg	76.6	76.6	80.65	95.5	133.075	144.7	144.7
Uridine PO 800 mg/kg	88.2	88.2	89.4	96.1	111.5	115.6	115.6
Uridine Triacetate PO 500 mg/kg	119.1	119.1	122.375	140.3	165.35	171	171
Vehicle Control	63.4	63.4	64.925	71.8	89.7	94.9	94.9

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Uridine IP 400 mg/kg	4	43.000	42.000	10.7500	0.047
Uridine PO 400 mg/kg	4	40.000	42.000	10.0000	-0.142
Uridine PO 800 mg/kg	4	40.000	42.000	10.0000	-0.142
Uridine Triacetate PO 500 mg/kg	4	72.000	42.000	18.0000	2.787
Vehicle Control	4	15.000	42.000	3.7500	-2.504

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
11.7000	4	0.0197*

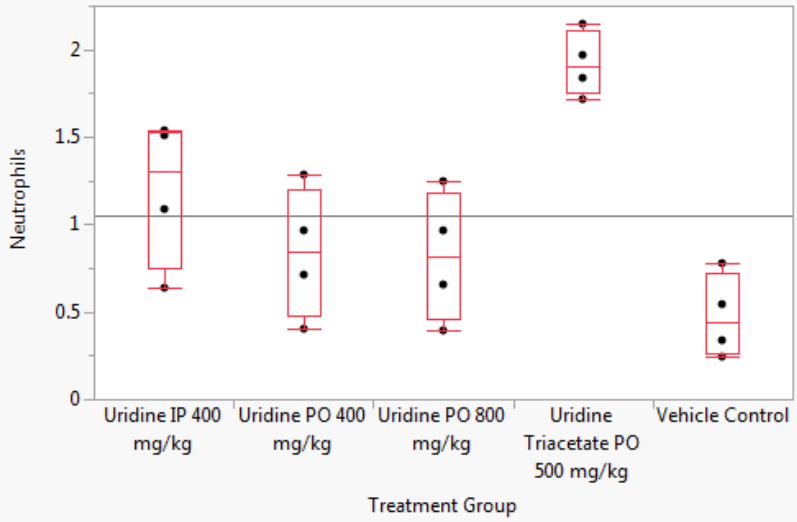
Table 9: Nonparametric Comparisons for Each Pair Using Wilcoxon Method for Spleen Wt. Day 12

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	3.75000	1.732051	2.16506	0.0304*	36.0500
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	3.75000	1.732051	2.16506	0.0304*	41.6000
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	2.75000	1.732051	1.58771	0.1124	40.9500
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	0.25000	1.732051	0.14434	0.8852	0.6000
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-0.25000	1.732051	-0.14434	0.8852	-8.7500
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	-0.25000	1.732051	-0.14434	0.8852	-4.0000
Vehicle Control	Uridine PO 400 mg/kg	-2.75000	1.732051	-1.58771	0.1124	-23.7000
Vehicle Control	Uridine PO 800 mg/kg	-2.75000	1.732051	-1.58771	0.1124	-24.1500
Vehicle Control	Uridine IP 400 mg/kg	-3.25000	1.732051	-1.87639	0.0606	-28.1500
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-3.75000	1.732051	-2.16506	0.0304*	-65.7500

Neutrophils

Neutrophil count also demonstrated a statistical difference among the dose groups as the following graph shows, again with the uridine triacetate group having the highest median. The p value for the Kruskal-Wallis analysis was 0.012.

Figure 12: Oneway Analysis of Neutrophils by Treatment Group on Day 12



Excluded Rows 1

Quantiles

Level	Minimum	10%	25%	Median	75%	90%	Maximum
Uridine IP 400 mg/kg	0.64	0.64	0.7525	1.3	1.5325	1.54	1.54
Uridine PO 400 mg/kg	0.4	0.4	0.4775	0.84	1.2025	1.28	1.28
Uridine PO 800 mg/kg	0.39	0.39	0.4575	0.815	1.18	1.25	1.25
Uridine Triacetate PO 500 mg/kg	1.72	1.72	1.75	1.905	2.105	2.15	2.15
Vehicle Control	0.24	0.24	0.265	0.44	0.72	0.78	0.78

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Uridine IP 400 mg/kg	4	49.000	42.000	12.2500	0.614
Uridine PO 400 mg/kg	4	36.500	42.000	9.1250	-0.473
Uridine PO 800 mg/kg	4	33.500	42.000	8.3750	-0.756
Uridine Triacetate PO 500 mg/kg	4	74.000	42.000	18.5000	2.978
Vehicle Control	4	17.000	42.000	4.2500	-2.316

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
12.8704	4	0.0119*

Table 10: Nonparametric Comparisons for Each Pair Using Wilcoxon Method for Neutrophils Day 12

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann
Uridine Triacetate PO 500 mg/kg	Uridine IP 400 mg/kg	3.75000	1.732051	2.16506	0.0304*	0.63500
Uridine Triacetate PO 500 mg/kg	Uridine PO 400 mg/kg	3.75000	1.732051	2.16506	0.0304*	1.07000
Uridine Triacetate PO 500 mg/kg	Uridine PO 800 mg/kg	3.75000	1.732051	2.16506	0.0304*	1.12000
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	-0.50000	1.721710	-0.29041	0.7715	-0.02000
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-1.75000	1.732051	-1.01036	0.3123	-0.32000
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	-1.75000	1.732051	-1.01036	0.3123	-0.36000
Vehicle Control	Uridine PO 400 mg/kg	-2.25000	1.732051	-1.29904	0.1939	-0.40000
Vehicle Control	Uridine PO 800 mg/kg	-2.25000	1.732051	-1.29904	0.1939	-0.37000
Vehicle Control	Uridine IP 400 mg/kg	-3.25000	1.732051	-1.87639	0.0606	-0.75500
Vehicle Control	Uridine Triacetate PO 500 mg/kg	-3.75000	1.732051	-2.16506	0.0304*	-1.45500

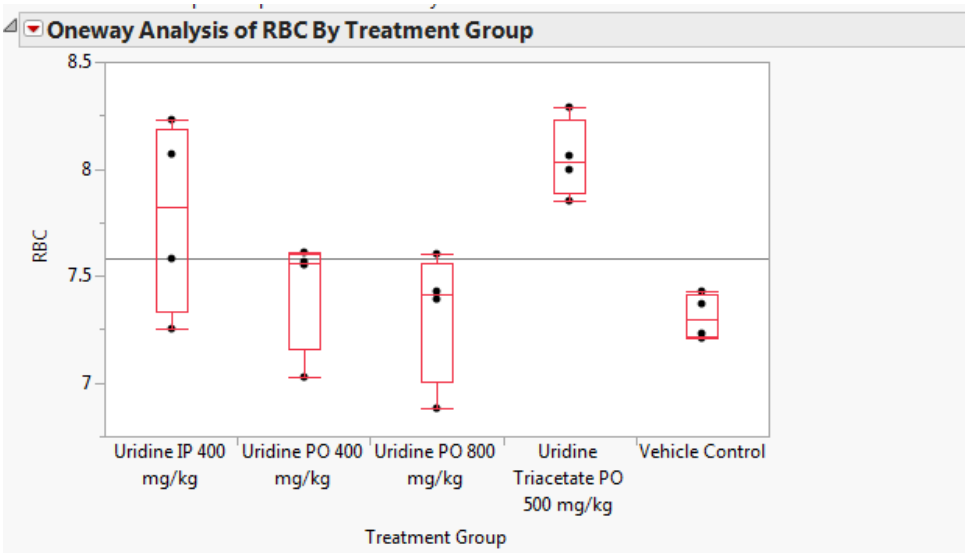
Lymphocytes – No statistical differences (not shown)

Platelets – No statistical differences (not shown)

RBCs

RBCs demonstrated a statistical difference among the dose groups as the following graph shows, again with the uridine triacetate group having the highest median. The p value for the Kruskal-Wallis analysis was 0.012.

Figure 13: Oneway Analysis of RBCs by Treatment Group on Day 12



Excluded Rows 1

Quantiles

Level	Minimum	10%	25%	Median	75%	90%	Maximum
Uridine IP 400 mg/kg	7.25	7.25	7.3325	7.825	8.19	8.23	8.23
Uridine PO 400 mg/kg	7.03	7.03	7.16	7.56	7.6	7.61	7.61
Uridine PO 800 mg/kg	6.88	6.88	7.0075	7.41	7.5575	7.6	7.6
Uridine Triacetate PO 500 mg/kg	7.85	7.85	7.8875	8.03	8.2325	8.29	8.29
Vehicle Control	7.21	7.21	7.215	7.3	7.415	7.43	7.43

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
Uridine IP 400 mg/kg	4	54.000	42.000	13.5000	1.087
Uridine PO 400 mg/kg	4	37.000	42.000	9.2500	-0.425
Uridine PO 800 mg/kg	4	29.500	42.000	7.3750	-1.134
Uridine Triacetate PO 500 mg/kg	4	68.000	42.000	17.0000	2.410
Vehicle Control	4	21.500	42.000	5.3750	-1.891

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
10.1612	4	0.0378*

Table 11: Nonparametric Comparisons for Each Pair Using Wilcoxon Method for RBCs Day 12

Nonparametric Comparisons For Each Pair Using Wilcoxon Method

q*	Alpha
1.95996	0.05

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Uridine Triacetate PO 500 mg	Uridine PO 400 mg/kg	3.75000	1.732051	2.16506	0.0304*	0.500000	.	.
Uridine Triacetate PO 500 mg	Uridine PO 800 mg/kg	3.75000	1.732051	2.16506	0.0304*	0.650000	.	.
Uridine Triacetate PO 500 mg	Uridine IP 400 mg/kg	0.75000	1.732051	0.43301	0.6650	0.245000	.	.
Vehicle Control	Uridine PO 800 mg/kg	-1.00000	1.721710	-0.58082	0.5614	-0.110000	.	.
Uridine PO 800 mg/kg	Uridine PO 400 mg/kg	-1.25000	1.732051	-0.72169	0.4705	-0.145000	.	.
Uridine PO 400 mg/kg	Uridine IP 400 mg/kg	-1.75000	1.732051	-1.01036	0.3123	-0.480000	.	.
Uridine PO 800 mg/kg	Uridine IP 400 mg/kg	-1.75000	1.732051	-1.01036	0.3123	-0.550000	.	.
Vehicle Control	Uridine PO 400 mg/kg	-1.75000	1.732051	-1.01036	0.3123	-0.190000	.	.
Vehicle Control	Uridine IP 400 mg/kg	-2.75000	1.732051	-1.58771	0.1124	-0.505000	.	.
Vehicle Control	Uridine Triacetate PO 500 mg	-3.75000	1.732051	-2.16506	0.0304*	-0.730000	.	.

The following table shows the median values for the parameters analyzed in this experiment. Values in bold red are significantly different from 5-FU treated control ($\alpha = 0.05$).

Table 12: Summary of Hematological Parameters after Antidote Treatment with Uridine or Uridine Triacetate

Dose Group	WBC (1000/µL)	Marrow (1000000/mg)	Spleen Wt. (mg)	Neutrophils (1000/µL)	Lymphocytes (1000/µL)	Platelets (1000/µL)	RBCs (1000000/µL)
Uridine IP 400 mg/kg	**4.7**	**5.8**	75.3	**0.56**	**3.48**	**745**	8.01
Uridine PO 400 mg/kg	**4**	1.6	73.6	0.52	3.53	488	7.73
Uridine PO 800 mg/kg	**4.2**	2.3	68.8	**0.76**	3.7	523	7.98
Uridine Triacetate PO 500 mg	**5.9**	3.2	78.4	**1.15**	**4.54**	**769**	8.23
Vehicle Control	3.4	1.6	71.5	0.27	3.23	346	8.07
(b) (4) Mean[1]	8.87		100	1.74	7.29	963	9.98
Low	5.69			0.74	3.6	476	9.16
High	14.84			3.01	11.56	1611	11.7

This experiment is deficient in numerous ways. First, it lacks an untreated control group making the results difficult to interpret. The investigators included a control treated with 5-FU and vehicle (vehicle control in the tables), but not one treated with only vehicle so it was necessary to compare the results to the (b) (4) controls.[1] These values are compared in the overall summary below. The values from (b) (4) are possibly not directly applicable to the normal values of the mice used in these experiments as the (b) (4) information represents their mouse colony from January 2008 to December 2012. Also, the investigators did not specify the colony from which the mice in this experiment originated. Nevertheless, these values should be reasonably close to those of the experimental mice and provide the best comparison available. The age and weights of the mice in the (b) (4) report are similar to those specified by the investigators.

The dose of 5-FU is relatively low and non-lethal, there is no way to judge if the findings are toxicologically significant or if they provide any evidence of human surrogacy. Lastly, N is too low to provide sufficient statistical power to differentiate uridine from uridine triacetate.

[1] (b) (4) website (accessible at: http://www (b) (4)

Indeed, for the most part uridine looks to be as good a treatment as uridine triacetate when adjusted for dose.

Experiment 2: Uridine Triacetate Dose-Response

Dose The following table shows the dose groups in Experiment 2

Table 13: Doses for Study 1, Experiment 2

Group	5-FU mg/kg IP	Antidote	Treatment dose mg/kg
1	150	Vehicle	0
2	150	Uridine Triacetate	100
3	150	Uridine Triacetate	250
4	150	Uridine Triacetate	500
5	150	Uridine Triacetate	1000

Schedule	All mice received 5-FU at 1:00 pm on Day 1. Treatments with uridine triacetate were administered 4 times on Day 1 (3:00 pm, 5:00 pm, 7:30 pm & 10:00 pm), 6 times on Day 2 (9:00 am, 11:00 am, 1:00 pm, 3:00 pm, 6:00 pm & 10:00 pm), and once on Day 3 (11:00 am).
Route	5-FU – intraperitoneal injection; uridine triacetate - oral
Dose Volume	0.2 to 0.4 mL
Formulation	Deionized water for oral administration
Species	Female BALB/C mice
Number	14 per dose group
Age	Not specified
Weight	About 20 grams
Necropsy	Seven animals on day eight and seven animals on day 12
Design	On day eight the investigators collected blood via the orbital sinus (0.2 – 0.3 mL) from seven mice per dose group and determined white blood cells, neutrophils, lymphocytes, platelets, and red blood cells. They did cell counts of the femoral bone marrow and weighed the spleens. On day 12 they did the same procedures for the remaining 7 mice.

Results

This experiment was designed to demonstrate a dose response with uridine triacetate treatment. The investigators measured the same parameters as in the previous experiment, but the treatments were increasing doses of uridine triacetate. The sample size was somewhat larger in this experiment, 7 per dose group, but that is still too small to warrant parametric analysis in the light of the fact that the data was again heteroscedastic with large variances (not shown). There were again two mice in the control group, C4 and C5, with somewhat anomalous findings in the day 12 group. The investigators say of these animals:

"CBC data from Mice ID# C4 and C5 in the group receiving vehicle p.o. were deemed to be unreliable, likely due to a sporadic infection and/or undocumented gavage accident, based on enlarged spleens noted as anomalous at necropsy, and neutrophil counts >3 standard deviations higher than the mean of the other mice in the group."

