CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208169Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 208169	Submission Date(s): 01/08/2015, 04/10/2015, 04/22/2015, 05/04/2015, 05/11/2015, 05/29/2015
Brand Name	Xuriden
Generic Name	Uridine Triacetate (PN401)
Reviewer	Sandhya Apparaju
Team Leader	Sue Chih Lee
OCP Division	DCP3
OND Division	DGIEP
Sponsor	WellStat Therapeutics
Relevant IND(s)	118,931
Submission Type	NME Original-Priority
Formulation; Strength(s)	Immediate release granules in 2 g, (b) (4)
Indication	Uridine replacement therapy (b) (4) with hereditary orotic aciduria (HOA)

NDA 208169 was discussed within OCP on June 02, 2015 from 11.30- 12.30 PM in White Oak, Building 51, and conference room 3300. Attendees were Drs. Issam Zineh, Shiew Mei Huang, Edward D. Bashaw, Hae Young Ahn, Lei Zhang, Brian Booth, Pengfei Song, Sue Chih Lee, Insook Kim, Dilara Jappar, Elizabeth Shang, Sarah Dorff, Mike Pacanowski, Christian Grimstein, Carla Epps and Sandhya Apparaju.

Table of Contents

1 Executive Summary 2
1.1 Recommendation 2
1.2 Phase IV Commitments 2
1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings 2
2 Summary of CPB Findings 4
2.1 General Attributes of the Drug 4
2.2 General Clinical Pharmacology 7
2.3 Intrinsic Factors 22
2.4 Extrinsic Factors 22
2.5 General Biopharmaceutics 23
2.6 Analytical Section 25

Reference ID: 3774715

1 Executive Summary

1.1 Recommendation

NDA 208169, Xuriden (uridine triacetate granules) is acceptable from a Clinical Pharmacology perspective.

1.2 Phase IV Commitments

None

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Proposed indication and dose: Xuriden (uridine triacetate) granules for oral administration are intended for the treatment of hereditary orotic aciduria (HOA), a rare genetic disorder. The proposed starting dose is 60 mg/kg/day, administered once daily up to a maximum dose of (b) (4) mg/kg/day (8 g maximum dose). (b) (4)

- Levels of orotic acid in urine remain above normal or increase above the usual or expected range for the patient
- Signs or symptoms (b) (4) worsening
- Laboratory values (e.g., red blood cell or white blood cell indices) affected by HOA show evidence of worsening

Dose selection- Relative bioavailability: uridine triacetate vs. uridine

The relative bioavailability of plasma uridine from uridine triacetate is approximately 4-fold on an equiweight (mg/kg basis) and 6-fold higher on an equimolar basis compared to that from oral uridine (uridine is not an FDA approved therapy but is available to patients via investigator INDs). This is attributed to the (b) (4) on the pro-drug, as well as less first-pass degradation of uridine from the pro-drug to uracil. Typical uridine dose in HOA is ~ 200 mg/kg/day. Based on the relative BA information, a starting dose of 60 mg/kg/day (translates to a conversion factor of ~ 3 for most patients on prior uridine therapy) of Xuriden was identified as a conservative means of achieving comparable systemic exposure of uridine from the pro-drug upon switching from oral uridine, while avoiding under-dosing.

Clinical data
Efficacy: In support of the current indication for Xuriden, the sponsor conducted one pivotal trial in n = 4 U.S., HOA patients (b) (6) years of age. Urinary orotic acid and patient-specific hematological endpoint remained stable in patients

Reference ID: 3774715

switching over from oral uridine to Xuriden. In a treatment naïve patient, hematological endpoint showed no evidence of worsening, if not improvement. All patients demonstrated evidence of systemic uridine delivery with the oral administration of the prodrug, and had urinary orotic acid levels that remained within or close to normal range. Patients' growth showed improvement during the 42 days main study and during the extension phase. Data suggests that uridine triacetate provides a reasonable means of oral uridine replacement therapy in patients with HOA.

Safety: At the doses administered, uridine triacetate appears to have been well tolerated in HOA patients. No serious adverse events occurred during the trial. Please see the medical officer's review for an in depth assessment of safety of uridine triacetate in HOA.

Drug-drug interaction potential: In vitro data suggests that uridine triacetate or uridine do not inhibit or induce major CYP450 enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A). The potential for uridine triacetate to act as a *p-gp* inhibitor at the gut level cannot be ruled out.

Food-effect on PK: Food did not impact the pharmacokinetics of uridine from a slightly different uridine triacetate granule formulation. While food-effect PK data from the to-be-marketed formulation is not available, at present there is no plan to request a new study in this regard as drug has a wide safety margin and is titrated to effect. In addition both formulations are primarily comprised of active ingredient (> 90 %). Thus Xuriden can be dosed (b) (4).

Reference ID: 3774715

2 Summary of CPB Findings

2.1 General Attributes of the Drug

What pertinent regulatory background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?

Uridine for oral replacement therapy in hereditary orotic aciduria is not FDA approved, but has been allowed under compassionate use INDs. Uridine was supplied by Repligen Corporation, which plans to not manufacture this drug anymore.

At the request of FDA, including the Office of Drug Evaluation III, Division of Gastroenterology and Inborn Errors Products, Office of Orphan Products Development, Office of Drug Shortages and the Office of Rare Diseases (22 March 2013 teleconference and 7 August 2013 pre-IND meeting), Wellstat developed uridine triacetate (company code PN401; 2', 3', 5'-tri-O-acetyluridine) as uridine replacement therapy in pediatric patients with hereditary orotic aciduria (HOA).

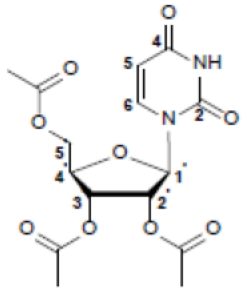
FDA granted Orphan Drug Designation for uridine triacetate in the treatment of hereditary orotic aciduria (#13-4010) on August 9, 2013 as well as designation of uridine triacetate as a Potential New Drug for a Rare Pediatric Disease (#13-4010V) for the treatment of hereditary orotic aciduria on August 9, 2013. On April 30, 2014 FDA granted Breakthrough Therapy Designation for uridine triacetate for uridine replacement in pediatric patients with hereditary orotic aciduria, thus resulting in a priority review.

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Chemically, uridine triacetate is 2',3',5'-tri-O-acetyluridine and has the empirical formula $C_{15}H_{18}N_2O_9$ and a molecular weight of 370.31 g/mole. The drug substance is a white to off-white solid. It is slightly to sparingly soluble in water. The solubility of uridine triacetate is unaffected by changes in pH.

The structure of uridine triacetate is as shown:

Reference ID: 3774715



The drug product "Uridine Triacetate Oral Granules" is composed of coated granules containing 95.08% uridine triacetate (the active substance) and inactive ingredients claimed to improve the palatability of the otherwise bitter drug substance. The composition of uridine triacetate granules is given below:

Component	Percent w/w	Function
Uridine Triacetate	95.08	Active
Opadry® (b) (4) (b) (4) (b) (4) Clear (b	(b) (4)	(b) (4)
(b) (4)		
Natural Orange Juice Flavor S.D. #80618	(b) (4)	Flavor
(b) (4)		

The drug product is packaged in individual (b) (4) sachets containing 2 g or (b) (4) of uridine triacetate.

