Clinical and Imaging Biomarker Trial of Uridine for Veterans With Suicidal Ideation: Protocol and Statistical Analysis Plan

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Uridine for Veterans with Suicidal Ideation: a Magnetic Resonance Spectroscopy Study

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A Introduction

A1 Study Abstract

Veteran suicides, attempts and suicidal ideation (SI) remain an urgent concern for the Veterans Health Administration (VHA). Research indicates that approximately half of veteran suicides take place within 1 month of the decedent's final VHA encounter, with one quarter occurring within 1 week. This provides a temporal window of opportunity to intervene, and necessitates development of a rapid-acting treatment for veterans with SI.

Intravenous ketamine has become the prototypical anti-suicidal drug, having been shown to rapidly reduce SI in some patients. However there are significant concerns regarding ketamine in both veterans, and military personnel. These include ketamine's potential for toxicity and misuse, and the brief duration of its anti-suicidal effect.

The ideal anti-suicidal treatment for use within the VHA system:

1) Would be administered orally, rather than requiring intravenous infusion;
2) Would achieve "target engagement" with the same neural substrates that ketamine does;
3) Would have lower risk of medical and psychiatric adverse events, particularly among veterans and active duty military personnel; and
4) Would have a more durable and lasting anti-suicidal effect, as the reduction in SI following ketamine administration lasts an average of 3 days, according to a recent systematic review of the ketamine literature.[1]

Therefore this study will test the novel intervention uridine as a rapid-acting oral treatment for veterans with SI. As described below, uridine's potential to fulfill this role lies in the broad overlap in the brain mechanisms and neural effects shared by uridine, ketamine and the anti-suicidal drug lithium. The reason for this surprising commonality may lie in the fact that ketamine's mechanism-of-action dependent on activation of *de novo* pyrimidine biosynthesis – and the fact that uridine is the endogenous, circulating pyrimidine in man.

To initiate testing of uridine for veterans with SI, we will conduct a four-week, double-blind, placebo-controlled clinical trial of uridine 2000 mg daily for veterans with SI. To make the study more informative, translational neuroimaging has been integrated into the protocol, in an attempt to identify some of the brain chemistry biomarkers of SI.

A2 Primary Hypotheses and Specific Aims

Specific Aim 1: **To Demonstrate that Uridine Decreases Suicidal Ideation in Veterans**: We hypothesize that 4 weeks of uridine 2000 mg daily will decrease the probability and severity of suicidal ideation, compared with placebo.

Specific Aim 2. **To Measure Rapid Changes in Brain GABA, in Uridine-Treated Veterans with Suicidal Ideation**: The hypothesis is that brain GABA levels, measured with magnetic resonance spectroscopy, will show a greater increase after 1 week, in uridine-treated vs. placebo-treated veterans with suicidal ideation.

Specific Aim 3. **To Examine the Durability of Uridine Treatment Response, in Veterans with Suicidal Ideation**: The hypothesis is that treatment responders will demonstrate a durable clinical response over 4 weeks,** in addition to acceptable patient compliance, satisfaction and engagement.

> ** A systematic literature review of ketamine as a treatment for suicidal ideation was recently published.[1] Rather than including only data from randomized, placebo-controlled trials, the authors included non-controlled data, open-label data, and case reports. The authors reported the reduction in suicidal ideation associated with ketamine treatment lasts an "average of 3 days" (see Reinstatler and Youssef, page 38).[1] Thus Specific Aim 3 covers a period of time (i.e. 28 days) that is roughly 10 times the mean duration-of-action of ketamine.[1]

A3 Purpose of the Study Protocol

The purpose of the study protocol is to describe the study's scientific rationale, and the procedures for acquisition and analyses of human subjects data.

B Background

B1 Prior Literature and Studies

Background and Prior Literature

Embedded in the VISN 19 Rocky Mountain Mental Illness Research, Education and Clinical Center (MIRECC) with its mission to reduce veteran suicide, this application pairs the investigational drug uridine with translational brain imaging, to address a critical unmet need: the lack of a rapid-acting treatment for veterans with suicidal ideation (SI).