This explanation seems unlikely. The counts for these animals and the spleen weights all appear normal, that is, they appear as if the animals did not receive 5-FU (spleen weight ~ 100 mg, WBC > 8.1). The Applicant excluded these animals from their analysis. Since they used a parametric ANOVA, this exclusion makes a large difference in the means. With a non-parametric analysis exclusion makes little difference in the median values. I have included analyses of this data both with and without the excluded values.

The following chart and tables show the analysis for WBC on day 8, the most comprehensive parameter. The results of other analysis are not shown. The Kruskal-Wallis analysis is significant with a p value of 0.0003. The median values appear to demonstrate a dose response, but only the 500 mg/kg group is significantly different from control and the median value for the 1000 mg/kg group is actually lower than that of the 500 mg/kg group. The 500 mg/kg group is significantly different from the 100 mg/kg and the 250 mg/kg groups but the 1000 mg/kg group is not. More may not be better.

Table 14: Oneway Analysis of WBC dose response

Level	Minimum	10%	25%	Median	75%	90%	Maximum
Control	2	2	2.2	2.4	2.7	3	3
100 mg/kg	2.3	2.3	2.4	2.5	2.6	2.6	2.6
250 mg/kg	2.1	2.1	2.3	2.5	3.3	3.5	3.5
500 mg/kg	3.4	3.4	3.8	4.1	4.4	4.4	4.4
1000 mg/kg	2.4	2.4	3.3	3.6	3.9	4.6	4.6

Chi^2 = 21, DF = 4, Probability > Chi^2 = 0.0003

Table 15: Nonparametric Comparisons for WBC Dose Response for All Pairs Using Dunn Method

Level	- Level	Score Mean Difference	Std Error Difference	Z	p-Value
500 mg/kg	100 mg/kg	18.7	5.46	3.42	**0.0062***
500 mg/kg	250 mg/kg	16.0	5.46	2.93	**0.034***
1000 mg/kg	100 mg/kg	13.0	5.46	2.39	0.168
500 mg/kg	1000 mg/kg	5.50	5.46	1.01	1.00
250 mg/kg	100 mg/kg	2.58	5.46	0.47	1.00
Control	100 mg/kg	-1.07	5.46	-0.196	1.00
Control	250 mg/kg	-3.78	5.46	-0.69	1.00
250 mg/kg	1000 mg/kg	-10.3	5.46	-1.89	0.58
Control	1000 mg/kg	-14.3	5.46	-2.61	0.089
Control	500 mg/kg	-19.9	5.46	-3.65	**0.0027***

The following table summarizes the median values for day 8 and day 12 for this experiment. An extra row is included to show that the median values for the control group on day 12 do not change significantly when the analysis excludes the mice, C4 and C5. Maximal values for each group are in **bold red**.

Table 16: Median Values for all Parameters as a Function of Dose on Days 8 and 12

Summary of Median Values on Day 8							
Dose Group	WBC	Marrow Count	Spleen Wt.	Neutrophils	Lymphocytes	Platelets	RBCs
Vehicle Control	2.4	1.5	57.3	0.02	2.38	270	8.36
100 mg UTA	2.5	1.7	62.4	0	2.45	420	8.23
250 mg UTA	2.5	2.9	60.7	0.02	2.5	608	8.54
500 mg UTA	**4.1**	3.5	63	0.03	**4.1**	**760**	8.29
1000 mg UTA	3.6	**4.75**	**63.1**	**0.04**	3.46	667	**8.69**
Summary of Median Values on Day 12							
Vehicle Control	**5.9**		83.2	0.24	**5.91**	1801	8.07
100 mg UTA	4		79	0.11	4.51	1723	7.84
250 mg UTA	4.7		**106.1**	0.51	4.46	**2354**	7.88
500 mg UTA	5.9		89.6	0.43	6.9	1737	**8.81**
1000 mg UTA	5.7		89.8	**0.72**	5.345	1404	8.58
Summary of Median Values on day 12 with excluded controls							
Vehicle Control	5.5		78.6	0.24	5.595	2000	7.88
(b) (4) Mean[1]	8.87		100*	1.74	7.29	963	9.98
Low	5.69			0.74	3.6	476	9.16
High	14.84			3.01	11.56	1611	11.7

[1] - See reference 4

On day 8, all parameters except RBCs appears to show some degree of dose response, but again all values, even the high dose group, are significantly below the (b) (4) normal values. Treatment with uridine triacetate does not reverse the damage that occurs during the first two hours, which is significant; it only prevents further damage. The experiment should have included an untreated control (no 5-FU) and a control in which uridine triacetate was given immediately after the 5-FU dose. For platelets and lymphocytes the values for the 500 mg/kg group are greater than those for the 1000 mg/kg group.

On day 12, all parameters show signs of further recovery, but the values are about the same as the control group. There were few statistical differences (not shown). Uridine triacetate prevents further damage after it is taken, but it does not hasten recovery from the damage that has already occurred. On day 12, the values in all groups are still well below the normal (b) (4) means.

The following table shows the p values for the overall Kruskal-Wallis analysis and for the different dose groups relative to control. For clarity, the table does not show the p values for the comparison between groups (Dunn's pairwise comparison, analysis not shown.

Table 17: One Way Kruskal-Wallis with Dunn's test on all pairs for Hematological Dose response for day 8 and day 12

Level	- level	WBC	Marrow Count	Spleen Wt	Neutrophils	Lymphocytes	Platelets	RBCs
p-Value Kruskal-Wallis		**0.003**	**0.001**	0.15	0.4	**0.0004**	0.0002	0.081
Vehicle Control	100 mg UTA	1.00	1	1	1	1	1	1
Vehicle Control	250 mg UTA	1.00	0.678	1	1	1	0.11	1
Vehicle Control	500 mg UTA	**0.0027**	**0.027**	0.142	1	**0.0029**	**0.0021**	1
Vehicle Control	1000 mg UTA	0.09	**0.0068**	1	1	0.11	**0.0021**	1
p Values on Day 12								
p-Value Kruskal-Wallis		0.079		**0.047**	**0.026**	**0.035**	**0.0022**	**0.012**
Vehicle Control	100 mg UTA	0.51		1	1	0.31	1	1
Vehicle Control	250 mg UTA	0.64		0.2171	1	0.067	1	1
Vehicle Control	500 mg UTA	1		1	1	1	1	0.51
Vehicle Control	1000 mg UTA	1		1	1	1	0.14	0.86
p Values on Day 12 excluded controls								
p-Value Kruskal-Wallis		0.12		**0.02**	**0.0024**	0.08	**0.0004**	**0.005**
Vehicle Control	100 mg UTA	1		1	1	1	1	1
Vehicle Control	250 mg UTA	1		**0.029**	0.32	0.36	1	1
Vehicle Control	500 mg UTA	1		1	0.25	1	0.39	0.12
Vehicle Control	1000 mg UTA	1		1	0.066	1	**0.0094**	0.21

This table again demonstrates that for the most part, the 500 mg/kg group has a better result than the 1000 mg/kg group.

To establish a dose response, I fit the WBC data to the following equation by non-linear regression analysis in Microsoft Excel.

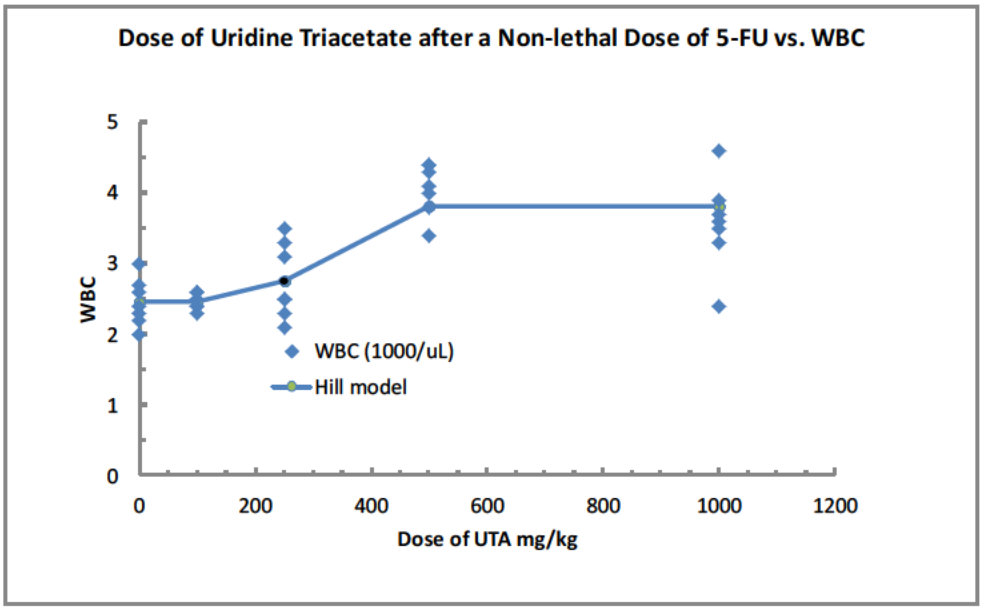
$$Y = Bottom + (Top - Bottom)/(1 + \ (1 + EXP((ED50 - Dose))$$

A standard dose response equation. The regression parameters determined by Microsoft Solver were:

Top	3.8
Bottom	2.5
ED50	251
Sum of Squared Error	6.7

The following graph shows a plot of dose vs. Day 8 WBC and the regression line that results from the non-linear analysis.

Figure 14: Plot of WBC with Increasing Uridine Triacetate Dose and a Non-Linear Regression Model



The parameter, ED50, is an estimate of the ED_{50} (half maximal effective dose) of 251 mg/kg. While the sample size is somewhat small and the data has a high variance the sum of squared error was small.

Analysis of the WBC day 8 data as a function of dose in JMP showed that that the regression about the mean of the data (3.06) gives an SSE of 20.3 which is considerably greater than the SSE for the non-linear regression of 6.7. The non-linear model above thus better describes the data than the mean. The regression SSE for a linear model of the data is 8.3 with an $r^2 = 0.4$ again greater than the SSE for the non-linear regression. Thus, the non-linear model above is superior to a simple line.

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2) Effects of Uridine Triacetate [PN401] in Two Models of 5-Fluorouracil (5-FU) Overexposure in Mice: 5-FU Overdose and Impaired 5-FU Elimination Due to DPD Inhibition

Study Number	R.401.14.03
Filename	r4011403-report-body.pdf, Module 4.2.1
Laboratory	Wellstat Therapeutics Corp., Gaithersburg, MD 20878
Study Date	March 2010
GLP	No
Audited	No
Drug	Uridine Triacetate, Lot# 1911-C-4P (manufacture 1995) [redacted](b)(4), Purity 101.3 %
	The investigators did not provide evidence that the Uridine Triacetate

used in these studies had not degraded over the 15 years between the issuance of a certificate of analysis and the initiation of the studies.

Methods

Animals: Female BALB/c mice (b) (4) greater than 20 g in weight, about 20-30 weeks old
Treated with antibiotics sulfamethoxazole and trimethoprim during the acclimation period. The investigators gave these antibiotics "to avoid early deaths due to opportunistic infections, which models supportive care in the typical clinical situation in patients at risk of excess 5-FU toxicity".

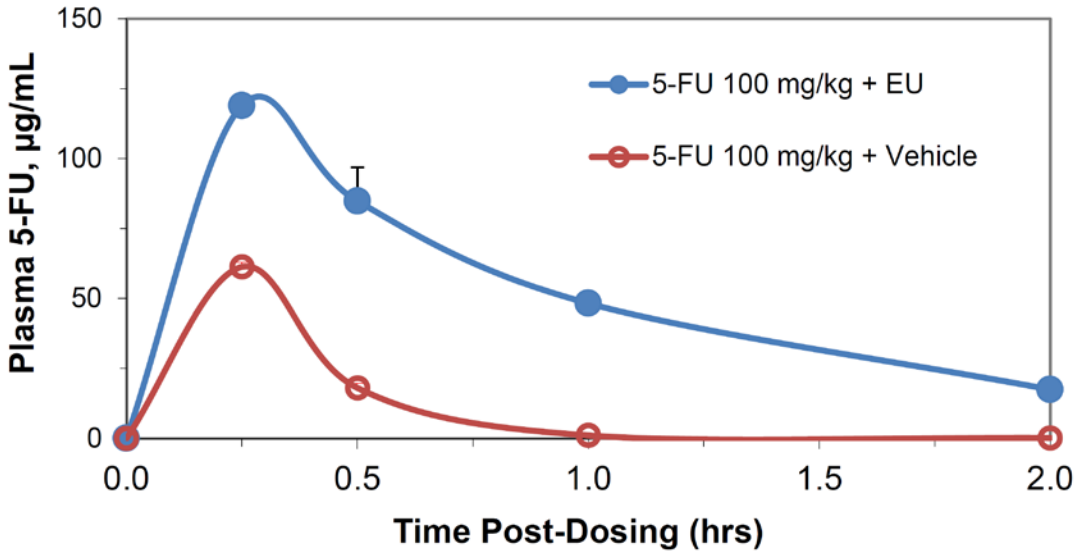
Dosing: See the individual experiments

Experiment 1: Impaired 5-FU Elimination Model –Confirmation of DPD Inhibition

Experiment 1A

The investigators did these experiments to determine the ability of ethynyluracil to inhibit 5-FU catabolism to dihydro-5-flurouracil via DPD *in vivo*. In Experiment 1A, they treated mice intraperitoneally with either vehicle (saline) or EU (2 mg/kg) one hour prior to treatment with 100 mg/kg of 5-FU. They sampled the animals by retro-orbital bleeding before dosing and at 0.25, 0.50, 1 and 2 hours post-dosing. The following figure from the Applicant's study report presents the results of this experiment graphically.

Figure 15: Mean Plasma 5-FU Concentrations Following 5-FU Administration in Mice Pretreated with EU or Vehicle



The graph shows that the elimination of 5-FU is biphasic in both cases. Pretreatment with 5-EU significantly increases the plasma concentration of 5-FU as has been confirmed in many other experiments by other investigators.[2] The following table presents the investigators calculated values for C_{max} and AUC in this experiment in molar units. Treatment with 5-EU increases exposure four-fold and maximal concentrations two-fold.