2.1.2. What are the proposed mechanism(s) of action and therapeutic indication(s)?

Indication: Xuriden (Uridine triacetate granules) is proposed for (b) (4) pyrimidine (b) (4) uridine, for replacement therapy in patients with hereditary orotic aciduria (HOA).

Disease: HOA is a rare congenital autosomal recessive disorder, with about 20 documented cases worldwide and four recognized cases in the U.S. It is caused by mutations reducing the activity of uridine monophosphate synthase (UMPS). UMPS is a bifunctional enzyme with two main domains (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase) and catalyzes the formation of uridine monophosphate (UMP), an energy-carrying molecule in many important biosynthetic pathways. Specifically, the orotate phosphoribosyl transferase catalyzes the conversion of orotic acid to orotate monophosphate, OMP while the orotidine 5`-decarboxylase catalyzes the conversion of OMP to uridine monophosphate, UMP. UMP is the fundamental precursor of all other pyrimidine nucleotides. Consequently patients with HOA cannot make

Reference ID: 3774715

uridine normally, and have orotic acid and orotidine accumulation (orotidine is formed by coupling of orotic acid to ribose prior to conversion to OMP). In addition, as orotic acid biosynthesis is normally subject to feedback inhibition by intracellular nucleotides, orotic acid is also overproduced in HOA. Thus the accumulated orotic acid, which is very poorly soluble is excreted in urine (hence the name of the disease), causing crystalluria and obstructive uropathy. The clinical consequences of HOA involve hematological abnormalities, developmental delays, failure to thrive, and if left untreated, early death. The type of clinical features in HOA may vary between patients and depend on duration and degree of disease. Because early intervention is important, and therapy is likely to be life-long, the patient population includes pediatric as well as adult patients.

Mechanism of action: Uridine replacement therapy in HOA provides plasma uridine, which then is available to enter cells and tissues (Uridine + ATP + Uridine-cytidine Kinase → UMP + ADP). Treatment restores intracellular uridine nucleotide concentrations, enabling the biosynthetic functions of pyrimidine nucleotides and their derivatives. It also suppresses orotic acid overproduction by inhibiting carbamoyl phosphate synthetase 2, the enzyme involved in the first step in pyrimidine nucleotide synthesis, reducing its urinary excretion and thereby renal consequences. For years, uridine therapy is reportedly used as the compensatory treatment for HOA based on published case reports, with apparent clinical benefit permitting relatively normal life if started early in life, at sufficient doses.

Rationale for the pro-drug: Uridine triacetate (MW 370 g/mole) is an orally bioavailable, acetylated prodrug of uridine (MW 244 g/mole). Oral uridine undergoes rapid first-pass catabolism by uridine phosphorylase in the intestine and liver to form uracil and therefore is poorly bioavailable (~ 6-10 %; Van Groeningen et al, 1991). (b) (4) The higher bioavailability of uridine from oral uridine triacetate is also attributed to the lower first pass degradation to uracil.

Rationale for uridine dose (as proposed by the sponsor): In adults, de novo synthesis has been estimated at 4 to 6 millimoles of orotic acid per day (Bono et al., 1964) or 450 to 700 mg of orotic acid per day (Smith, 1973; Weissman et al., 1962). Orotic acid is quantitatively converted to UMP, with no other metabolic fate. A quantity of 450 to 700 mg of orotic acid (MW 156) corresponds to 700 to 1100 mg of uridine (MW 244). In a 60 kg adult this translates to at least 12 to 18 mg/kg of systemic uridine per day to provide sufficient intracellular UMP to satisfy normal turnover and maintenance of pyrimidine pools. The oral bioavailability of uridine has been measured at 6.9 to 10% in humans (van Groeningen et al., 1991). Delivery of 12 to18 mg/kg/day uridine systemically would therefore require oral administration of uridine at a minimum range from 120 mg/kg/day to 270 mg/kg/day. This dose range of oral uridine in the clinical setting has been reported to show improvements in orotic aciduria, hematologic parameters and failure to thrive. As uridine triacetate provides more bioavailable uridine than does oral uridine itself, by a factor of approximately 4 to 6 (Report No. 401.09.001-PK), on the basis of previous HOA patient experience and from estimates of daily de novo pyrimidine synthesis, a starting dose of 60 mg/kg/day uridine triacetate was determined for uridine replacement

Reference ID: 3774715

therapy in HOA, anticipating that this dose would deliver at least as much systemic uridine as an oral dose of 200 mg/kg uridine and would deliver at least as much systemic uridine as is produced by de novo synthesis in normal subjects.

2.1.3. What are the proposed dosage(s) and route(s) of administration?

Uridine Triacetate Granules (Xuriden) are indicated for oral administration in the treatment of HOA. The recommended starting dose is 60 mg/kg/day. The sponsor proposes that dose can be increased (b) (4) to a maximum of (b) (4) mg/kg/day (not to exceed 8 grams per day) for insufficient efficacy as shown by any of the following:

- Levels of orotic acid in urine remain above normal or increase above the usual or expected range for the patient
- Signs or symptoms (b) (4) of worsening
- Laboratory values (e.g., red blood cell or white blood cell indices) affected by HOA show evidence of worsening

(b) (4)

Uridine Triacetate Oral Granules are intended to be mixed with foods that are easily swallowed, such as applesauce, yogurt or pudding immediately prior to administration. Drug is proposed to be given once daily.

The proposed labeling includes body weight-based dosing tables for the 60, 120, (b) (4) and (b) (4) mg/kg/day dose levels to customize daily dosing. Dosing unit is in fractions or

(b) (4)

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

Reference ID: 3774715

Study 401.13.001: The primary component of this submission is a single, pivotal, clinical efficacy and safety trial (401.13.001) in HOA patients (IND 118931). This was an open-label trial in all four known HOA patients in U.S. ages (b) (6) and (b) (6) years, respectively at study initiation. The first three patients were on their regular dose of uridine (150 or 200 mg/kg/day), while the youngest patient was treatment naïve. Two of the three patients with prior oral uridine experience were on a once-daily uridine regimen. Subject (b) (6) was on a t.i.d. regimen of uridine, but for the purposes of baseline PK assessments, (b) (6) total daily dose was administered as a single dose in the morning on day 0. Two patients ((b) (6), and (b) (6) at site (b) (6) had genetically confirmed HOA, while two patients ((b) (6) and (b) (6) at site (b) (6) were not genetically confirmed to have HOA at the time of this review but were enrolled based on signs and symptoms of HOA including macrocytosis, high urinary orotic acid concentrations and failure to thrive. Patient (b) (6) was newly diagnosed and was treatment naïve at study entry.