Suicidal behavior remains a concern for the Veterans Administration (VA), yet there is no proven treatment and few VA studies of SI have been conducted. Thus by testing a novel and hypothesis-based intervention, while concurrently pursuing 'biomarkers' of SI to inform future research, this proposal addresses 2 of 3 "issues for Congress" identified in the 2016 Congressional Research Office report [R42340] on suicide prevention in VA[2]: "building the evidence base," and "increasing access to evidence-based mental healthcare."[2]

Recent VA suicide research points to a window of opportunity for intervention. First was the report that 23% of veteran suicides occur within just 7 days of the decedent's final

VA visit, while over half (51%) occur within 30 days.[3] This was followed by the finding that the two key 'warning signs' which increase the risk of suicide death within 7 days of the final visit are psychosis, and documented SI (Odds Ratio 3.46).[4] A rapid-acting, well-tolerated and easily-administered SI treatment would thus provide a powerful, impactful tool for VA prescribers.

Intravenous (I.V.) ketamine has become the prototypical antisuicidal drug, with a rapid albeit temporary effect lasting 10 days or less.[5] However ketamine is a phencyclidine derivative, which gives it dissociative and psychotomimetic properties, significant abuse liability,[6] and ketamine is associated with permanent gastrointestinal and genitourinary damage. More concerning, in the setting of acute SI in VHA patients, are ketamine-associated mania, tolerance and addiction, and even dysphoria with new-onset SI[7] – all in psychiatric patients who received ketamine. In addition, a survey of military anesthesia clinicians found concerns regarding frank delirium, when ketamine is administered to combat veterans, or soldiers with any of the following: anxiety, traumatic brain injury (TBI), or posttraumatic stress disorder (PTSD).[8] For use within VA, then, an alternative to ketamine – which ideally does not require an I.V. infusion every 10 days – is required.

The serendipitous observation of ketamine's rapid anti-suicidal effect provides an opportunity to investigate the neural substrates of SI. In this regard, neuroimaging can provide important insights. In line with this, the 2010 NIMH Advisory Council's report, *From Discovery to Cure*, emphasized that treatment development should be guided by emerging scientific understanding of psychiatric disorders. Further, the Research Domain Criteria (RDoC) initiative advocates the study of specific symptoms, such as SI. Converging lines of evidence implicate disruptions in amino acid neurotransmission in the pathogenesis of suicidal behavior, including in veterans. Investigation is focused on alterations to gamma-Aminobutyric acid (GABA) and glutamine (Gln). To measure these *in vivo*, proton-1 magnetic resonance spectroscopy (^{1}H-MRS) brain imaging is the sole current methodology. Importantly for this proposal, studies have used ^{1}H-MRS to show that levels of GABA[9] and Gln[10] are increased in response to ketamine administration. Meanwhile *Preliminary Data* from our ^{1}H-MRS studies of uridine (detailed below), suggest uridine may also be capable of engaging these two neurochemical targets.

Uridine, the subject of this proposal, is a pyrimidine required for normal brain function.[11] Notably, ketamine's rapid antidepressant effect depends on activation of the mechanistic target of rapamycin (mTOR) pathway,[12] and two publications in *Science* report that the function of mTOR is to activate *de novo* pyrimidine synthesis.[13, 14] Because uridine is the circulating human pyrimidine, this means that ketamine is an obligate up-regulator of uridine. In addition as shown in **Table 1**, the literature reflects a remarkably diverse overlap in the neural effects and brain mechanisms shared by uridine, ketamine and lithium, another drug believed to possess anti-suicidal properties. mTOR acts to phosphorylate CAD, the multifunction enzyme complex that catalyzes the first three steps of pyrimidine synthesis, the final product of which is uridine triphosphate (UTP).[15] Three other facts support uridine's rationale as a treatment for SI: first, the discovery of pyrimidine neurotransmission;[16] second, the fact that the CAD gene is down-regulated in the brains of suicide victims;[17] and third, ketamine[18] and the uridine prodrug PN401[19] both increase the concentration of brain-derived neurotrophic factor (BDNF).