Table 18: Pharmacokinetic Parameters for the Elimination of 5-FU in Mice Treated with 5-EU

Treatment	$AUC_{0 \text{ to } 2 \text{ hr}}$ µM*hr	C_{max} µM	T_{max} hr
Vehicle	177	471	0.25
5-EU	819	915	0.25

Experiment 1B

Like 5-FU, uracil, but not uridine, is a substrate for dihydropyrimidine dehydrogenase (DPD). Excess uridine causes plasma concentrations to increase because the activity of pyrimidine-nucleoside phosphorylase (EC 2.4.2.2) is reversible catalyzing the reaction:

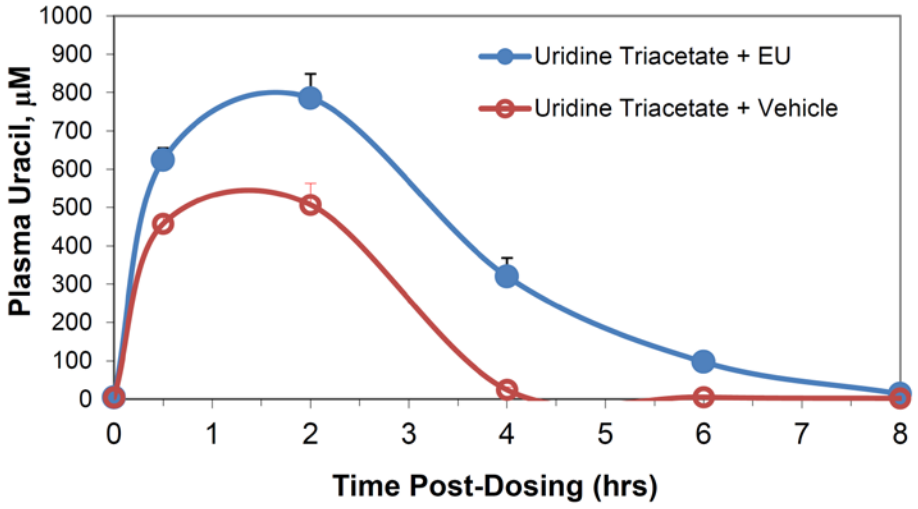
Uridine + phosphate $\rightleftharpoons$ Uracil + alpha-D-ribose 1-phosphate

In experiment 1B, the investigators treated mice with either vehicle (saline) or 5-EU. They then gave both groups of mice an oral dose of 2000 mg/kg of uridine triacetate and determined plasma uracil as a function of time.

They dosed three to four mice per time point. The following figure from the study report shows the time course of uracil exposure in both groups.

[2] R.L. Schilsky and H.L. Kindler, 2000, Eniluracil: an irreversible inhibitor of dihydropyrimidine dehydrogenase, Expert Opin Investig Drugs, 9(7):1635-49.

Figure 16: Mean Plasma Uracil (µM) Concentrations Following Oral Uridine Triacetate in Mice Pretreated with EU Vehicle



The shape of the curve suggests that the elimination of uridine from plasma is more complicated than biphasic. The data is too sparse to adequately characterize the kinetics of uracil, but the experiment does show that inhibition of DPD does significantly increase the concentration of uracil and delay its elimination. In humans, using an oral dose of 500 mg/m^2 of uracil, a dose comparable to the dose of uridine triacetate here, 300 mg/m^2 in mice, van Staveren *et al.* found that elimination of uridine in humans was zero order suggesting saturation of the DPD activity. DPD is clearly not saturated in mice at 300 mg/m^2.[3] This may be because the administration of uridine triacetate in mice as opposed to uridine in humans delays the absorption of the dose. The absorption phase in the graph above is long, lasting for nearly two hours. This could prevent saturation of DPD. The following table shows the pharmacokinetic parameters for uracil elimination in mice that the investigators calculated from the uracil measurements. In this experiment, treatment with 5-EU increased the AUC of uridine by about 20% (not shown). This is probably because the elevated concentrations of uracil lead to increased uridine synthesis.

[3] MC van Staveren, B Theeuwes-Oonk, HJ Guchelaar, AB van Kuilenburg, and JG Maring, 2011, Pharmacokinetics of orally administered uracil in healthy volunteers and in DPD-deficient patients, a possible tool for screening of DPD deficiency, Cancer Chemother Pharmacol, 68(6):1611-1617.

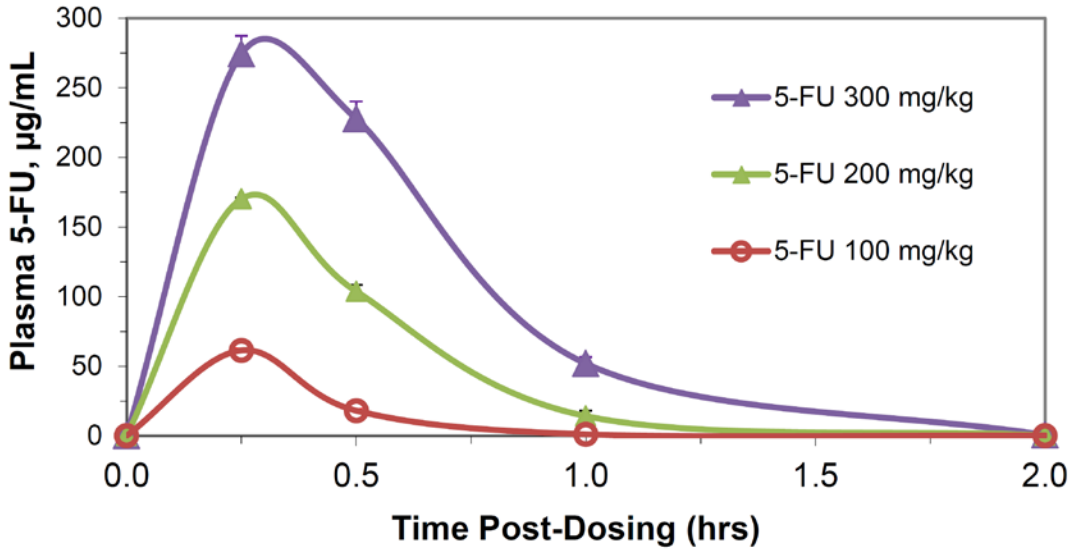
Table 19: Plasma Uracil in Mice after treatment with uridine triacetate plus saline or Ethynyluracil.

Treatment	AUC 0 to 2 hr µM*hr	C_{max} µM	T_{max} hr
Vehicle + Uridine Triacetate	1408	507	2
EU + Uridine Triacetate	2848	786	2

Experiment 2: 5-FU Overdose Model – Dose Proportionality Studies with 5-FU

The investigators designed Experiment 2 to characterize their 5-FU overdose model. They treated mice with a single IP dose of 5-FU of 100, 200 or 300 mg/kg in the absence of EU pretreatment. The figure below from the study report shows the exposure to 5-FU as a function of dose.

Figure 17: Mean Plasma 5-FU Concentrations Following 5-FU Administration in mice as a function of 5-FU dose



The following table the increase in AUC with increasing dose. The data is too sparse to calculate half-lives for 5-FU, but H. Yi *et al.* have determined it to be about 9 minutes.[4]

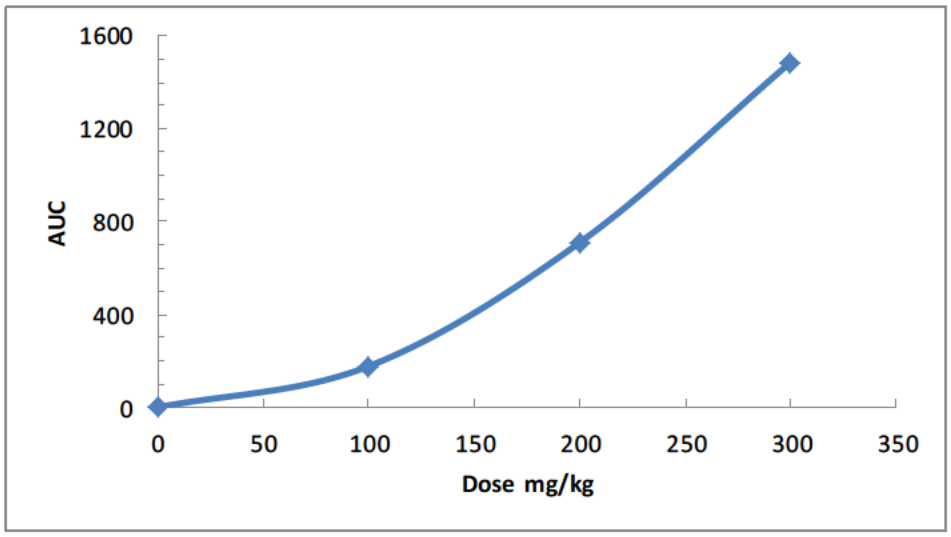
[4] Yi, H, HJ Cho, SM Cho, DG Lee, A Abd El-Aty, SJ Yoo, GW Bae, K Nho, B Kim, CH Lee, JS Kim, MG Bartlett, and HC Shin, 2010, Pharmacokinetic properties and antitumor efficacy of the 5-fluorouracil loaded PEG-hydrogel, BMC Cancer, 10:211-218.

Table 20: Pharmacokinetic Parameters for the Elimination of Different Doses of 5-FU in the Presence of 5-EU Inhibition of DPD.

5- FU dose mg/kg	$AUC_{0\ to\ 2\ hr}$ µM*hr	C_{max} µM	T_{max} hr
100	177	471	0.25
200	709	1308	0.25
300	1484	2109	0.25

The increase in AUC is linear with dose by regression analysis ($r^2 = 0.99$), but, this linearity is somewhat deceiving as the increases in AUC are much greater than dose proportional. This suggests that plasma DPD is saturated at doses above 100 mg/kg. The shape of the curve at higher doses cannot be determined as higher doses are lethal. The investigators did not report control values for 5-FU; presumably they were zero. The following graph shows that the curve is not linear when the data are forced through zero.

Figure 18: Dose of 5-FU vs AUC_{0to2hr} of 5-FU in Mice Pretreated with 5-EU



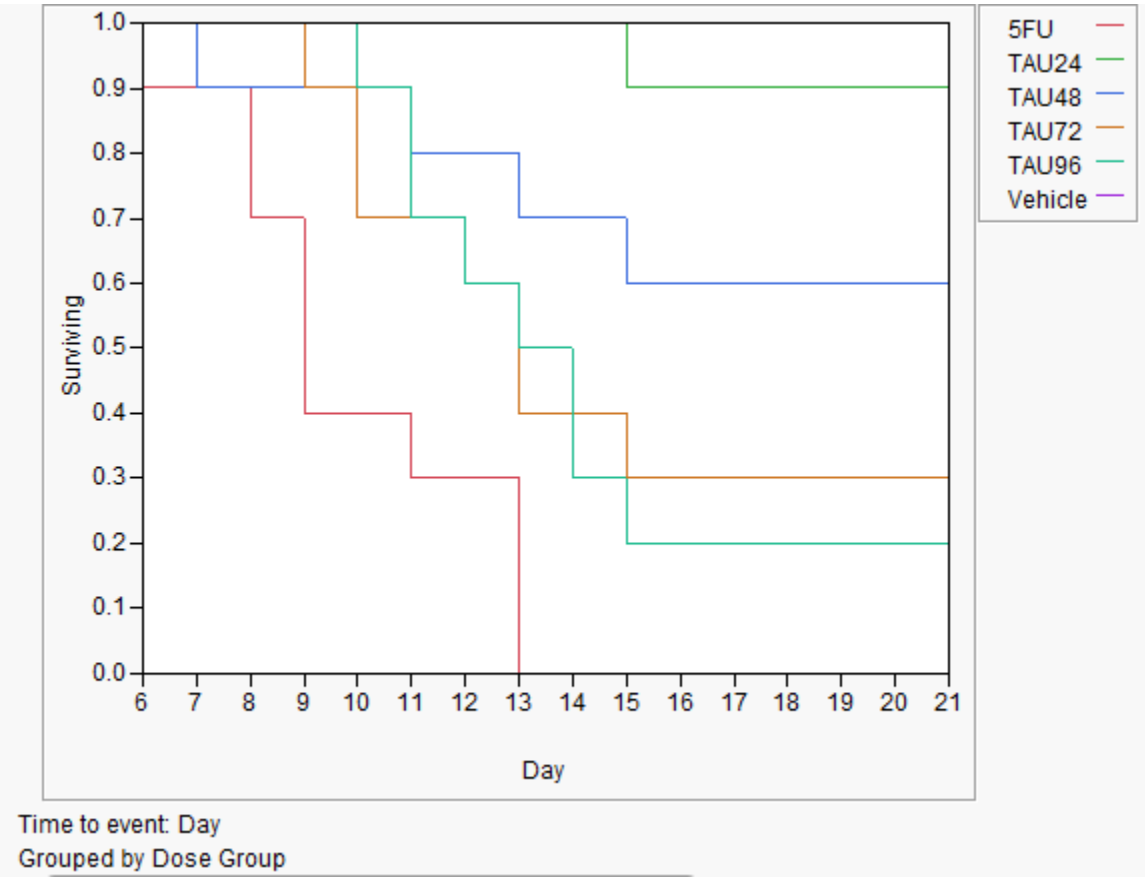
In Experiment 2, the investigators did not report the fate of any of the animals. Presumably, they dispatched the animals after obtaining blood by retro-orbital bleeding. They also report no signs of toxicity. Nevertheless, they say that an IP dose of 300 mg/kg is an LD_{100}. They provide no time course for lethality.

Experiment 3: 5-Fu Overdose Model – Effects of Uridine Triacetate on Survival and Body Weight

The investigators designed experiment 3 to evaluate the effects of uridine triacetate in a model of lethal 5-FU overdose in otherwise normal animals by assessing survival and body weight changes. They gave groups of 10 female mice a single IP dose of 300 mg/kg of 5-FU, a

known lethal dose. They then gave the groups of mice 2000 mg/kg of oral uridine triacetate three times daily for five days for a total of 15 doses starting at different time intervals from the initial 5-FU dose. Controls received vehicle starting at 24 hours. Treated animal groups received uridine triacetate beginning at 24, 48, 72 or 96 hours. The Applicant provided Kaplan-Meier plots, but did not provide a Kaplan-Meier analysis. The following graph shows the Kaplan-Meier survival curves for the results of this experiment that I calculated using SAS JMP.

Figure 19: Kaplan-Meier Survival Curves for Mice Treated with Uridine Triacetate beginning at Different Times after Treatment with a lethal dose of 5-FU



The following table shows the parameters for the data calculated in JMP.