The trial consisted of a main study period (6 weeks) during which patients were dosed with 60 mg/kg/day uridine triacetate granules. Pharmacokinetic (PK) assessments for plasma uridine were obtained over 8 hours at baseline (day 0 on uridine), and on day 1 and day 28 after switching to uridine triacetate. Urinary orotic acid and orotidine were evaluated as pharmacodynamic (PD) endpoints on day 0, day 1, day 28 and day 42. Since, in addition to orotic acid, orotidine may also accumulate in HOA, it was also measured in this trial. However, there was no historic documentation of orotidine levels from these patients. During the study, orotidine levels in patients remained within normal range (after switching from uridine). Clinical endpoints included patient-specific hematological endpoint of interest, and growth (height and weight) information. These were collected at baseline and during treatment. After the 42-day main study, patients continued to be dosed under the treatment extension period. The two patients at site (b) (6) received 120 mg/kg/day dose during the extension phase. Additional PK, PD and hematological/growth data were obtained at this dose level. As per information provided in May 2015 (120 day safety update), one patient (b) (6)) from site (b) (6) received dose increase from 60 to 100 mg/kg/day. PD, hematology, and clinical information is available from this patient.

In vitro DDI studies: Sponsor also submitted results from in vitro drug-drug interaction studies for evaluating CYP450 inhibition and induction potential and p-glycoprotein interaction potential for uridine triacetate and uridine.

Additional clinical pharmacology and biopharmaceutics information was reviewed from studies conducted with uridine triacetate under different INDs:

Study PN401.09.001-PK: This was an open label, phase II trial for patients with pyrimidine responsive syndrome of neurological dysfunction and susceptibility to infection. Uridine triacetate (known as Vistonuridine at the time) was supplied in this study as non-scored, caplet shaped 500 mg tablet, and was available as a powder. The powder was to be mixed with a non-citrus liquid. Each patient initially received orally administered uridine (333 mg/kg/dose given 3 times daily (tid) for a total dose of 1000 mg/kg on the first day of treatment (Day 0). On Day 1, each patient then started daily treatment with oral uridine triacetate at 33 mg/kg tid (100 mg/kg/day). The dosage of

Reference ID: 3774715

uridine triacetate was periodically increased in these patients over ~1.5 years to 100 mg/kg tid (300 mg/kg/day). Serial plasma samples for up to 8 hours post-dosing were taken after the first daily dosage.

Study PN401.07.002: This was an open-Label, randomized, single-Dose, 2-way crossover study to evaluate the effect of food on uridine pharmacokinetics and relative bioavailability in healthy volunteers. A single 6 g dose of uridine triacetate granule formulation ((b) (4) % active) was administered under fasted or fed conditions. For fed conditions, following overnight fasting, subjects received a standardized high fat/high calorie meal 30 minutes prior to dose. Drug product was sprinkled on ½ cup of apple sauce and immediately administered to subject. This was followed by water.

Publications: Finally, a compilation of published case reports of historic uridine usage in HOA patients was submitted by the sponsor on April-10, 2015 which helped to understand the clinical features of HOA, doses of uridine historically used, hematologic, and pharmacodynamic endpoints, response to treatment, and their inter-relationship if available.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

The primary efficacy endpoints in the trial are hematologic in nature and individualized for each patient. Secondary endpoints are urinary orotic acid (PD) and plasma uridine concentrations (PK). For patients who were on oral uridine at study entry, stability of their principal hematological parameter of concern following the switch to oral uridine triacetate was considered the primary efficacy endpoint. For the treatment naïve patient, improvement and stability of their principle hematological endpoint at day 28 and day 42 relative to baseline was evaluated instead.

The criteria for stability were as follows:

• If the baseline (Day 0) principal hematologic parameter was within the laboratory reference range (LRR) and remained within the LRR at Days 28 and 42 of treatment with uridine triacetate, the response was considered stable.

• If the baseline principal hematologic parameter was outside the LRR and improved or worsened no more than 15% to 30% when compared to Day 0 at Day 28 and Day 42, the response was considered stable.

The following principal hematological endpoints were identified for each patient in the pivotal HOA clinical trial:

Reference ID: 3774715

(b) (6)	
	Neutrophils
	White blood cell count
	Mean corpuscular volume
	Mean corpuscular volume

In addition, growth (weight and height) was followed during treatment.

PK and PD: As this is intended to be a replacement therapy for uridine, bioavailability of systemic uridine was confirmed through plasma sampling and pharmacokinetics following oral administration of uridine at baseline and uridine triacetate from day 1 of therapy. Since, HOA occurs as a result of mutations in the enzyme (UMPS) that converts orotic acid to UMP, increased orotic acid excretion in the urine is a natural consequence of the disease and a known disease diagnostic. It is also of clinical concern due to its potential to cause crystalluria and obstructive uropathy. Hence treatment that reduces urinary orotic acid excretion is also of interest in HOA therapy. Extent of urinary orotic acid can also serve as an indicator of therapeutic benefit. When systemic availability of uridine through exogenous dosing restores intracellular nucleotides, it would also suppress the overproduction of orotic acid by feedback inhibition. Thus, urinary orotic acid levels were measured as the pharmacodynamic endpoint in this trial.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Plasma uridine (PK) was determined using validated HPLC method. Urinary orotic acid (PD) concentrations were determined using validated electrospray tandem mass spectrometric methods. An assay for uridine triacetate (prodrug) was not validated by the sponsor claiming instability of the drug substance in biological fluids. This was based on feasibility studies (60 minute incubations in plasma or whole blood at various temperatures) that suggested rapid degradation of the drug. Please refer to the bioanalytical section of this review for additional information.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

In the pivotal HOA trial 401.13.001, three of the four patients were already on oral uridine and therefore stability of their specific hematological endpoint and urinary orotic acid values after switching to uridine triacetate were of primary interest. Thus, their PD

Reference ID: 3774715

information for the most part while demonstrated stability of response after switching the source of uridine, doesn't provide further information on time to clinical benefit.

Patient (b) (6), whose clinical feature of interest was low neutrophils (%) prior to treatment, however appears to demonstrate a greater improvement on uridine triacetate suggesting that this patient may have needed higher exposure of uridine than (b) (6) was receiving from his current oral uridine dose of ~ 200 mg/kg/day at the time. Neutrophils (%) in this patient, improved over baseline by 47 % during the 6 month extension period. In addition, this patient continued to gain height and weight with treatment. While in other patients the hematological endpoints remained stable after switching to the pro-drug, there was no worsening either. No time to onset of clinical benefit or resolution can be obtained from this information, although (b) (6) and (b) (6) demonstrated increase in their height and weight during the 6 month extension phase when they received an increased dose of 120 mg/kg/day. Doubling of dose in these patients doubled their exposure (AUC) but provided no further hematological or PD benefit.

Information regarding clinical features of HOA disease, doses of uridine and time to onset of clinical benefit with treatment etc., can be gleaned from 14 published case studies of oral uridine therapy in HOA. Overall, published data suggests that the use of oral therapy to deliver uridine to plasma of HOA patients restores their hematological endpoints into normal ranges, thus providing evidence of clinical benefit. Limited information available from these case studies also suggests long-term beneficial effects in terms of overall growth and development and potential for a normal life in patients who continue to take uridine into adulthood. High pre-treatment urinary orotic acid (UOA) levels were noted which were reduced at post-treatment. In addition, with a reduction in dose, worsening of hematological outcomes occurred and in parallel, an increase in the urinary orotic acid levels also was seen, supporting the use of UOA as a PD endpoint. While data available data is small, there appears to be rapid improvement in hematological endpoints within 1 to 4 weeks (Becroft and Phillips, 1965; Hagarrd and Lockhart, 1967; Rogers et al, 1968; Bensen et al, 1991; Tubergen et al, 1969; Fox et al, 1969; Fox et al, 1973) and PD outcome within ~1 week upon initiation of uridine treatment:

Disease characteristics: 14 published cases had information on uridine dose, pre- and post-treatment urinary orotic acid excretion (PD), hematological and clinical (growth) endpoints. Anemia (low hemoglobin; 13/14 cases) and leukopenia (low WBCs; 8/14 cases) appear to be the usually noted hematological abnormalities.
Urinary orotic acid (UOA) excretion was higher at pre-treatment (data from 9/14 patients), with some also showing symptoms such as crystalluria, hematuria, ureter obstruction etc. n = 5 patients did not have quantitative UOA information, but had reported symptoms such as crystalluria, and obstructive uropathy.