Preliminary Studies at the University of Utah

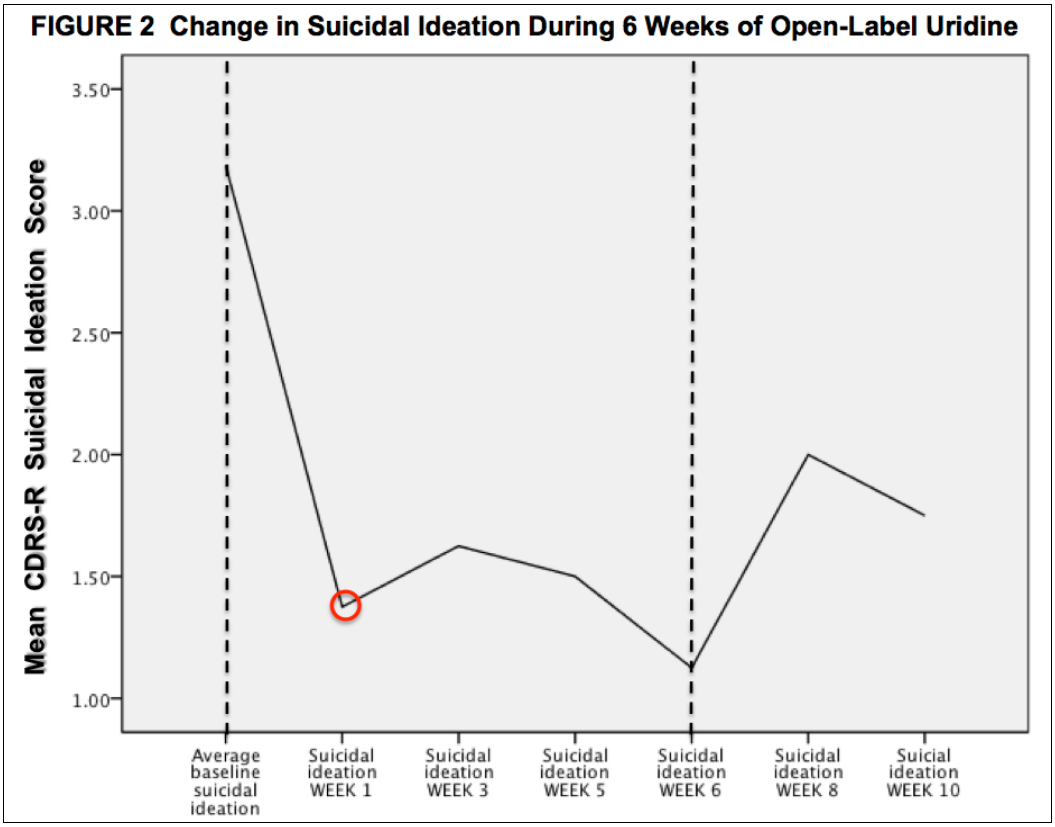
<u>Preliminary Study #1</u>: Open-Label Uridine Increases Serum Levels of Uridine, and Rapidly Decreases Suicidal Ideation in Bipolar Depression.

Methods: Twenty-four adolescents with bipolar depression were enrolled for a 6-week open-label study of uridine 1000 mg daily. Pre- and post-treatment serum uridine levels were measured. Suicidality was measured with Item #13 of the Children's Depression Rating Scale-Revised (CDRS-R).[20]

Serum Uridine Results: The normal range for serum uridine is 1.9-8.4 nmol/ml.[21] The mean baseline uridine was 5.4 nmol/ml; at week 6 the mean was 8.8 nmol/ml (Cohen's *d*=1.86; Effect Size=0.68).

Suicidal Ideation Results: As shown in **Figure 1**, suicidal ideation decreased over 6 weeks, with the majority of change occurring during the first week of treatment (mean week 1 score=1.37; mean week 6 score=1.33).

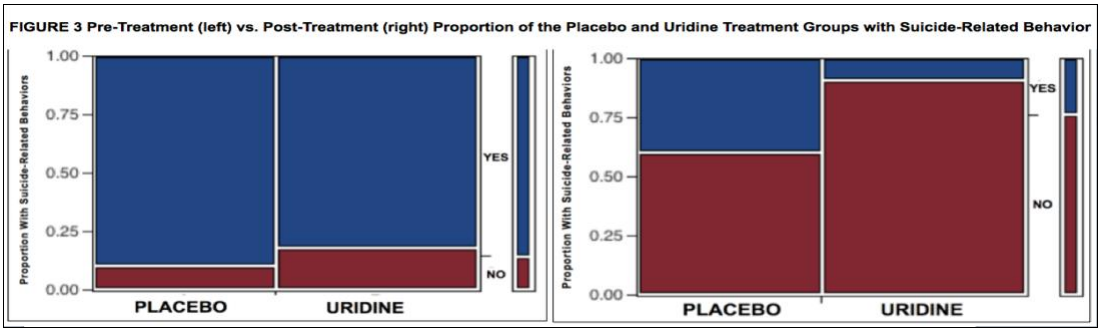
FIGURE 2 Change in Suicidal Ideation During 6 Weeks of Open-Label Uridine



Conclusions: These data demonstrate that oral uridine is absorbed systemically, and increases serum uridine values. Furthermore, 97.6% of the decrease in SI was observed at the end of week 1.