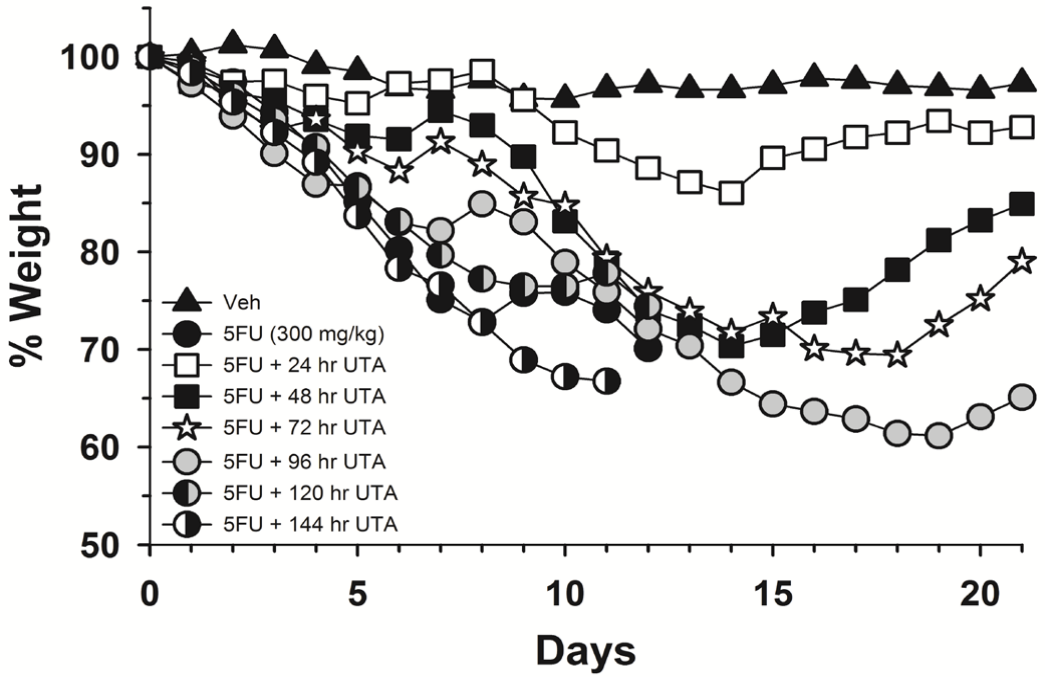
Table 21: Mean Survival and % Survival in Mice Treated with Uridine Triacetate at Different Times after a Lethal Dose of 5-FU

Treatment Group	Mean Survival (Days)	Std Error	% Survival at Day 25
Vehicle	25	0.0	100
5-FU	9.9	0.8	0
5-FU + UTA 24	24	1.0	90
5-FU + UTA 48	19.6	2.3	60
5-FU + UTA 72	14.5	1.5	0
5-FU + UTA 96	14.2	1.2	0
5-FU + UTA 120	10.6	0.5	0
5-FU + UTA 144	10.5	0.3	0

The tests for differences between groups, Log-Rank and Wilcoxon, both indicated significant differences with a p value of less than 0.0001 (not shown).

The following graph from the study report shows the time course of body weight loss in this experiment. Animals treated within 24 hours only lost about 15% of their body weight with a nadir at day 14. Animals in all the other treatment groups lost about 30% of their body weight irrespective of when treatment began, again with a nadir around day 14.

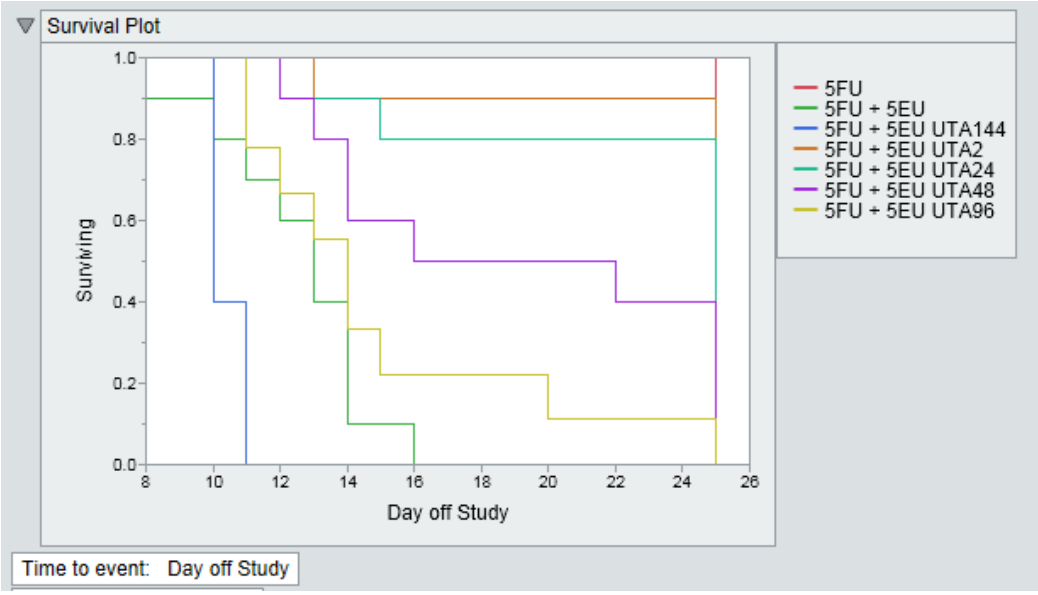
Figure 20: Effects on Body Weight in Mice Treated with Uridine Triacetate at Different Times after a Lethal Dose of 5-FU



Experiment 4 Impaired 5-Fu Elimination Model – Effects of Uridine Triacetate on Survival and Body Weight

The investigators designed Experiment 4 to demonstrate the effects of uridine triacetate (2000 mg/kg/dose given TID for a total of 15 doses over 5 days) in their 5-EU model. They first treated with 2 mg/kg of 5-EU IP. They followed this treatment with a single dose of 100 mg/kg 5-FU, an LD_{100} in the presence of 5-EU. They then treated the animals with uridine triacetate starting at different times, 2, 4, 8, 12, 24, 48, 72, 96, 120 and 144 hours post dosing. The following graph generated by JMP analysis demonstrates Kaplan Meyer survival.

Table 22: Mean Survival and % Survival in Mice Treated with Uridine Triacetate at Different Times after a Lethal Dose of 5-EU plus 5-FU



This graph does not include all the time groups, some are omitted for clarity. Nevertheless, the graph demonstrates the effect of delayed treatment. The tests for differences between groups, Log-Rank and Wilcoxon, both indicated significant differences with a p value of less than 0.0001 (not shown).

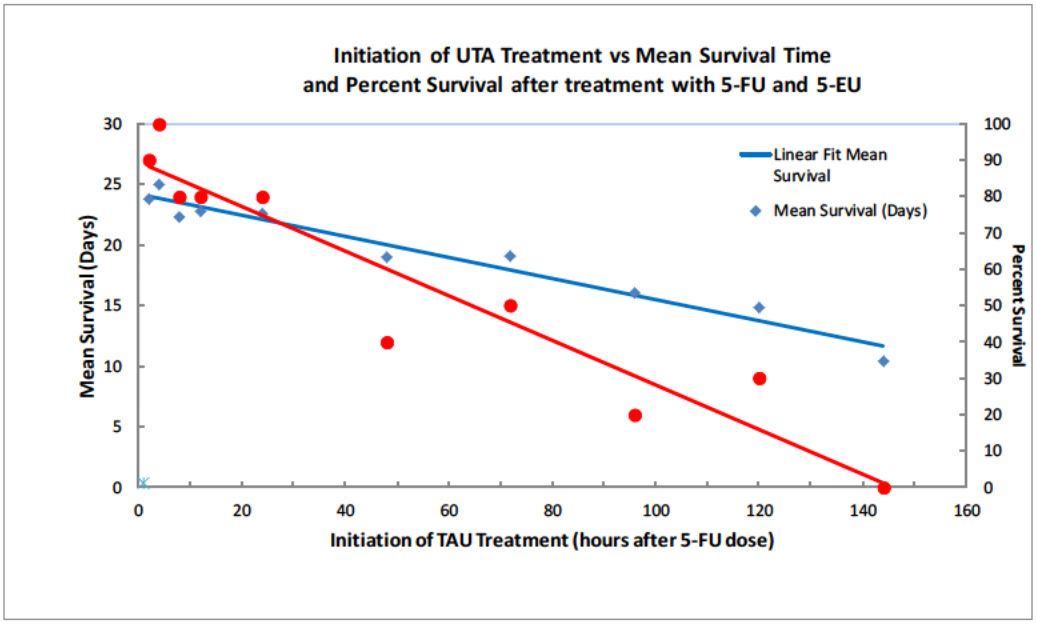
The following table shows the results for mean survival for all groups.

Table 23: Mean Survival and % Survival in Mice Treated with Uridine Triacetate at Different Times after a Lethal Dose of 5-EU plus 5-FU

Group	*Mean Survival Time (Days)*	*Std. Error*	*% Survival at 25 days*
5-FU	25	0.0	100
5-FU + 5-EU	12.5	0.7	0
5-FU + 5-EU uridine triacetate 2 hr	23.8	1.2	90
5-FU + 5-EU uridine triacetate 4 hr	25	0.0	100
5-FU + 5-EU uridine triacetate 8 hr	22.3	1.8	80
5-FU + 5-EU uridine triacetate 12 hr	22.7	1.5	80
5-FU + 5-EU uridine triacetate 24 hr	22.7	1.5	80
5-FU + 5-EU uridine triacetate 48 hr	19.1	1.8	40
5-FU + 5-EU uridine triacetate 72 hr	19.1	2.0	50
5-FU + 5-EU uridine triacetate 96 hr	16	1.7	20
5-FU + 5-EU uridine triacetate 120 hr	14.8	2.2	30
5-FU + 5-EU uridine triacetate 144 hr	10.4	0.2	0

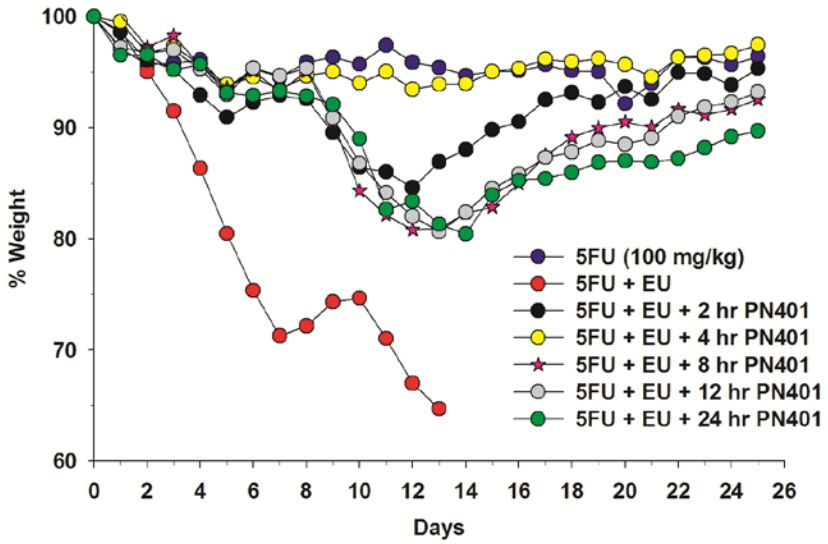
The following graph shows that percent survival decreased linearly with increasing interval between the 5-FU dose and uridine triacetate treatment ($r^2 = 0.90$). Mean survival also decreased linearly ($r^2 = 96$).

Figure 21: Initiation of Uridine Triacetate Treatment vs Mean Survival Time and Percent Survival after Treatment with 5-EU and 5-EU



The following chart from the study report shows that the body weight of treated animals decrease for animals treated within 24 hours after the injection of 5-FU. The Applicant also plotted the body weights for dose groups treated more than 24 hours after 5-FU treatment (not shown). The body weights follow a similar pattern with diminished survival.

Figure 22: Effects on Body Weight in Mice Treated with Uridine Triacetate at Different Times after a Lethal Dose of 5-EU plus 5-FU



With the exception of animals that received treatment beginning at 4 hours, weight loss was comparable in all treated groups ranging from about 15 to 20 percent in animals treated within 24 hours. Body weight in these groups reached a nadir around day 12. Untreated animals lost up to 35 percent of their body weight before they expired. The group that received treatment at 4 hours is significantly anomalous; the body weight profile is indistinguishable from that of animals treated with a non-lethal dose of 5-FU. Also, no animals died in the 4 hour group, while one animal in the group that received treatment beginning at 2 hours died and the animals in that group lost a significant amount of weight. These results strongly suggest a dosing error in the 4 hour group. It appears likely that this group did not receive either 5-FU, 5-EU or both. As both the 5-FU alone and the 4 hour group lost about 6 percent of their body weight in the first five days of the experiment, it appears most likely that the 4 hour group did not receive 5-EU. While this anomaly does not affect the interpretation of the experimental results it does call into question the quality of the experimentation.

All treated animals lost between about 6 and 9 percent of their body weight by day five, then their weight stabilized until about day 9 when a precipitous decrease began. This suggests that the initial weight loss was related to 5-FU induced thymidine deficiency and that the second weight loss was related to the substitution of 5-FU into RNA with the subsequent derangement of protein synthesis.

Experiment 5: Impaired 5-Fu Elimination Model – Effects of Uridine Triacetate on Pathological Changes to the Intestinal Mucosa

The investigators did this experiment to evaluate the ability of uridine triacetate to protect the gastric mucosa of mice against the toxic effects of 5-FU. They dosed the mice intraperitoneally with 2 mg/kg of 5-EU, and then two hours later gave the mice 100 mg/kg of 5-FU intraperitoneally as in experiment 4 with appropriate controls. They then gave the mice oral uridine triacetate thrice daily with a dose of 2000 mg/kg/dose in two different groups. The first group received drug every eight hours for a total of 12 doses. These animals were necropsied on day 4. The second group received drug every eight hours for a total of 15 doses. These animals were necropsied on day 10 to demonstrate the extent of recovery. The investigators resected 2 cm sections of the duodenum and fixed them in paraformaldehyde. They then then took 3 consecutive 10 micron sections at 100 micron intervals from each duodenal sample along the length of the duodenum and stained them. They measured luminal diameter by microscopic planimetry, using the NIH ImageJ software.[5] Likewise, they determined the villus area within the intestinal cross section by planimetry, and then calculated the ratio of villus area to total intestinal cross section presented as % Villus Area. They then compared %Villus Area by ANOVA. The experiment included the following dose groups with N = 5 mice for a total of 15 cross sectional measurements per dose group.

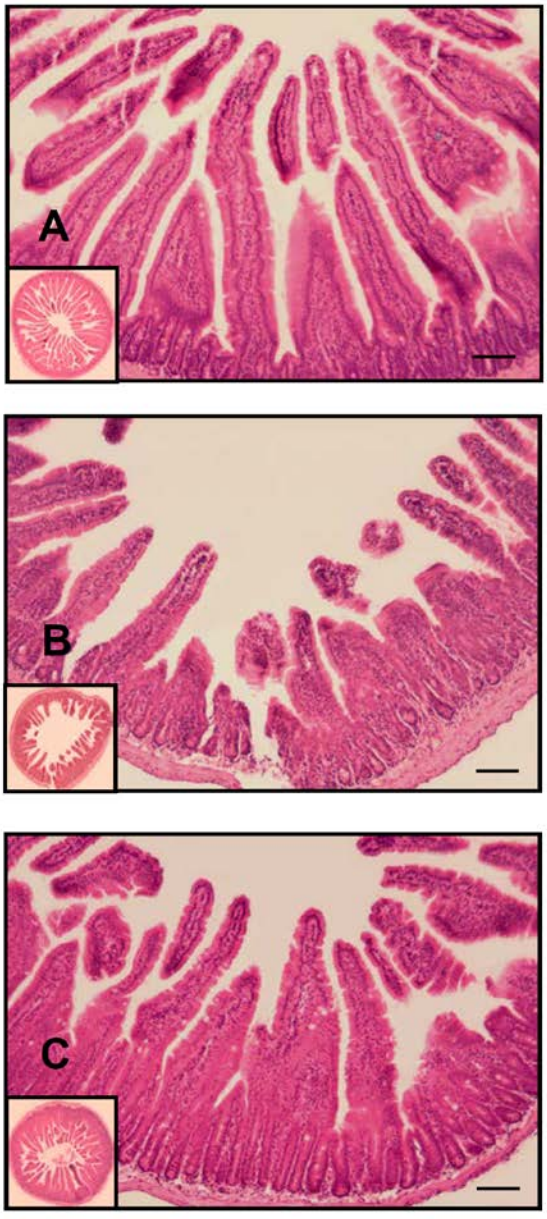
1) Saline (control)
2) 5-FU plus Vehicle (control)
3) 5-FU plus uridine triacetate (control)
4) 5-FU plus 5-EU without uridine triacetate (treatment control, lethal at day four or thereafter, *v.s.*)
5) 5-FU plus 5-EU with uridine triacetate beginning at 2 hours (treatment)
6) 5-FU plus 5-EU with uridine triacetate beginning at 24 hours (treatment)

The Applicant did not include groups 2 or 3 in the original submission and there were missing data points that the text of the original submission did not explain. We issued an information request in the filing letter asking the Applicant to address these omissions. The Applicant says they did not include these groups because they did not show any difference in response from controls. Nevertheless, they are essential controls and these values contributed to the determination of the normality of the samples.