Treatment: Oral uridine dose generally ranged from 75 to 300 mg/kg/day, with most patients receiving 150 mg/kg/d.

Reference ID: 3774715

Clinical findings: Anemia typically resolved within 1 to 4 weeks after treatment initiation (time to resolution was available in n = 6 cases; anemia was also noted as resolved in 5 more patients, but time to resolution was unavailable). Data in one patient suggested worsening of hemoglobin within 3 weeks after stopping uridine therapy and was accompanied by increased urinary orotic acid. No post-treatment hemoglobin data was available in n = 3 patients. WBC count was restored to normal in n = 5 patients, although the time to normalization was not noted. In one case, resolution was descriptively noted as 'rapid'. WBC count was 'unresolved' or 'below normal' in n = 3 patients. WBC count was unavailable at post-treatment in n = 6 patients.

PD findings: Relative to pre-treatment, the urinary orotic acid levels were lower in n = 8 patients after initiation of uridine therapy, but time to resolution was not noted in these cases. In one patient, it was noted that urinary orotic acid levels 'decreased within one week' after restoring uridine dose to 150 mg/kg/d after a dose-reduction, suggesting a rapid effect of treatment on this PD endpoint (Becroft et al, 1969). No post-treatment UOA excretion data were available in n = 6 patients. The units for urinary orotic acid excretion varied across publications, but mostly it was reported in mg/day (in 6/8 cases with reported UOA).

Relationship between dose, PD and clinical endpoints: Post-treatment UOA levels ranging 23 - < 300 mg/day were associated with positive response in terms of improvements in hematological outcomes (Hb, WBC). In patients (n = 2), where multiple dose levels of uridine were attempted there appeared to be a dose-response trend with respect to urinary orotic acid excretion (decreasing UOA was noted with increasing dose; Becroft and Phillips, 1965, Becroft et al, 1969) and with respect to resolution of anemia/leukopenia (hemoglobin increase and leucocytes within normal range occurred with increasing doses; Suchi et al, 1997; Yazaki et al, 1987). However, with the exception of one patient who demonstrated no reported hematological improvement with post-dose UOA levels at 740 mg/d, there is not enough UOA data in patients or at dose levels of uridine that failed to provide adequate therapeutic response. Hence, quantitative correlation between PD endpoint and clinical outcomes was not possible with this limited information.

Developmental outcomes: Increase in BW percentile was noted within 6- 8 months of oral uridine therapy (data from n = 3). In 2 patients, growth/developmental issues remained unresolved. In n = 9 patients, specific growth information was unavailable. However, descriptions of positive treatment outcomes were noted in several patients at follow-up, years after treatment initiation. One patient was reportedly able to walk unaided (after initially unable to crawl or walk at diagnosis/Treatment initiation) after 9 months of treatment with uridine. One patient remained 'seizure-free' on uridine for 8 years at last follow up. One patient did not need frequent transfusions to combat anemia.

Safety and efficacy of chronic use: N= 3 patients (one male, two females) were noted to have become parents to children without HOA and continue to be on uridine therapy in adulthood. Limited data also suggests that in pregnant HOA patients, substantial

Reference ID: 3774715

increases to uridine dose occurred (up to ~ 24 g/day; titrated to avoid anemia) and was reportedly well tolerated.

2.2.4.2 Does this drug prolong the QT or QTc interval?

Sponsor submitted a QT study-waiver request, which was granted by the QT-IRT team (see review in DARRTs dated 12/17/2014).

2.2.4.3 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

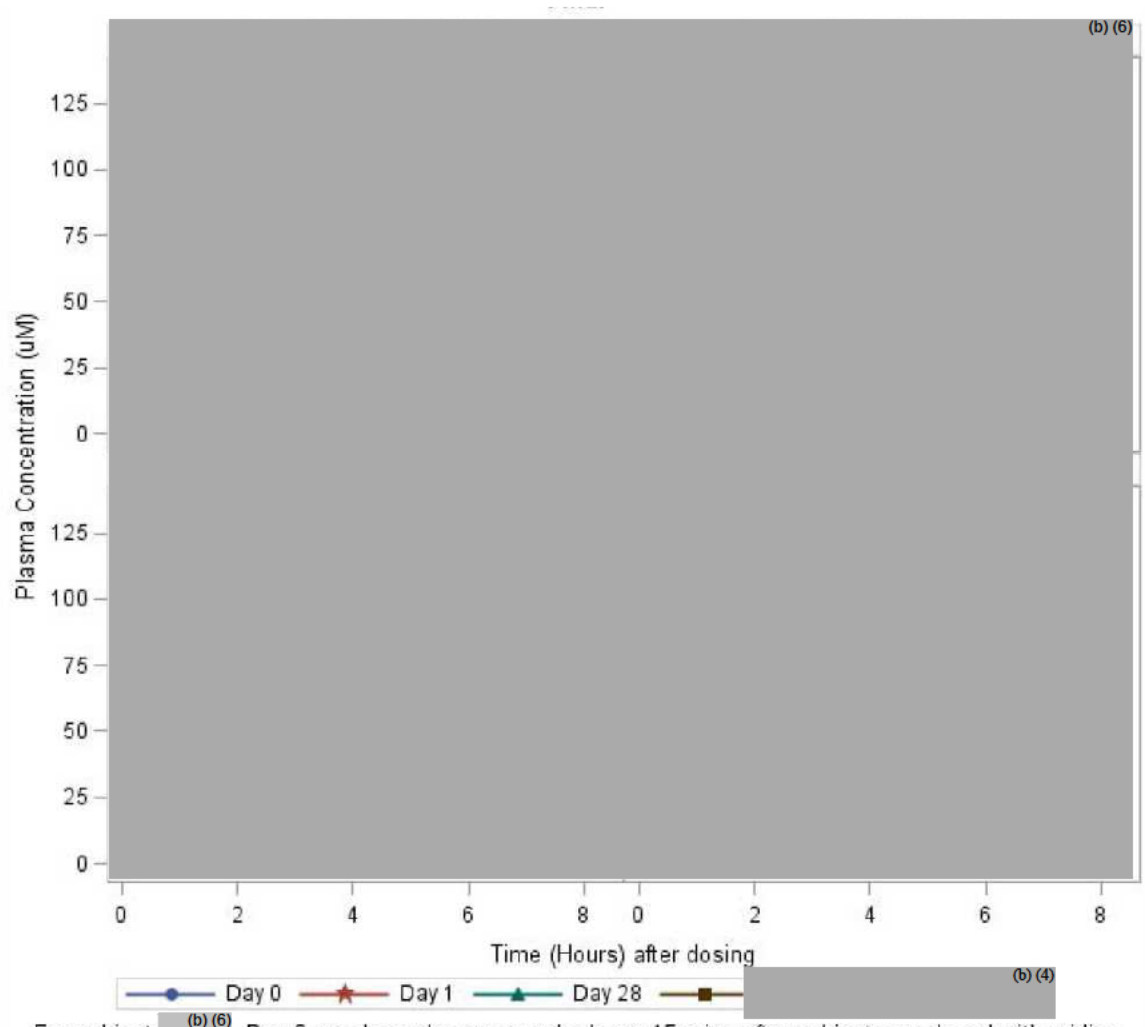
The proposed dose and dosing regimen are acceptable. The starting dose of the pro-drug is approximately 1/3rd the typical (historic) dose of oral uridine (on mg/kg basis), owing to the higher bioavailability of uridine from the prodrug as previously established. Switching from oral uridine to uridine triacetate was able to deliver bioavailable uridine as evidenced by PK data, maintain hematological and PD endpoints at the patient's baseline (on oral uridine) levels in the patients of the pivotal HOA trial. The dosing regimen used in this trial for the acetylated pro-drug was once-daily and the drug will be labeled as such. While oral uridine itself was given in multiple daily doses (primarily to handle the large doses required, as well as to reduce incidence of osmotic diarrhea), with the overall lower dose load for the pro-drug and low incidence of diarrhea, a once-daily dose appears to be adequate. Although the plasma levels decline to baseline by 8 h after once-daily dosing due to short half-life of ~ 1.5 to 2 h, uridine that is taken up and trapped intracellularly is primarily responsible for restoring pyrimidine nucleotides and their derivatives, and alleviating disease symptoms. There are no unresolved dosing issues from a Clinical Pharmacology perspective with the proposed starting dose, dose range, and frequency of uridine triacetate.