[5] This software is available on the Internet at http://rsb.info.nih.gov/nih-image/.

The Applicant included three micrographs to demonstrate the changes in the appearance and surface area of the villi.

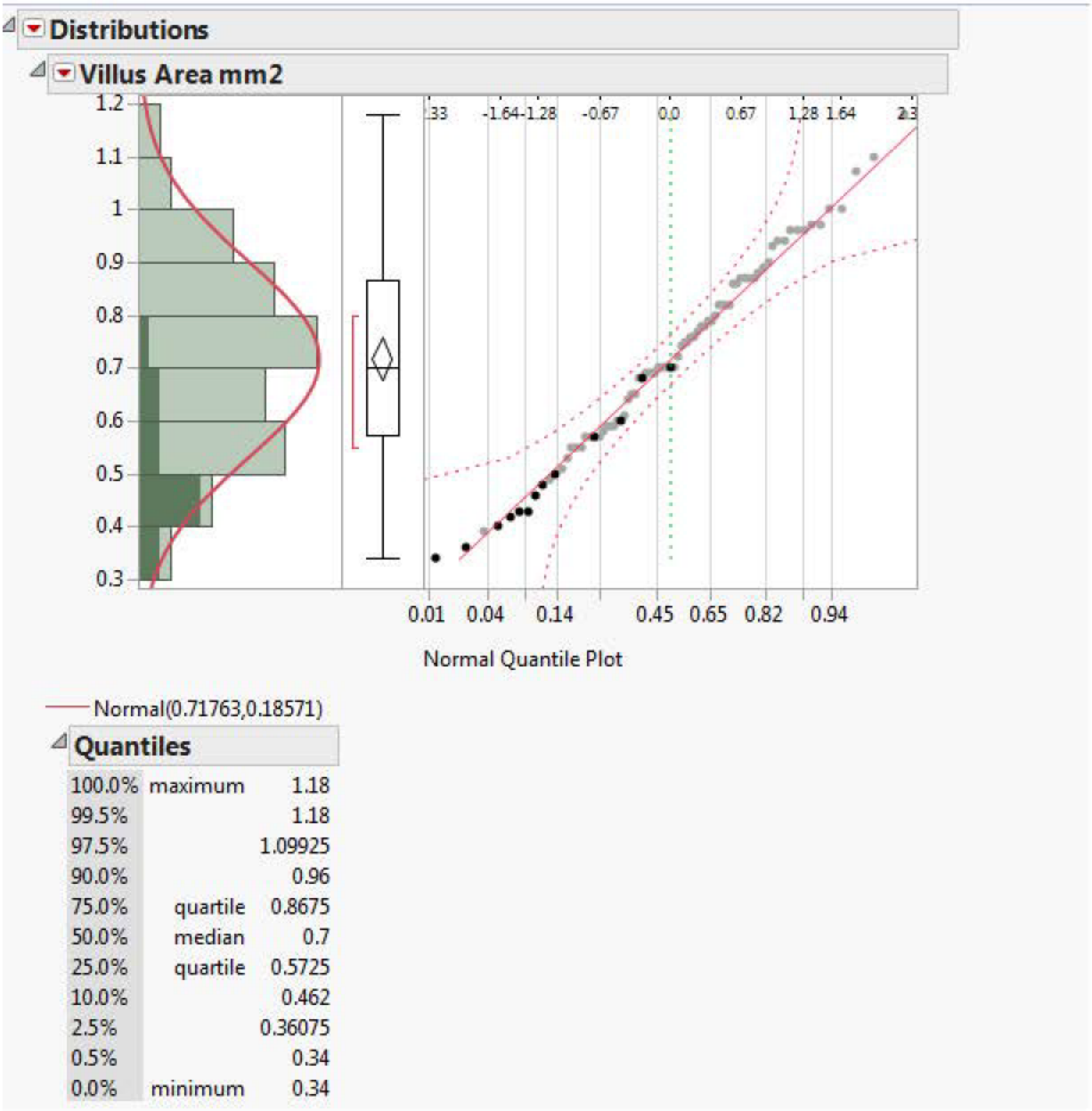
Figure 23: Micrographs of Villus Changes in Mice Treated with 5-FU plus 5-EU with or without Uridine Triacetate

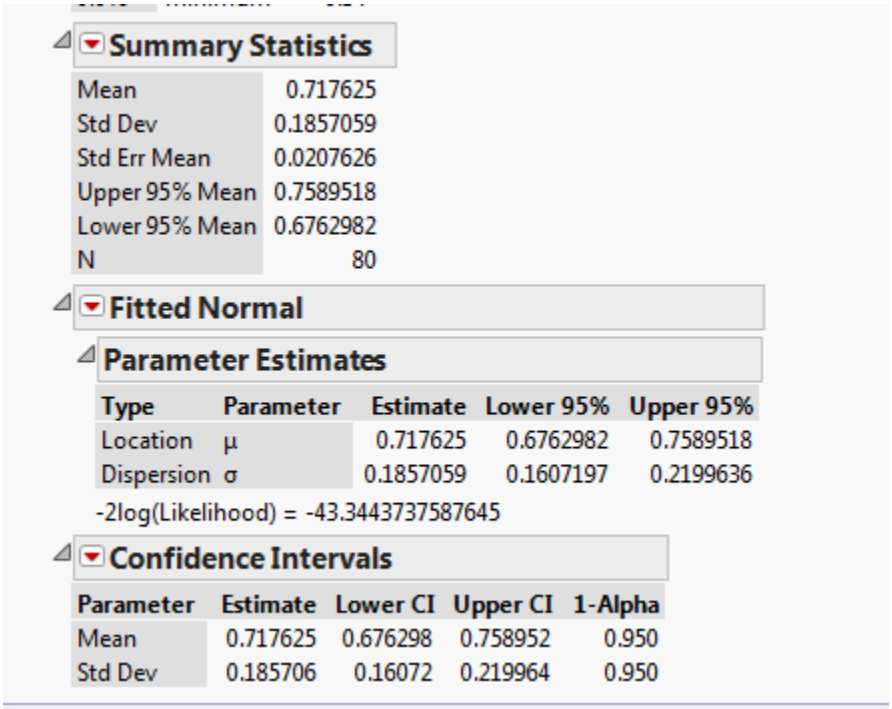


A. Vehicle (negative control), Day 4 sacrifice
B. 5-FU + EU (positive control), Day 4 sacrifice
C. 5-FU + EU + Uridine Triacetate (initiated 2 hours after 5-FU), Day 4 sacrifice.

I examined the villus area and total intestinal cross sectional area in JMP for normality. The following graphs show the results for villus area across all groups.

Figure 24: Test of Villus Area Measurements for Normality





Summary Statistics

Mean	0.717625
Std Dev	0.1857059
Std Err Mean	0.0207626
Upper 95% Mean	0.7589518
Lower 95% Mean	0.6762982
N	80

Fitted Normal

Parameter Estimates

Type	Parameter	Estimate	Lower 95%	Upper 95%
Location	μ	0.717625	0.6762982	0.7589518
Dispersion	σ	0.1857059	0.1607197	0.2199636

-2log(Likelihood) = -43.3443737587645

Confidence Intervals

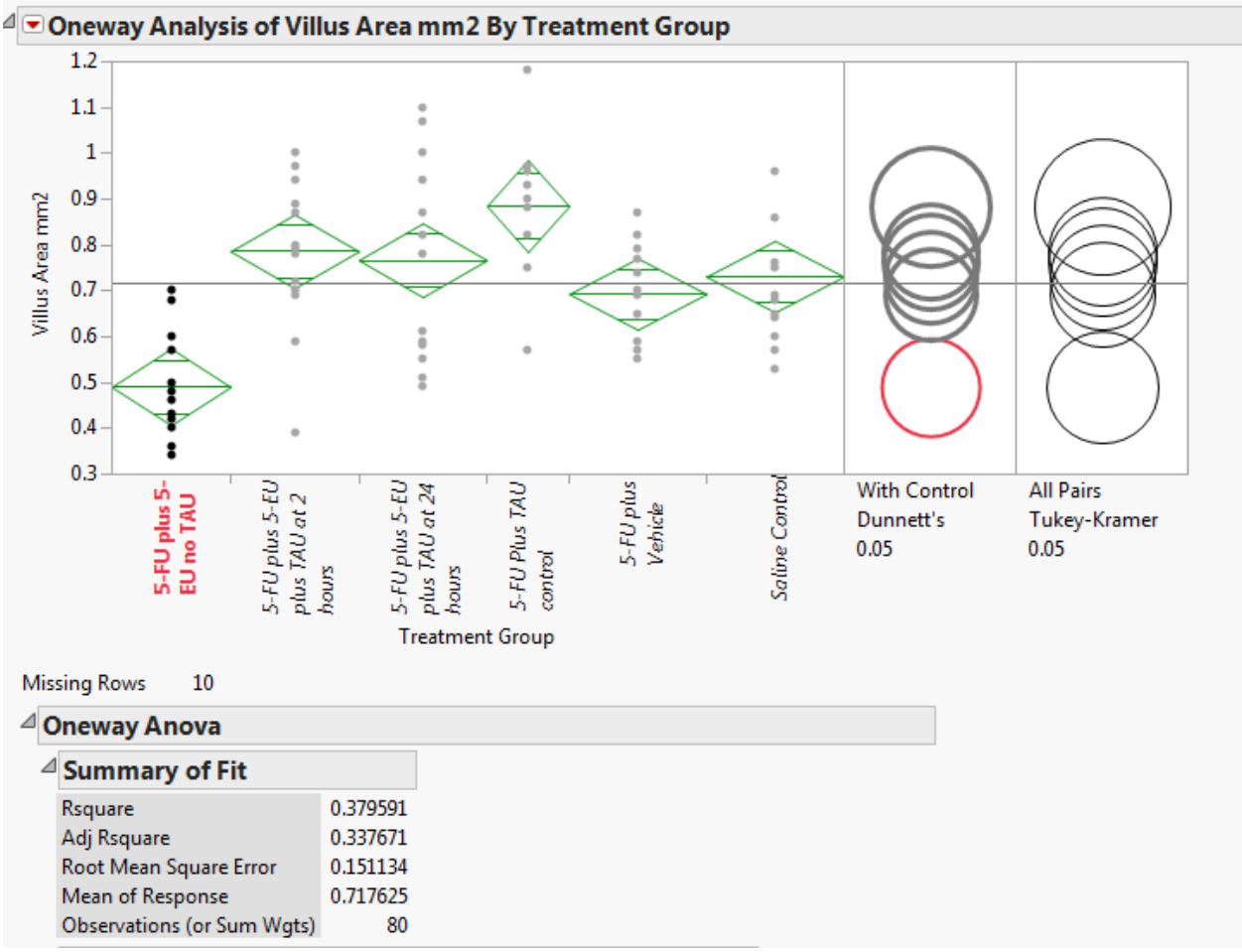
Parameter	Estimate	Lower CI	Upper CI	1-Alpha
Mean	0.717625	0.676298	0.758952	0.950
Std Dev	0.185706	0.16072	0.219964	0.950

Villi area appears normally distributed except for one group (darker shade). This group is the 5-FU plus 5-EU without uridine triacetate (treatment control). Normality improves considerably when this group is excluded (not shown).

Similarly, the total duodenal luminal area was also normal (not shown). The treatment control again expanded the distribution. Normality improved again when this group was excluded (not shown).

Since both samples are appear to be normal, ANOVA is an appropriate analysis for both villus area and total intestinal area. The following graph and tables show my analysis of variance for villus area.

Figure 25: Oneway Analysis of Villus Area by Treatment Group



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	5	1.0341756	0.206835	9.0552	<.0001*
Error	74	1.6902732	0.022842		
C. Total	79	2.7244488			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
5-FU plus 5-EU no TAU	13	0.490000	0.04192	0.40648	0.57352
5-FU plus 5-EU plus TAU at 2 hours	14	0.785714	0.04039	0.70523	0.86620
5-FU plus 5-EU plus TAU at 24 hours	14	0.766429	0.04039	0.68595	0.84691
5-FU Plus TAU control	9	0.884444	0.05038	0.78406	0.98482
5-FU plus Vehicle	15	0.692667	0.03902	0.61491	0.77042
Saline Control	15	0.730667	0.03902	0.65291	0.80842

Std Error uses a pooled estimate of error variance

The ANOVA was significant ($p < 0.0001$) so the analysis continued with a comparison of means to the treatment control value by Dunnet's Method.

Figure 26: Comparison of Villus Area by Treatment Group with Dunnet's Method

Means Comparisons

Comparisons with a control using Dunnett's Method

Control Group = 5-FU plus 5-EU no TAU

Confidence Quantile

|d|	Alpha
2.56773	0.05

LSD Threshold Matrix

Level	Abs(Dif)-LSD	p-Value
5-FU Plus TAU control	0.226	<.0001*
5-FU plus 5-EU plus TAU at 2 hours	0.146	<.0001*
5-FU plus 5-EU plus TAU at 24 hours	0.127	<.0001*
Saline Control	0.094	0.0003*
5-FU plus Vehicle	0.056	0.0032*
5-FU plus 5-EU no TAU	-0.15	1.0000

Positive values show pairs of means that are significantly different.

In the ANOVA graph the Treatment Control shows this group as the lowest circle, the red one. All other groups were significantly different from this Treatment Control group by $p = 0.0032$ or less. This warranted testing the means by the Tukey-Kramer method.