2.2.5 What are the PK characteristics of the drug and its major metabolite?

<u>Pharmacokinetics in HOA patients</u>: Uridine concentrations were measured in the four patients enrolled into the pivotal HOA trial 401.13.001, all of whom received approximately 60 mg/kg/day once daily doses during the main study (6 weeks). Plasma uridine was assessed at baseline (day 0) in n = 3 patients who were receiving oral uridine (150 or ~200 mg/kg/day) at study entry. Plasma uridine following oral uridine triacetate was assessed in all four patients on day 1, and on day 28. Additional PK data was obtained from [redacted] subjects who received dose escalation to 120 mg/kg/day during the extension phase.

Plasma uridine concentration (μM)-time profiles are shown for each of the 4 patients:

Reference ID: 3774715



For subject (b) (6), Day 0 pre-dose plasma sample drawn 15 mins after subject was dosed with uridine

Starting dose of uridine triacetate was selected to account for higher bioavailability of uridine from the pro-drug as noted in previous PK studies (see PK information from pediatric patients with mitochondrial disease patients). The emphasis was primarily to avoid under-dosing. A dose of 60 mg/kg/day (~ 1/3rd the daily dose of uridine) was expected to provide systemic uridine concentrations at least comparable to those noted on oral uridine, if not greater.

PK following uridine triacetate in individual patients are shown in the table below from reviewer calculations:

Baseline (uridine)	(b) (6)	(b) (6)	(b) (6)	(b) (6)
Cmax (µM)	49.14	44	75	NA
Tmax (h)	2	1	4	NA
AUCall (µM.h)	171	134	430	NA
Thalf (h)	1.18	1.3	NA	NA

Reference ID: 3774715

Day 1 (Xuriden)	(b) (6)	(b) (6)	(b) (6)	(b) (6)
Cmax (µM)	91	137	69	69
Tmax (h)	2	2	2	1
AUCall (µM.h)	301	547	270	185
Thalf (h)	1.06	2.01	2.03	1.18
Day 28 (Xuriden)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Cmax (µM)	119	126	33	77
Tmax (h)	1	1	2.5	1.5
AUCall (µM.h)	344	480	169	175
Thalf (h)	1.11	1.99	4.65	1.59

Following oral administration of uridine triacetate at 60 mg/kg/day, two patients demonstrated higher plasma uridine exposure ((b) (6), (b) (6) compared to their baseline PK on oral uridine at ~ 200 mg/kg/day, while one patient (b) (6) had lower exposure to uridine after uridine triacetate compared to their baseline levels on oral uridine. Inexplicably, the baseline $AUC_{0\text{-}8\ h}$ on uridine therapy was highest in patient (b) (6) (at uridine dose of 150 mg/kg/day), compared to subjects in site who were receiving a comparable mg/kg/day [200 mg/kg/day] total dose of uridine.

In addition, despite receiving comparable or higher total daily doses, patients (b) (6) and (b) (6) in study site had lower systemic exposure of uridine, after the starting dose of ~60 mg/kg/day, compared to patients in site . While this could be due to normal variability in a small population, other factors such as dosing and sampling errors were considered. Dosing was standardized by the use of digital weighing spoon in all patients. In the treatment-naïve patient (b) (6), the uridine levels fell to baseline sooner, compared to patients who were on uridine therapy, thus resulting in overall lower AUC. Sponsor attributes this to potentially uridine-starved cells taking up uridine more rapidly. Protocol deviations with respect to PK sampling times were noted on day 28 for subjects at site All PK samples from 1 to 8 h post-dose were delayed by 30 minutes. PK was calculated using the appropriate time points. The delayed sampling may explain some of the PK differences as the peak may have been underestimated on day 28 in patients (b) (6) and (b) (6).

The average relative bioavailability based on $AUC_{0\text{-}8}$ was 3.6-fold on an equiweight (mg/kg) basis, and 5.5-fold on an equimolar basis. The treatment-naïve patient (b) (6) demonstrated evidence of systemic delivery of uridine from Xuriden. Doubling of the dose during the extension phase in two patients (b) (6), (b) (6)) approximately doubled their AUC. Accumulation of uridine was minimal following once-daily administration in individual patients.

Mean plasma uridine PK parameters are provided below:

Reference ID: 3774715

	Baseline (Day 0); n = 3 [150-200 mg/kg/d Uridine]	Day 1 n = 4 [60 mg/kg/d Xuriden]	Day 28 n = 4 [60 mg/kg/d Xuriden]	Extension phase n = 2 [120 mg/kg/d Xuriden]
Cmax (µM)	56 (16.6)	91.55 (32.15)	88.68 (43.2)	95, 67
AUC0-8 (uM.hr)	245 (161)	326 (155)	292 (149)	532, 398
Tmax (Range)	2.00 [1 – 4]	2.00 [1 – 2]	1.25 [1 – 2.5]	4.00, 2.00
T1/2 (h)	1.24 (0.1)	1.57 (0.5)	2.3 (1.6)	3.2, 12.5

Inspection findings: Subsequent to FDA data audit, a Form FDA 483 (voluntary action indicated, VAI-no response requested) was issued for inadequate and/or inaccurate records for 2 instances, both pertaining to discrepancy in documenting the time of dosing for one of the PK days (day 28) as noted:

For subject (b) (6), there were two different documents that had two different times for dosing: 7:55 (dosing log) and 7:45 (parent's own log).
For subject (b) (6), there were no source documents to support a case report form change from 8:30 to 8:43 made to the dosing time for the Day 28 dose.

Reviewer recalculated the pharmacokinetic parameters with both dosing times, and did not find the PK parameters to be affected by the dosing time differences.

Plasma uridine triacetate was not assayed in this trial. Per sponsor, the parent drug is not expected in systemic circulation due to its rapid conversion to uridine by the ubiquitous esterases. Sponsor determined based on a feasibility study (one hour incubations of low and high concentrations of uridine triacetate in human plasma and whole blood) that an assay for uridine triacetate determination couldn't be developed due to instability of the prodrug in the biological matrices (see bioanalytical section). Although an assay may potentially be developed and validated (for e.g. esterase activity can be inhibited during the development phase e.g. by pre-treatment with sodium fluoride), absence of PK information for the pro-drug and/or documentation of its absence from the systemic circulation as claimed, are not considered deterrents for the review of this drug's potential to provide clinical benefit in this rare, potentially life-threatening disease and its potential to address current drug shortages for oral uridine. Our colleagues in the oncology division have been alerted to the absence of uridine triacetate assay as a rolling NDA has been submitted to that Division. For any planned clinical trials, we recommend that the sponsor consider developing an assay to evaluate the systemic concentrations of the prodrug.

Reference ID: 3774715

PK from Mitochondrial disease patients (Study 401.09.001-PK):

Treatment	Dose (mg/kg) *	N	AUC_{0-8} (µM·hr)	C_{max} (µM)	Trough (µM)	C_{ss} (µM)
Uridine	333	4	644.8	119.5	28.9	80.6
Vistonuridine	33	8	269.0	64.6	12.6	33.6
	50	4	374.1	95.9	11.5	46.8
	67	4	517.5	148.8	16.7	64.7
	83	4	579.5	157.6	22.5	72.4
	100	4	932.3	205.1	52.7	116.5

Average PK data suggest approximately dose related increases in Cmax and AUC, with the exception of the highest dose (100 mg/kg), which demonstrated greater than dose proportional increase in AUC. Based on the average data presented, uridine triacetate at ~ 1/3rd the dose of oral uridine, provided approximately 45 % higher AUC_{0-8}, 72 % higher C_{max}, and 82 % higher C_{trough} values following t.i.d. dosing. Excluding an outlier, the plasma uridine from UTA was higher by ~3.8 fold on an equiweight basis and ~ 5.8 on an equimolar basis, compared to baseline uridine treatment. This is comparable to the relative bioavailabilty information from the HOA patients.

2.2.5.1 What are the ADME characteristics of the drug?

Absorption
Following oral administration, uridine triacetate is deacetylated by nonspecific esterases, yielding plasma uridine. The prodrug delivers 4- to 6-fold more uridine into the systemic circulation compared to equimolar doses of uridine itself. This is believed to be due to [redacted] (b) (4), and lower extent of inactivation of uridine to uracil when administered as a prodrug. Maximum concentrations of uridine in plasma following oral uridine triacetate are generally achieved within 2 to 3 hours, and the half-life ranges from approximately 2 to 2.5 hours.

Distribution
Circulating uridine is taken up into various cells via specific nucleoside transporters, and also is capable of crossing the blood brain barrier.

Metabolism
Uridine triacetate is deacetylated by nonspecific esterases present throughout the body, yielding uridine in the circulation. Uridine enters into intermediary metabolism pathways that are present and active in mammalian cells. Uridine uptake into cells via nucleoside transporters is generally linked to its phosphorylation by uridine kinase, forming intracellular uridine monophosphate (UMP), which is further anabolized to uridine diphosphate and uridine triphosphate.

Excretion

Reference ID: 3774715

Uridine can be excreted via the kidneys, but is also metabolized by normal pyrimidine catabolic pathways present in most tissues.

2.2.6. What are the PD characteristics of the drug?

As indicated by the name of the disease, one of the hallmark characteristics of HOA is the increased urinary excretion of orotic acid, which results in crystalluria and obstructive uropathy. Thus, urinary orotic acid concentrations were assessed in all four patients at baseline, on day 1 (at pre-dose), and at day 28 and day 42 of oral uridine triacetate treatment. The urinary OA concentrations were assayed in (b) (4) . Orotidine, an orotic acid derivative was also assayed. Urinary orotic acid is an important PD endpoint. Individual patient's urinary orotic acid levels both pre- and post- uridine triacetate are shown in the table below. The normal range and each patient's documented historic high value for orotic acid level in urine is also provided as reference:

Patient ID/ Urinary orotic acid (mmol/mol of	Orotic acid Normal range	Pre-uridine (historic high)	Baseline (on oral uridine)	Day 1 (pre-dose)	Day 28 (on oral UTA)	Day 42 (on oral UTA)	6 mo extension$ (on oral UTA)
(b) (6)	0.3 – 2.82	125	2.9	3	2.8	3.1	N/A 3.2#
	0.3 - 2.82	17.6	1.8	1.5	2.1	2	2.1
	0.3 – 2.82	9.5	0.5	0.3	0.9	0.3	0.3
	0.3 - 2.82	19.3	1*	0.4	0.5	0.5	0.5

*treatment-naïve; value pre-treatment on day 0
$patients (b) (6), (b) (6) received 120 mg/kg/d of Xuriden in the extension phase
patient (b) (6) received a dose increase to ~ 100 mg/kg/day

Historic high orotic acid concentrations in each patient (untreated) suggest a significant deviation from the normal range of 0.3 -2.82 mmol/mol of creatinine). In the first three patients who were on oral uridine at study enrollment, orotic acid values were much lower and within or close to the normal range. Orotic acid concentrations after switching to uridine triacetate continued to remain stable and did not show worsening suggestive of treatment adequacy after switching to the triacetate form. In the treatment naïve patient, orotic acid value was normal prior to uridine triacetate and after treatment. Therefore, the relevance of this finding is not clear.

Plots for urinary orotic acid (UOA) concentrations in individual patients of study 401.13.001 are shown below. Also shown are the individual hematological endpoints. The reference lines for upper normal UOA or normal hematological ranges are shown in red. Data from the oral uridine-treated patients suggests decrease in UOA from their pre-treatment (historic) levels. This is best exemplified in patient (b) (6). In addition, this patient demonstrated an increase in UOA during a uridine drug holiday suggesting a correlation between uridine replacement and orotic acid excretion in urine. In patient (b) (6)

Reference ID: 3774715

(b) (6), urinary concentrations of orotic acid were markedly higher prior to uridine therapy. The UOA concentrations appear to have reduced markedly when uridine was initiated but still remained above normal range. At baseline (day 0 on oral uridine) this patient demonstrated a UOA value within the normal range, which was then maintained at this normal level after patient was switched over to uridine triacetate. Right-hand panels show changes in hematology.

(b) (4), (b) (6)

Reference ID: 3774715

(b) (4), (b) (6)

A representative plot of PD and hematological endpoint changes overtime are shown for patient (b) (6):

Reference ID: 3774715

(b) (4), (b) (6)

At the120 day safety update (submitted May 11, 2015), patient (b) (6) received a dose increase from 60 mg/kg/day to ~ 100 mg/kg/day in an attempt by the investigator to bring the orotic acid levels (3.1 mmol/mol of creatinine) to within normal range (0.3- 2.8); no additional benefit on orotic acid was noted in this patient with dose increase. Patient (b) (6) continues on 60 mg/kg/day with stable clinical response. Patients (b) (6), and (b) (6) continue on 120 mg/kg/day with stable response. All patients show increase in growth and stability of hematological endpoints. Orotidine levels (not shown) show an increase in patients (b) (6) and (b) (6) but remained within normal range (0.06-2.0). No new pharmacokinetic information is available at this update.