Figure 27: Comparison of Villus Area by Treatment Group using the Tukey-Kramer Method

Comparisons for all pairs using Tukey-Kramer HSD

Confidence Quantile

q*	Alpha
2.92572	0.05

LSD Threshold Matrix

Abs(Dif)-HSD

	5-FU Plus TAU control	5-FU plus 5-EU plus TAU at 2 hours	5-FU plus 5-EU plus TAU at 24 hours	Saline Control	5-FU plus Vehicle	5-FU plus 5-EU no TAU
5-FU Plus TAU control	-0.20844	-0.09019	-0.07090	-0.03266	0.00534	0.20270
5-FU plus 5-EU plus TAU at 2 hours	-0.09019	-0.16713	-0.14784	-0.10927	-0.07127	0.12540
5-FU plus 5-EU plus TAU at 24 hours	-0.07090	-0.14784	-0.16713	-0.12856	-0.09056	0.10612
Saline Control	-0.03266	-0.10927	-0.12856	-0.16146	-0.12346	0.07311
5-FU plus Vehicle	0.00534	-0.07127	-0.09056	-0.12346	-0.16146	0.03511
5-FU plus 5-EU no TAU	0.20270	0.12540	0.10612	0.07311	0.03511	-0.17344

Positive values show pairs of means that are significantly different.

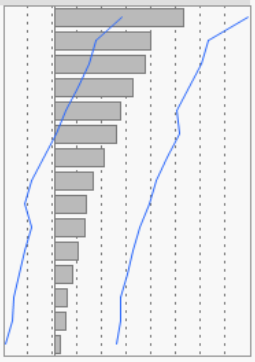
Connecting Letters Report

Level		Mean
5-FU Plus TAU control	A	0.88444444
5-FU plus 5-EU plus TAU at 2 hours	A B	0.78571429
5-FU plus 5-EU plus TAU at 24 hours	A B	0.76642857
Saline Control	A B	0.73066667
5-FU plus Vehicle	B	0.69266667
5-FU plus 5-EU no TAU	C	0.49000000

Levels not connected by same letter are significantly different.

Ordered Differences Report

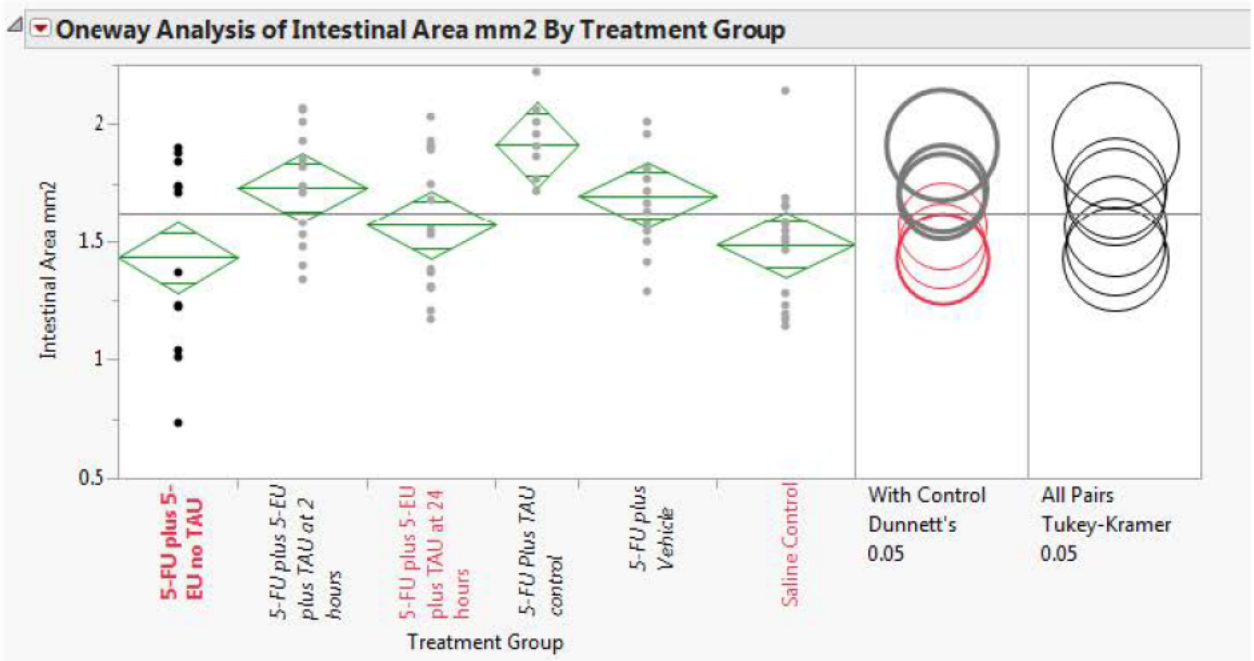
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
5-FU Plus TAU control	5-FU plus 5-EU no TAU	0.3944444	0.0655362	0.202704	0.5861850	<.0001*
5-FU plus 5-EU plus TAU at 2 hours	5-FU plus 5-EU no TAU	0.2957143	0.0582115	0.125404	0.4660249	<.0001*
5-FU plus 5-EU plus TAU at 24 hours	5-FU plus 5-EU no TAU	0.2764286	0.0582115	0.106118	0.4467392	0.0001*
Saline Control	5-FU plus 5-EU no TAU	0.2406667	0.0572696	0.073112	0.4082216	0.0010*
5-FU plus Vehicle	5-FU plus 5-EU no TAU	0.2026667	0.0572696	0.035112	0.3702216	0.0088*
5-FU Plus TAU control	5-FU plus Vehicle	0.1917778	0.0637238	0.005340	0.3782157	0.0402*
5-FU Plus TAU control	Saline Control	0.1537778	0.0637238	-0.032660	0.3402157	0.1651
5-FU Plus TAU control	5-FU plus 5-EU plus TAU at 24 hours	0.1180159	0.0645716	-0.070902	0.3069342	0.4547
5-FU Plus TAU control	5-FU plus 5-EU plus TAU at 2 hours	0.0987302	0.0645716	-0.090188	0.2876485	0.6469
5-FU plus 5-EU plus TAU at 2 hours	5-FU plus Vehicle	0.0930476	0.0561632	-0.071270	0.2573655	0.5643
5-FU plus 5-EU plus TAU at 24 hours	5-FU plus Vehicle	0.0737619	0.0561632	-0.090556	0.2380798	0.7768
5-FU plus 5-EU plus TAU at 2 hours	Saline Control	0.0550476	0.0561632	-0.109270	0.2193655	0.9228
Saline Control	5-FU plus Vehicle	0.0380000	0.0551864	-0.123460	0.1994600	0.9827
5-FU plus 5-EU plus TAU at 24 hours	Saline Control	0.0357619	0.0561632	-0.128556	0.2000798	0.9878
5-FU plus 5-EU plus TAU at 2 hours	5-FU plus 5-EU plus TAU at 24 hours	0.0192857	0.0571233	-0.147841	0.1864127	0.9994



As the ordered differences report shows again, all groups are statistically different from the 5-FU plus 5-EU (no TAU) treatment control by p = 0.0088. Additionally, 5-FU plus uridine triacetate control is significantly different from the 5-FU plus vehicle control. This shows the effect of uridine triacetate in the absence of 5-EU. The experiment would have been much stronger had it included groups receiving uridine triacetate plus different doses of 5-FU. All other groups are statistically equivalent, suggesting that uridine triacetate treatment either at 2 or 24 hours prevents toxicity almost to the level of control (animals not treated with 5-FU). This effect is best seen graphically (*v.s.*). Indeed the mean value for luminal area for animals treated with only 5-FU and uridine triacetate at 2 hours is greater than the means of all other groups though this increase does not reach significance due to variability and missing values (N = 9 instead of 15, this was one of the groups the Applicant did not include in the original submission).

The following graph and tables show my analysis of variance for total intestinal cross sectional area values reported in the study report.

Figure 28: Oneway Analysis of Intestinal Cross-Sectional Area by Treatment Group



Missing Rows 10

Oneway Anova

Summary of Fit

Rsquare	0.246033
Adj Rsquare	0.195089
Root Mean Square Error	0.272975
Mean of Response	1.623875
Observations (or Sum Wgts)	80

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	5	1.7993610	0.359872	4.8295	0.0007*
Error	74	5.5141378	0.074515		
C. Total	79	7.3134987			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
5-FU plus 5-EU no TAU	13	1.43231	0.07571	1.2815	1.5832
5-FU plus 5-EU plus TAU at 2 hours	14	1.73214	0.07296	1.5868	1.8775
5-FU plus 5-EU plus TAU at 24 hours	14	1.57214	0.07296	1.4268	1.7175
5-FU Plus TAU control	9	1.91444	0.09099	1.7331	2.0957
5-FU plus Vehicle	15	1.69867	0.07048	1.5582	1.8391
Saline Control	15	1.48800	0.07048	1.3476	1.6284

Std Error uses a pooled estimate of error variance

Here too the ANOVA was positive with a p value of 0.0007. This again warranted comparison of the means with Dunnet's Method. The following tables show the continuation of my JMP analysis. The results of this analysis are also shown demonstrated in the graph of overlapping circles above.

Figure 29: Comparison of Villus Area by Treatment Group with Dunnet's Method

Means Comparisons

Comparisons with a control using Dunnett's Method

Control Group = 5-FU plus 5-EU no TAU

Confidence Quantile

|d|	Alpha
2.56773	0.05

LSD Threshold Matrix

Level	Abs(Dif)-LSD	p-Value
5-FU Plus TAU control	0.178	0.0005*
5-FU plus 5-EU plus TAU at 2 hours	0.03	0.0240*
5-FU plus Vehicle	0.001	0.0491*
5-FU plus 5-EU plus TAU at 24 hours	-0.13	0.5405
Saline Control	-0.21	0.9761
5-FU plus 5-EU no TAU	-0.27	1.0000

Positive values show pairs of means that are significantly different.

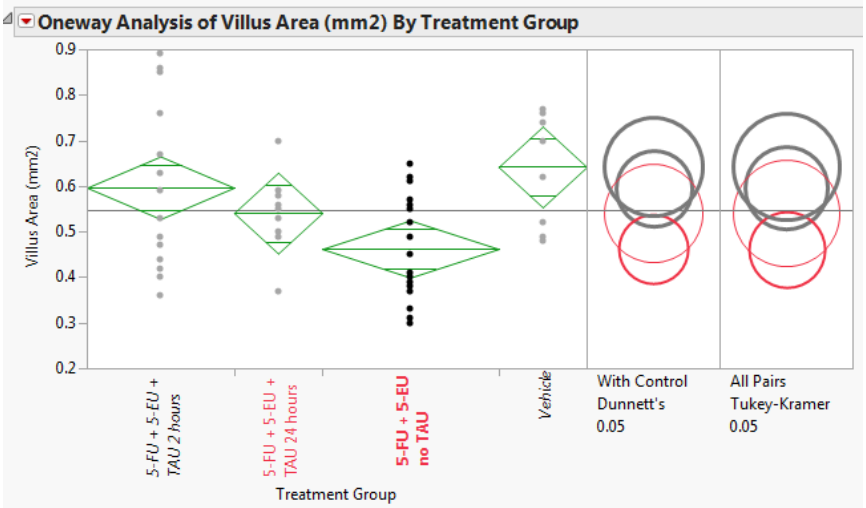
Again in this analysis, each value is compared to the treatment control, 5-FU plus 5-EU no uridine triacetate. The differences do not reach significance for the Saline Control or for the 5-FU plus 5-EU plus uridine triacetate at 24 hours. But the other three groups are significantly different from the treatment control with $p < 0.05$ for each group including 5-FU plus vehicle. Again, in the 5-FU plus uridine triacetate control had the highest mean value and was significantly different from Treatment Control. Tukey-Kramer comparison of individual means yielded similar results (not shown).

In this case of intestinal area, several competing factors are likely at work. 5-FU is causing an inflammatory response initially and then destruction of the villi at the cellular level. The former would likely cause expansion of the luminal volume while the latter would cause contraction. Again, uridine triacetate treatment appears either protective or it is adding to the inflammatory response. The latter is possible because at such high doses a considerable amount of acetate is being released into the trans-luminal cells as the uridine triacetate is hydrolyzed. Whatever the mechanism, luminal area is not invariant under the conditions of this experiment so analysis of the data by comparison of percentage of villus area relative to total luminal cross-sectional area, the metric used by the Applicant, is inappropriate. I have also not included the Applicant's analysis because they tested the percentage villus area using parametric analysis of variance. The transformation of the data to a percentage requires that the data be analyzed by a non-parametric ANOVA such as Kruskal-Wallis analysis. The

investigators allowed a group of mice to recover to day 10 after the 5-FU dose in order to demonstrate recovery. The dose groups were the same except that two groups were, unfortunately, omitted. These were the 5-FU plus Vehicle (control) and the 5-FU plus uridine triacetate (control). These groups possibly would have demonstrated the efficacy of uridine triacetate without the use of the 5-EU dosing. Again, the inclusion of several dose groups of 5-FU would have been useful.

Again, the distribution appeared fairly normal but the values for the treatment control are skewed to the lower end of the distribution (not shown). I analyzed this data by ANOVA as shown in the graphs and tables that follow. The ANOVA showed a significant difference among the groups (p = 0.0058) warranting further testing with Dunnett's Method and Tukey-Kramer analysis.

Figure 30: Oneway Analysis of Villus Area by Treatment Group on Day 10



Oneway Anova

Summary of Fit

Rsquare	0.232044
Adj Rsquare	0.183025
Root Mean Square Error	0.132222
Mean of Response	0.547451
Observations (or Sum Wgts)	51

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment Group	3	0.2482797	0.082760	4.7338	0.0058*
Error	47	0.8216889	0.017483		
C. Total	50	1.0699686			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
5-FU + 5-EU + TAU 2 hours	15	0.596667	0.03414	0.52799	0.66535
5-FU + 5-EU + TAU 24 hours	9	0.541111	0.04407	0.45245	0.62978
5-FU + 5-EU no TAU	18	0.462222	0.03117	0.39953	0.52492
Vehicle	9	0.642222	0.04407	0.55356	0.73089

Std Error uses a pooled estimate of error variance

Dunnet's method shows that all groups were different from the treatment control by a p value of 0.0045. Analysis by the Tukey-Kramer method showed that the values for animals treated within two hours were statistically the same as controls by day 10 while the other two

groups had not recovered (not shown). Analysis of the Day 10 data for total intestinal volume showed similar results (not shown).

3) Anti-Tumor Efficacy of 5-Fluorouracil with and without Uridine or Uridine Triacetate in the CD8F1 Murine Mammary Carcinoma System

Study Number	R.401.15.01
Filename	r4011501-report-body.pdf, Module 4.2.1.2
Laboratory	Wellstat Therapeutics Corp., Gaithersburg, MD 20878
Study Date	December 1991
GLP	No
Audited	No
Drug	Uridine Triacetate, Lot# 1911-C-4P (manufacture 1995) (b) (4), Purity 101.3 %

Experiment 1, Comparison to Uridine

Method

Dose The following table shows the dose groups in Experiment 1.

Table 24: Dose Groups for Tumor Growth Delay Experiment

Group	5-FU mg/kg IP	Antidote	Route	Treatment dose mg/kg
1	Saline	None	PO	
2	150	None	PO	
3	150	Vehicle	PO	0
4	150	Uridine	IP	3500
5	150	Uridine	PO	5000
6	150	Uridine triacetate	PO	7582

5000 mg/kg uridine and 7582 mg/kg uridine triacetate are molar equivalent doses.

Schedule	5-FU or saline control was given once weekly × 3 Antidote was given was given 2 hours and 22.5 hours after each weekly dose of 5-FU
Vehicle	1:1 corn oil: distilled water emulsion + 2.5% Tween 80
Route	See table above
Species	Female BALB/C x DBA/8 mice
Tumor model	First generation transplants of CD8F1 spontaneous mammary adenocarcinoma
Tumor injection	"Female mice of this strain develop spontaneous mammary tumors; for a study, 3 or 4 spontaneous tumors are combined and made into a brei with a tissue grinder and screen, and the brei is injected into test animals, yielding relatively uniform syngeneic tumors derived directly from

	primary tumors." Tumors were allowed to grow to about 155 mg prior to treatment
Tumor measure	Two axis, longest and shortest (two dimensional) Tumor weight was estimated using the formula: [L (mm) × W (mm)2] ÷ 2
Number	10 per dose group
Age	Not specified
Weight	Not specified
Parameters	Survival and tumor size in groups with > 50% survival one week after the third weekly injection of 5-FU

Results of experiment 1

The following table from the study report shows the results of this study.

Table 25: Tumor Growth Delay and Survival in Mice Treated with 5-FU plus Antidote

Group	Treatment 1 (i.p.) *	Treatment 2 ** (Rescue Strategies)	Survival (%)	Average Tumor Weight (mg)
1	Saline	None	70%	7391
2	5-FU (150 mg/kg)	None	10%	§
3	5-FU (150 mg/kg)	Vehicle (oral) ***	0%	§
4	5-FU (150 mg/kg)	Uridine (i.p.), 3500 mg/kg	90%	1604
5	5-FU (150 mg/kg)	Uridine (oral), 5000 mg/kg ****	100%	896
6	5-FU (150 mg/kg)	Uridine triacetate (oral) 7582 mg/kg ****	90%	1013
(Martin et al., 1983)	*5-FU (100 mg/kg)*	*Historical data (5-FU alone at MTD)*****	*90-100%*	*1900-2600*

* Treatment 1 was given once weekly × 3
** Treatment 2 was given every 8 hours × 5 starting 2 hours after each weekly dose of 5-FU
*** 1:1 corn oil: distilled water emulsion + 2.5% Tween 80
**** 5000 mg/kg uridine and 7582 mg/kg uridine triacetate are molar equivalent doses.
***** For mice with $CD8F_1$ tumors starting at 150-160 mm^3 prior to treatment
§ Not meaningful due to low survival (<50%).

The investigators evidently included the historical control data with a lower dose of 5-FU because of the high mortality in the treated controls. The reference in the table is incorrect; it should refer to Martin *et al.* (1982, the date in the applicant's table above is incorrect).[6] The experiment demonstrates that both uridine and uridine triacetate groups maintain some degree of 5-FU efficacy in slowing tumor growth. As this is a spontaneous mouse tumor model it is impossible to determine the relevance of this finding to the clinical situation.

Experiment 2 describes a combination chemotherapy regimen. It is uncontrolled due to high mortality. It has no bearing on this NDA.

[6] DS Martin, RL Stolfi, RC Sawyer, S Spiegelman, and CW Young, 1982, High-Dose 5-Fluorouracil with Delayed Uridine "Rescue" in Mice, Cancer Research, 42(10):3964-3970.

11 Integrated Summary and Safety Evaluation

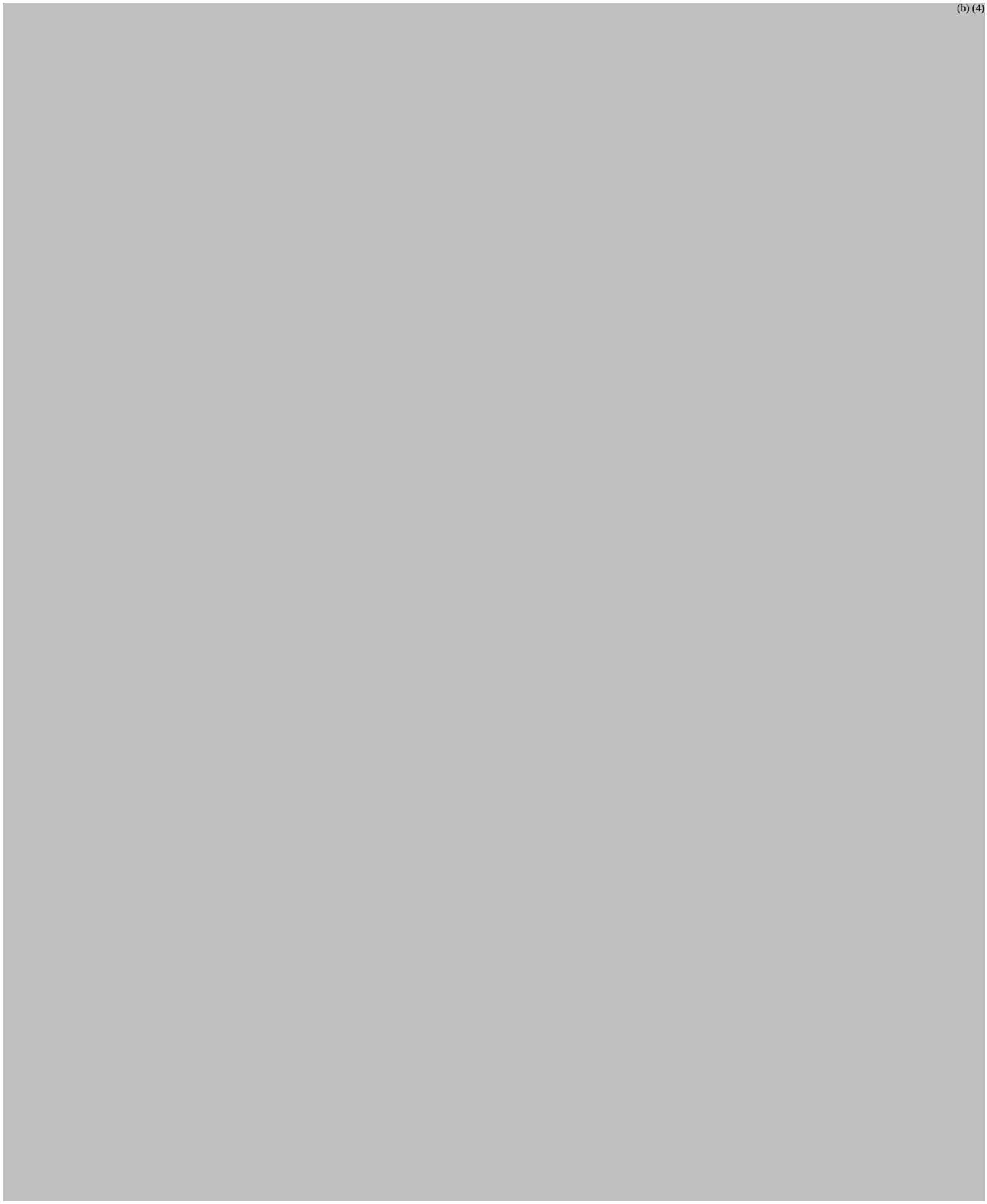
Biochemical Mechanism

This summary will encompass only the studies reviewed above. For a complete summary of the submission, including nonclinical safety studies, see Dr. Sruthi T. King's review of NDA 208169 dated June 18, 2015.

Uridine (CAS 58-96-8) is a pyrimidine composed of (b) (4)

(b) (4)

(b) (4)



(b) (4)

Toxicology

I have adapted the following from Dr. King's review of NDA 208169

In vitro, uridine triacetate did not inhibit the slow potassium rectifier channel (hERG) at physiologically relevant concentrations. In the repeat-dose toxicology studies, uridine triacetate caused no observable cardiac toxicity in dogs or rats.

(b) (4)

Uridine triacetate is absorbed readily after oral administration and 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 four to seven times greater than uridine, which has poor oral bioavailability (6-10%) and produces dose-limiting diarrhea at high doses. In toxicology studies, peak plasma uridine and uracil concentrations occurred about two hours after dosing 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.

In repeat-dose toxicology studies in rats (3 months and 6 months), animals tolerated doses as high as 2000 mg/kg/day, the maximum feasible dose. This dose was a no observed adverse effect level (NOAEL) dose in the 6-month repeat-dose toxicity study in rats.

When given orally for three months, beagle dogs well tolerated doses of uridine triacetate up to 1500 mg/kg/day (administered in 2 equal doses, 6 h apart). This high dose was a NOAEL.

Uridine triacetate was not genotoxic in the standard battery of *in vitro* and *in vivo* assays. Wellstat has not done carcinogenicity with uridine triacetate and DGIEP did not require them. According to Dr. King "no findings suggested that the compound was tumorigenic 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 in the study. Wellstat has agreed to a post-marketing requirement with DGIEP to do a Segment 3 pre- and postnatal development study.

Efficacy in Mouse Models

Study 1

Experiment 1

The first study of the efficacy of uridine triacetate included two experiments in mice. In the first experiment, investigators compared uridine triacetate to uridine after a non-lethal injection of 5-FU. The investigators measured marrow count, spleen weight, WBC, neutrophils, lymphocytes, platelets and RBC on days 8 and 12 after a single dose of 5-FU of 150 mg/kg IP. The mice then received various antidote treatments at a schedule that roughly approximates the clinical schedule. An analysis of the variance of the data demonstrated heteroscedasticity and N was small in each group necessitating non-parametric analysis. By day 8, all the medians for all the measured parameters were below the cumulative means for Balb/C mice in a dataset published by (b) (4) The investigators included a control treated with 5-FU and vehicle (vehicle control below), but not one treated with only vehicle so it was necessary to compare the results to the (b) (4) controls. The following table shows the median values for the parameters analyzed in this experiment. Values in bold red are significantly different from 5-FU treated control ($\alpha = 0.05$).

Dose Group	WBC (1000/µL)	Marrow (1000000/mg)	Spleen Wt. (mg)	Neutrophils (1000/µL)	Lymphocytes (1000/µL)	Platelets (1000/µL)	RBCs (1000000/µL)
Uridine IP 400 mg/kg	**4.7**	**5.8**	75.3	**0.56**	**3.48**	**745**	8.01
Uridine PO 400 mg/kg	**4**	1.6	73.6	0.52	3.53	488	7.73
Uridine PO 800 mg/kg	**4.2**	2.3	68.8	**0.76**	3.7	523	7.98
Uridine Triacetate PO 500 mg	**5.9**	3.2	78.4	**1.15**	**4.54**	**769**	8.23
Vehicle Control	3.4	1.6	71.5	0.27	3.23	346	8.07
(b) (4) Mean[1]	8.87		100	1.74	7.29	963	9.98
Low	5.69			0.74	3.6	476	9.16
High	14.84			3.01	11.56	1611	11.7

That all values were lower than (b) (4) historical controls demonstrates that treatment only stopped damage after it was initiated. It did not affect damage that had already occurred or hasten recovery by day 8. Nevertheless, all the antidote treatments resulted in improvements in WBC, neutrophils, lymphocytes and platelets compared to 5-FU treated controls, though some of the increases did not reach significance due to the small sample size. These findings should not be over interpreted as the experiment was underpowered. The three uridine treatments were for the most part as effective as uridine triacetate and were in most cases statistically indistinguishable from the uridine triacetate group, though as one would expect, the response to uridine given intraperitoneally was usually most similar to that of uridine triacetate due to better bioavailability and the low dose of uridine given orally was usually least similar. While treatment with uridine triacetate is beneficial, it does not prevent a considerable degree of toxicity even when given expeditiously. The uridine triacetate dose to mice, 500 mg/kg per treatment, is about 1500 mg/m^2. The human dose is 10 grams per administration, or about 5500 mg/m^2. On a molar basis, the 500 mg/kg UTA dose is approximately equivalent to the 400 mg/kg uridine dose. The 800 mg/kg dose is about twice the UTA dose on a molar basis.

The following table shows the values for these parameters after four more days of recovery, on day 12. The values in red are significantly different from the 5-FU treated vehicle control.

Dose Group	WBC	Marrow (not done)	Spleen Wt.	Neutrophils	Lymphocytes	Platelets	RBCs
Uridine IP 400 mg/kg	4.9		104	1.3	3.72	1704	7.82
Uridine PO 400 mg/kg	4.4		95.5	0.84	**3.49**	1825	7.56
Uridine PO 800 mg/kg	5		96.1	0.82	4.07	2170	7.41
Uridine Triacetate PO 500 mg	5.6		**140**	**1.95**	**3.5**	1423	**8.03**
Vehicle Control	5.1		71.8	0.44	4.6	2299	7.3
(b) (4) Mean*	8.87		100*	1.74	7.29	963	9.98
Low	5.69			0.74	3.6	476	9.16
High	14.84			3.01	11.56	1611	11.7

*The normal spleen weight for a 20 gram mouse is about 100 milligram.[21]

[21] B. Davies and T. Morris, Physiological Parameters in Laboratory Animals and Humans. *Pharmaceutical Research, Vol. 10, No. 7, 1993*

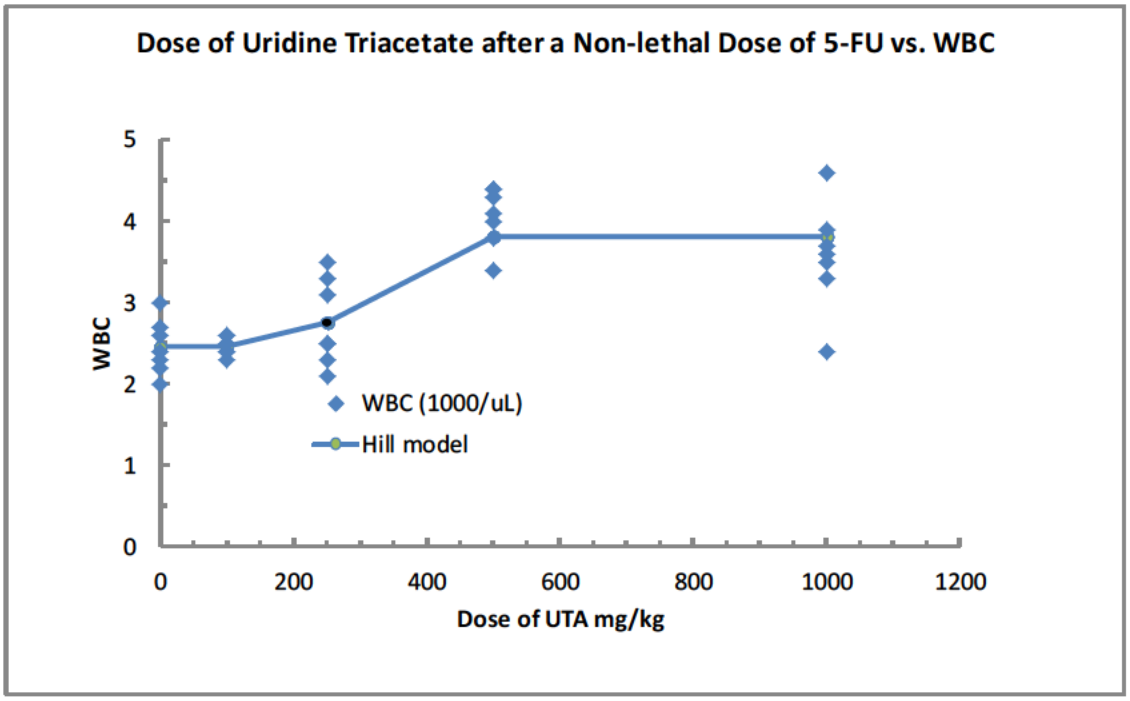
The investigators did not examine bone marrow on day 12. At this time, spleen weight had recovered in all groups except treated controls. The increase in the UTA treatment group is possibly due to a rebound response. WBC was little changed from day 8 except that the control group had recovered to approximately the same values as the antidote treated groups. This was also true of RBCs. Neutrophils in the controls remained low whilst lymphocytes and platelets showed signs of rebound recovery. The control lymphocytes were higher than the antidote treated groups and platelets were higher than the (b) (4) mean. All other parameters remained below the (b) (4) means.