Please see the clinical review of this NDA for additional interpretation of the hematological and other clinical findings. Two patients ((b) (6), (b) (6)) had higher than normal mean corpuscular volume (MCV; their pre-determined hematological endpoint of concern). With uridine triacetate therapy, these values did not fall into the normal range, but were stable, with no worsening. All patients demonstrated growth (height and weight increase) on uridine triacetate. This is summarized in the table below.

Patient	Growth	Baseline (uridine)	Day 1 (UTA)	Day 28 (UTA)	Day 42 (UTA)	6 mo extension
(b) (6)	Height (cm)					(b) (6)
	Weight (kg)					
	Height (cm)					
	Weight (kg)					
	Height (cm)					
	Weight (kg)					
	Height (cm)					

Reference ID: 3774715

	Weight (kg)	(b) (4), (b) (6)

2.3 Intrinsic Factors

No dedicated renal or hepatic impairment PK studies have been conducted with this drug.

2.4 Extrinsic Factors

No in vivo drug-drug interaction studies have been conducted. Based on in vitro studies, CYP450 enzyme induction and inhibition by the test drug appears unlikely. The potential for uridine triacetate to act as a p-gp inhibitor at the gut level cannot be ruled out.

2.4.1. *Is the drug a substrate of CYP enzymes?*

CYP450 enzymes are not involved in the metabolism of the prodrug or uridine. Uridine triacetate is deacetylated by plasma esterases into uridine. Uridine is then metabolized via the normal mammalian pathways for pyrimidines.

2.4.2. *Is the drug an inhibitor and/or an inducer of CYP enzymes?*

<u>CYP450 enzyme inhibition potential:</u> Uridine does not cause clinically relevant inhibition of CYP450 enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A). Although IC50 values for enzyme inhibition could be estimated for the effect of uridine on CYP2C19 and CYP3A (5.1 and 2.0 mM, respectively), these values are markedly high compared to the clinically relevant Cmax values of uridine noted in the pivotal HOA trial (ranging 32- 140 uM). Thus the potential for systemic enzyme inhibition by uridine appears remote. The IC50 values of uridine triacetate for CYP2C19 and CYP3A4 inhibition were 6.6 and 8.3 mM, respectively. The potential for uridine triacetate to cause clinically relevant CYP450 inhibition appears remote.

The gut concentrations of uridine triacetate are expected to be high (~ 37 mM, estimated using a dose of 60 mg/kg/day of uridine triacetate (MW: 370 g/mole) administered to a 60 kg individual in a 250 mL volume). However, the potential for inhibition of CYP2C19 and CYP3A4 by uridine triacetate doesn't appear to be clinically relevant, based on $[I_{gut}]/IC50$ i.e. $R_{alternate}$ estimates of < 10. For uridine, the potential for CYP inhibition at the gut level seems minimal as uridine is not likely to be present at significant levels pre-systemically following oral administration of uridine triacetate.

<u>CYP450 enzyme induction potential:</u> Uridine doesn't cause CYP450 enzyme induction. In vitro experiments suggest that uridine (at 50, 500 and 5000 uM concentrations) and uridine triacetate (at 5, 50 and 500 uM concentrations) do not induce CYP1A2, 2B6 and 3A4 in human hepatocyte cultures. Acceptable performance by positive control inducers was noted in these experiments. Cell viability assay performed at the end of these induction experiments did not suggest any hepatocyte cytotoxicity by uridine or by uridine triacetate at the concentrations used in this study. Although gut level

Reference ID: 3774715

concentrations would exceed than the 500 uM highest concentration of uridine triacetate used in these induction experiments, hepatocyte viability issues were noted at 5 mM concentrations in preliminary experiments, which prevented the sponsor from evaluating higher concentrations of uridine triacetate. However, across the uridine triacetate concentrations evaluated in this study (5, 50 and 500 uM), there was no evidence/trend for enzyme induction despite a 100-fold range in concentrations evaluated. Thus enzyme induction appears unlikely at higher concentrations.

2.4.3 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

Uridine triacetate appears to be a weak substrate of P-gp. The P-gp substrate potential of uridine couldn't be assessed. While uridine is not a p-gp inhibitor, uridine triacetate has a potential to be a P-gp inhibitor at concentrations anticipated locally in the gut.

P-gp substrate potential: The P-gp substrate assessment of uridine was not conducted in the Caco-2 monolayer system. No test drug could be recovered from the receiver chamber (and ~ 50 % reduction of test material was also noted in the donor chamber) during independent experiments for non-specific binding with uridine in cell-free collagen-coated transwell plates. No such issues were noted with the triacetate and hence bidirectional permeability assays were successfully conducted for the pro-drug. Uridine triacetate, appears to be a weak P-gp substrate (efflux ratios in Caco-2 cell monolayers were 3.08 and 2.05 at 5 uM, and 50 uM concentrations, which were reduced in presence of known p-gp inhibitor valspodar).

P-gp inhibition potential: Uridine doesn't appear to be a P-gp inhibitor. Uridine triacetate appears to inhibit P-gp with an IC50 value of 344 uM (using digoxin as a probe substrate). Due to high uridine triacetate concentrations at the gut level following oral dosing, a potential inhibitory effect on p-gp substrate drugs cannot be ruled out.

2.5 General Biopharmaceutics

2.5.1 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

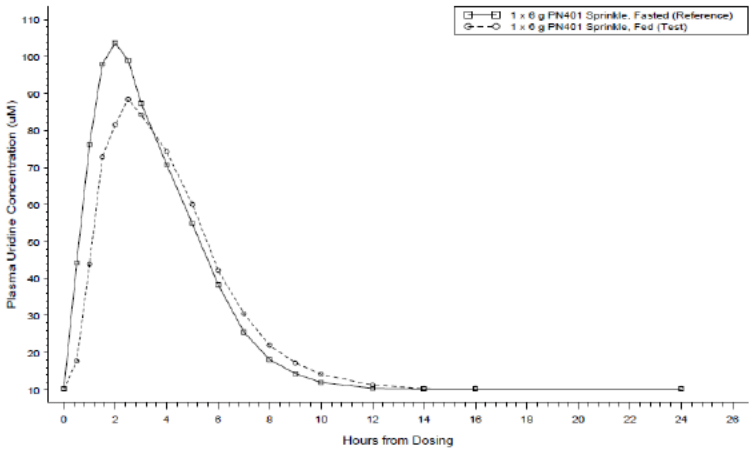
An open-label, randomized, single dose, two way crossover, food-effect PK study (with 7 days washout) was conducted in 20 healthy males and females. The study was conducted using an older granule formulation [containing (b) (4) % active ingredient (uridine triacetate) vs. 95 % active ingredient in the pivotal HOA trial/ proposed commercial formulation].

Reference ID: 3774715

(b) (4)

The study findings suggest absence of significant food-effect on PK of uridine following oral administration of uridine triacetate granules.