Experiment 2

A second experiment in this study was designed to demonstrate a dose response to the antidote treatment with uridine triacetate. Mice were again treated with 150 mg/kg of 5-FU IP followed two hours later by 100, 250, 500 or 1000 mg/kg of uridine triacetate treatment over the course of three days. Again the results were heteroscedastic and required non-parametric analysis. The following table shows the results of this experiment.

Dose Group	WBC (1000/µL)	Marrow (1000000/mg)	Spleen Wt. (mg)	Neutrophils (1000/µL)	Lymphocytes (1000/µL)	Platelets (1000/µL)	RBCs (1000000/µL)
Vehicle Control	2.4	1.5	57.3	0.02	2.38	270	8.36
100 mg UTA	2.5	1.7	62.4	0	2.45	420	8.23
250 mg UTA	2.5	2.9	60.7	0.02	2.5	608	8.54
500 mg UTA	**4.1**	**3.5**	63	0.03	**4.1**	**760**	8.29
1000 mg UTA	3.6	**4.75**	63.1	0.04	3.46	**667**	8.69

While the median values appear to show a dose effect in WBC, marrow, lymphocytes and platelets, only values in the upper two dose groups reach significance. There is no statistical difference between these two highest dose groups. But, in several cases the values for the 1000 mg/kg uridine triacetate group are actually lower than those of the 500 mg/kg group. The following graph shows the day 8 WBC data and a line generated by non-linear regression against that data.



The data better fit this non-linear regression better than it fit a linear regression or regression against the mean as measured by the sum of squared error. This strongly indicates a dose response to the antidote effects of uridine triacetate. The ED_{50} in this model was about 250 mg/kg and dose of about 500 mg/kg (1500 mg/m^2) showed a maximal response. The human dose is 10 g or about 5500 mg/m^2. This suggests that the human dose may be higher than that needed to achieve the maximum clinical benefit. Nevertheless, the median value for the 1000 mg/kg dose group is lower than that of the 500 mg/kg value. If this difference is real, there is some other phenomenon involved and the curve cannot completely account for the data. And if the difference is real, it suggests that the high dose may be inferior to the lower dose in protecting against 5-FU toxicity. This would suggest the possibility of a bell shaped dose response curve and perhaps minor toxicity from excess uridine. Such toxicity was not evident in the toxicology studies, but those studies were done in healthy animals, not animals dosed with 5-FU.

The experiment also determined the results for these parameters on day 12. Again, control values were about the same as those in the treatment group by day 12, showing that treatment with uridine triacetate does not hasten recovery, but only prevents further damage after it is administered. All values except spleen weight and platelets remained well below the (b) (4) reference means.

Study 2

A second study was designed to show the effects of uridine triacetate on 5-FU pharmacokinetics, mouse survival, and intestinal toxicity in mice treated with ethynyluracil (5-EU) to inhibit the metabolism of 5-FU. The intent was to model the response to 5-FU seen in

patients who express below normal concentrations of dihydropyrimidine dehydrogenase (DPD), the enzyme that metabolizes 5-FU. The study includes five components.

Experiment 1

In experiment 1A, the investigators determined the extent to which ethynyluracil to inhibited 5-FU catabolism to dihydro-5-flurouracil via DPD *in vivo* in a mouse model. They treated mice intraperitoneally with either vehicle or EU one hour prior to treatment with a non-lethal dose of 5-FU. The elimination of 5-FU is biphasic in both cases. Pretreatment with 5-EU significantly increases the plasma concentration of 5-FU. 5-EU pretreatment increased the plasma AUC four-fold, whilst increasing C_{max} about two-fold. 5-EU treatment did not affect T_{max}.

Experiment 1B was designed to show the effect of uridine acetate on uracil plasma uracil concentrations. Like 5-FU, uracil, but not uridine, is a substrate for dihydropyrimidine dehydrogenase (DPD). Excess uridine causes plasma uracil concentrations to increase because the activity of pyrimidine-nucleoside phosphorylase (EC 2.4.2.2) is reversible. In experiment 1B, the investigators treated mice with either vehicle (saline) or 5-EU. They then gave both groups of mice an oral dose of 2000 mg/kg of uridine triacetate and determined plasma uracil as a function of time. The data is too sparse to adequately characterize the kinetics of uracil, but the experiment does show that inhibition of DPD at these doses increases plasma uracil AUC almost two fold and delays its elimination.

Experiment 2:

The investigators designed Experiment 2 to characterize their 5-FU overdose model. They treated mice with a single IP dose of 5-FU of 100, 200 or 300 mg/kg in the absence of EU pretreatment. The following table the increase in AUC with increasing dose. The data is too sparse to calculate half-lives for 5-FU, but H. Yi, et al. have determined it to be about 9 minutes.[4] The increase in AUC is linear with dose, but this linearity is somewhat deceiving as the increases in AUC are much greater than dose proportional. This suggests that plasma DPD is saturated at doses above 100 mg/kg. The shape of the curve at higher doses cannot be determined as higher doses are lethal.

Experiment 3

The investigators designed experiment 3 to evaluate the effects of uridine triacetate in a model of lethal 5-FU overdose in otherwise normal animals by assessing survival and body weight changes. They gave groups of 10 female mice a single IP dose of 300 mg/kg of 5-FU, a known lethal dose. They then gave the groups of mice 2000 mg/kg of oral uridine triacetate three times daily for five days for a total of 15 doses starting at different time intervals from the initial 5-FU dose. Controls received vehicle starting at 24 hours. Treated animal groups received uridine triacetate beginning at 24, 48, 72 or 96 hours. The following table shows survival as a function of the time of antidote administration. Earlier administration of uridine triacetate clearly improves survival.

Dose Group	Mean Survival days	Std Error	Survival at 21 days
Vehicle	21	0	100%
5-FU alone	9.9	0.78	0%
uridine triacetate at 24 hours	20.4	0.6	90%
uridine triacetate at 48 hours	17.2	1.7	60%
uridine triacetate at 72 hours	14.5	1.5	30%
uridine triacetate at 96 hours	14.2	1.2	20%

Animals treated within 24 hours only lost about 15% of their body weight with a nadir at day 14. Animals in all the other treatment groups lost about 30% of their body weight irrespective of when treatment began, again with a nadir around day 14.

Experiment 4

The investigators designed Experiment 4 to demonstrate the effects of uridine in their 5-EU model. They first treated with 2 mg/kg of 5-EU IP. They followed this treatment with a single dose lethal dose of 5-FU. They then treated the animals with uridine triacetate starting at different times, 2, 4, 8, 12, 24, 48, 72, 96, 120 and 144 hours post dosing. The following table shows the effect of treatment on survival. Again, earlier treatment conveyed significantly more benefit.

Group	Mean Survival Time (Days)	Std. Error	% Survival at 25 days
5-FU	25	0.0	100
5-FU + 5-EU	12.5	0.7	0
5-FU + 5-EU uridine triacetate 2 hr	23.8	1.2	90
5-FU + 5-EU uridine triacetate 4 hr	25	0.0	100
5-FU + 5-EU uridine triacetate 8 hr	22.3	1.8	80
5-FU + 5-EU uridine triacetate 12 hr	22.7	1.5	80
5-FU + 5-EU uridine triacetate 24 hr	22.7	1.5	80
5-FU + 5-EU uridine triacetate 48 hr	19.1	1.8	40
5-FU + 5-EU uridine triacetate 72 hr	19.1	2.0	50
5-FU + 5-EU uridine triacetate 96 hr	16	1.7	20
5-FU + 5-EU uridine triacetate 120 hr	14.8	2.2	30
5-FU + 5-EU uridine triacetate 144 hr	10.4	0.2	0

The decrease in mean survival and percent survival both decreased linearly with increasing interval between antidote therapy. All treated animals lost between about 6 and 9 percent of their body weight by day five, then their weight stabilized until about day 9 when a precipitous decrease began reaching a nadir around day 14. After this surviving animals began to steadily recover their body weight. Animals treated 24 hours after 5-FU treatment weighed about 90% of their pretreatment body weight by day 25.

Experiment 5

The investigators did this experiment to evaluate the ability of uridine triacetate to protect the gastric mucosa of mice against the toxic effects of 5-FU. They dosed the mice intraperitoneally with 2 mg/kg of 5-EU, and then two hours later gave the mice 100 mg/kg of 5-FU intraperitoneally as in experiment 4. They then treated the mice with uridine triacetate as in experiment 4 at 2 and 24 hours after the 5-FU dose. They necropsied animals on day 4 and 10 and examined duodenal sections measuring the cross-sectional area of the villi and the total intestinal cross-sectional area. Micrographs demonstrated the deterioration of the intestinal villi with 5-FU treatment and diminished damage in the presence of uridine triacetate. All treatment groups had greater villus area than the treatment control (5-FU plus 5-EU without uridine triacetate). The following table presents these the mean villus area for each treatment group on day 4 and day 10.

	Day 4						Day 10				
Treatment Group	N	Mean	Std Error	Significance			N	Mean	Std Error	Significance	
5-FU Plus UTA control	9	0.88	0.05	A							
5-FU plus 5-EU plus UTA at 2 hours	14	0.79	0.04	A	B		15	0.60	0.03	A	
5-FU plus 5-EU plus UTA at 24 hours	14	0.77	0.04	A	B		9	0.54	0.04	A	B
Saline Control	15	0.73	0.04	A	B		9	0.64	0.04	A	
5-FU plus Vehicle	15	0.69	0.04		B						
5-FU plus 5-EU no UTA (treatment control)	13	**0.49**	0.04			C	18	**0.46**	0.03		B

Treatment groups not connected by the same letter are significantly different from each other. The value in bold red is the minimum for the series.

The results of the ANOVA show that on day 4, all groups have a larger villus area than the treatment control. The groups treated at 2 or 24 hours with uridine triacetate are statistically the same as vehicle control demonstrating protection of the intestinal villi from progressive damage due to 5-FU exposure. Oddly the villus area on day 10 is less than on day on day 4 in all groups. Some other factor must be involved so that this parameter does not demonstrate recovery. The following table shows the results for total intestinal cross sectional area.

	Day 4						Day 10				
Treatment Group	N	Mean	Std Error	Significance			N	Mean	Std Error	Significance	
5-FU Plus UTA control	9	1.91	0.09	A							
5-FU plus 5-EU plus UTA at 2 hours	14	1.73	0.07	A	B		15	1.24	0.07	A	
5-FU plus 5-EU plus UTA at 24 hours	14	1.57	0.07		B	C	9	1.19	0.08	A	
Saline Control	15	1.49	0.07			C	9	1.30	0.08	A	
5-FU plus Vehicle	15	1.70	0.07	A	B						
5-FU plus 5-EU no UTA (treatment control)	13	**1.43**	0.08			C	18	**1.07**	0.06	A	

Treatment groups not connected by the same letter are significantly different from each other. The value in bold red is the minimum for the series.

Again, all groups are different form the treatment control, but here groups treated with uridine triacetate at 2 or 24 hours are statistically the same as the 5-FU plus Vehicle control. Again, the total intestinal area on day 10 is less than that on day 4 for all groups. Some uncontrolled factor in the experiment is influencing these factors. Thus, in this last experiment, the best evidence for a treatment effect by uridine triacetate is the increased villus area at two and 24 hours relative to the treatment control.

Study 3

In a final study, investigators examined the antitumor effect of 5-Fluorouracil with and without Uridine or Uridine Triacetate in the CD8F1 Murine Mammary Carcinoma System. The study was poorly controlled and Wellstat did not provide the study data. In this experiment, treatment with 5-FU in combination with molar equivalent oral doses of uridine or uridine triacetate both slowed tumor growth as measured by tumor weight. Because of the lack of controls no other conclusions can be drawn from this experiment.

Conclusion

The toxicology studies previously reviewed under NDA 208169 are adequate to support the indication under NDA 208159. Based on available data, there are no signification toxicology concerns with the use of uridine triacetate for the proposed indication.

Though the animal efficacy experiments are poorly designed and in some places poorly controlled and missing data the total body of evidence indicates that uridine triacetate prevents further damage from high exposures to 5-FU once it is administered. Uridine triacetate treatment does not appear to significantly hasten recovery. (b) (4) hese animal efficacy studies support the evidence of clinical efficacy in this treatment setting.

W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T.

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/s/

WILLIAM D MCGUINN
12/01/2015

TODD R PALMBY
12/01/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA/BLA Number: 208159 **Applicant: Wellstat Therapeutics Corp** **Stamp Date: July 10, 2015**

Drug Name: VISTOGARD (Uridine Triacetate) **NDA Type: 505 b1**

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

	Content Parameter	Yes	No	Comment
1	s the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	S		
2	s the pharmacology/toxicology section ndexed and paginated in a manner allowing substantive review to begin?	S		
3	s the pharmacology/toxicology section egible so that substantive review can begin?	S		
4	Are all required and requested IND studies in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	S		
5	f the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route ntentionally and by desire of the FDA).	S		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	S		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?		S	The pharmacology study reports submitted to support the efficacy of uridine triacetate do not contain a statement that these studies were conducted in accordance with GLP or an explanation of deviations;

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
				however, the Division previously agreed that these studies may be acceptable and agreed to review them as part of the NDA submission. Therefore, the adequacy of the pharmacology studies and the resulting data will be determined during the review of the NDA.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	S		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed n either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		S	The units are not correct but can be easily corrected. The label will be revised during the review cycle.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	S		
11	f this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable
12	f the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

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

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

The following request for information should be sent to the Applicant.

"Please submit all individual animal data from each of the Primary Pharmacology studies you have submitted in support of the efficacy of Uridine Triacetate in animals (Study # R.401.14.01 and Study # R.401.14.03)."

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/s/

WILLIAM D MCGUINN
08/17/2015

TODD R PALMBY
08/18/2015