Statistical analyses of the systemic exposure data under fasted and fed conditions suggests no significant food-effect on uridine PK as indicated by 90 % CI surrounding the T/R ratios within 80- 125 % bioequivalence bounds. Based on the T/R ratios, the maximal effect of food was only Cmax, which reduced by ~ 14 %. Overall difference in AUC parameters was ~ 5 % under fed vs. fasted conditions.



Reviewer calculations (b) (4))

Parameter	Fed/Fasted (T/R) Ratio (%)	90 % CI
Cmax	87.55	81.91 – 93.58

Reference ID: 3774715

AUCt	95.58	88.68 – 103.01
AUCinf	96.29	89.32 – 103.80

Currently we are not planning on requesting a new food-effect PK study with the proposed formulation due to the following reasons: the drug has a wide safety margin, it is titrated to effect in the clinical setting, and patients are monitored regularly. Finally both food-effect and proposed formulations are primarily (> 90 %) comprised of active ingredient. Data from the food-effect PK study formulation which had higher amounts of excipients [redacted] (b) (4)) showed absence of food-effect on PK. Thus it appears unlikely that one would expect to see a substantial food-effect on PK when these excipients levels are decreased, as in the proposed formulation. [redacted] (b) (4)

2.6 Analytical Section

Plasma Uridine Assay: Plasma uridine concentrations were determined by [redacted] (b) (4) A high performance liquid chromatography method with ultraviolet absorbance detection at a wavelength of 260 nm was used for the determination of uridine and uracil concentrations in human plasma. The method used protein precipitation and liquid/liquid extraction with ethyl acetate to extract the analytes from 250 µL of plasma. The extract was evaporated, reconstituted and analyzed with an HPLC assay using a [redacted] (b) (4). An internal standard (IS) method of quantitation was used with [redacted] (b) (4) as the IS. Calibration samples containing 0.250 to 20.0 µg uracil/mL plasma and 2.50 to 100 µg uridine/mL plasma were prepared by dilution of the 20.0 µg uracil/mL and 200 µg uridine/mL calibration sample with blank plasma. The peak area ratios of the analytes/IS and the theoretical concentrations of the calibration samples were fit to the linear function with 1/x2 weighting, excluding the origin. QC samples containing 0.300 to 15.0 µg uracil/mL plasma and 3.00 to 150 µg uridine/mL plasma were prepared in triplicate and included in the analyses. System suitability and dilution standards were also prepared.

Recovery of primary analytes: The recovery of uridine was ~ 70 % for the low, medium and high QC samples. The recovery of the internal standard was 86.2%.

Reference ID: 3774715

Analyte Concentration	% Recovery Uracil	% Recovery Uridine
Low	77.8	70.0
Middle	76.2	68.3
High	82.5	68.2

Sensitivity: Lower limit of quantitation LLOQ (lowest calibration standard that meets acceptance criteria) was 2.5 ug/mL (10.2 µM) for uridine, and 0.25 µg/mL for uracil. The % RSD and % RE were < 5% at this LLOQ.

Specificity/Selectivity: The ability of method to differentiate analytes of interest from potentially interfering compounds was established.

Calibration standards: Based on %RSD and %RE of the back calculated concentrations for uridine and uracil, the reproducibility of the calibration data was deemed acceptable:

Calibration Samples

	Relative Standard Deviation (%)	Relative Error (%)
Uracil	0.57 to 1.6	-4.8 to 3.2
Uridine	2.0 to 3.2	-1.3 to 2.7

Accuracy (%RE or Bias) and Precision (%RSD or %CV): Precision and accuracy of the uridine and uracil assay were deemed acceptable as seen in the table:

Quality Control Samples

	Relative Standard Deviation (%)	Relative Error (%)
Uracil	1.2 to 2.4	-2.8 to 2.7
Uridine	3.0 to 4.0	-2.7 to 2.0

Stability: Uracil, uridine and internal standard in stock solutions were considered stable for up to 14 days of refrigerated storage. Uracil, uridine and the internal standard in stock solutions were considered to be stable for 7.5 hours of room temperature storage. Uracil and uridine in processed human plasma samples are stable for up to 15 days of refrigerated storage. Uracil and uridine in fortified frozen human plasma were considered to be stable for approximately 4 hours of room temperature storage after being frozen at either -20°C or -70°C. Uridine and uracil (with the exception of second F/T cycle) are stable for at least 3 freeze/thaw cycles when frozen at -20°C and -70°C.

Long term stability: Human plasma samples were fortified at 0.300 µg uracil/mL and 3.00 µg uridine/mL and 15.0 µg uracil/mL and 150 µg uridine/mL. The samples were stored frozen at approximately -70°C or -20°C for 10, 29 and 101 days, processed, and analyzed. Triplicate samples at each concentration level were analyzed at each time point.

Reference ID: 3774715

The mean measured uracil and uridine concentrations in human plasma samples from 0 to 101 days were compared to their corresponding target concentration.

	Long-Term Stability (% Of Target)	
Storage Conditions	Uracil	Uridine
Frozen at -70°C	96.1% to 104%	87.0% to 108%
Frozen at -20°C	95.2% to 115%	69.9% to 105%

The results met the acceptance criteria for stability with the exception of the uridine results for 29 days at -20°C storage (uridine stability at -70°C was acceptable at all QC concentrations). Since uridine was stable in plasma samples stored at -20°C for 101 days, uridine in human plasma was considered to be stable through 101 days.

Conclusions: The HPLC method for analyses of uridine and uracil was adequately validated. Sufficient sensitivity, selectivity, reproducibility, stability, precision and accuracy have been demonstrated. Thus the method is suitable for plasma sample analyses for uridine and uracil from human clinical trials.

Uridine triacetate assay: Sponsor attempted to validate an assay for detection of uridine triacetate. To evaluate feasibility, initially (b) (4) (bioanalytical facility) conducted stability studies in human plasma and whole blood (b) (4) (b) (4) However, considering the safety of a relatively large dose range of the pro-drug, and the urgent need for a uridine replacement therapy in the HOA population, we do not consider this a major deterrent for reviewing this application. The assay for the primary active moiety in plasma i.e. uridine, has been adequately validated. Our colleagues in the oncology division were alerted regarding the lack of a validated assay for the prodrug (email dated 04/28/2015), as a rolling NDA for this drug has also been submitted to that Division.

Urinary orotic acid and orotidine assay: Urinary orotic acid and orotidine were assessed by electrospray tandem mass spectrometry at (b) (4) Per sponsor, the method was based on a publication by Ito et al (Rapid screening of high-risk patients for disorders of purine and pyrimidine metabolism using HPLC-electrospray tandem mass spectrometry of liquid urine or urine-soaked filter paper strips; Clinical Chemistry 46:4; 2000) and subsequently

Reference ID: 3774715

modified. The methodology and transitions obtained (b) (4) In addition, this publication provides evidence of good recovery of the analytes and internal standards, low % CV, reproducibility and lack of carryover. The method used by (b) (4) laboratory is briefly described:

(b) (4)

1 Page has been Withheld in Full as b4 (CCI/TS) immediately following this page

Reference ID: 3774715

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

/s/

SANDHYA K APPARAJU
06/04/2015

SUE CHIH H LEE
06/05/2015