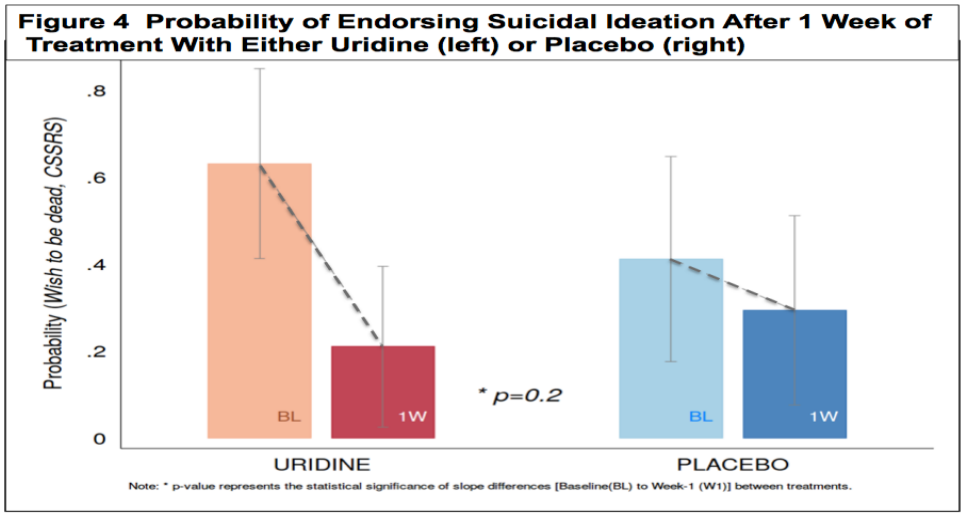
<u>Preliminary Study #2</u>: In a Placebo-Controlled Trial, Uridine Reduced Suicidal Ideation, and Neuroimaging Suggests 'Target Engagement' with Anterior Cingulate GABA and Glutamine.

Methods: N=34 adolescents with bipolar depression were enrolled in a follow-up study to our open-label protocol. This was a randomized, double-blind, placebo-controlled study of uridine 1000 mg daily. ^{1}H-MRS scans were performed at baseline, and repeated following 6 weeks of randomized treatment.



Suicidal Ideation Results: The presence of suicidal behavior, defined as either an attempt, plan, or SI, was determined with the Columbia-Suicide Severity Rating Scale (C-SSRS). As shown in **Figure 3**, at baseline 9 of 10 subjects in the placebo group endorsed suicidal behavior, compared to 9 of 11 in the uridine group (Chi Square=0.292; p=0.59). Below right, after 6 weeks we found a trend favoring uridine: 4 of 10 placebo-treated patients endorsed suicidal behavior on the day of their 2nd brain scan, versus 1 of 11 in the uridine group (Chi Square 2.89; p=0.08). Another way to compare treatments is by calculating relative risk (RR) of suicidal behavior. The RR of suicidal behavior in placebo vs. uridine was 2.27 (95% confidence interval, 1.04–4.96; p=0.04). In addition, the number needed to treat (NNT) was 1.96 (95% confidence interval, 1.18–5.91).

We next analyzed the probability that subjects endorsed SI <u>after just 1 week of uridine or placebo treatment</u>. As shown in **Figure 4**, the between-group difference was non-significant (p=0.2). However, inspection of the slope of the treatment group data suggests our results were limited by the sample size.



^{1}H-MRS Imaging Results: Subjects were scanned before and after 6 weeks of randomized treatment. The analyses focused on amino acid neurotransmitters including GABA. Direct measurement of GABA poses technical challenges, due to other ^{1}H-MRS metabolites that overlap the GABA peak and are present at higher concentrations. To overcome this, and to reduce confounding due to the inter-individual differences in human brain chemistry, within-subject metabolite ratios are often employed for analysis.

One such ratio is GABA/Glx, which is referred to as the inhibition/excitation ratio. The entity Glx (glutamate + glutamine) is a peak in ^{1}H-MRS spectra that contains overlapping contributions from Glu and Gln.

As shown in **Figure 5**, we found that GABA/Glx was significantly increased in the uridine group, compared with placebo (p=0.01).

FIGURE 5 Change in the Anterior Cingulate Inhibition / Excitation Ratio (GABA/Glx) Associated with Uridine or Placebo Treatment for 6 Weeks

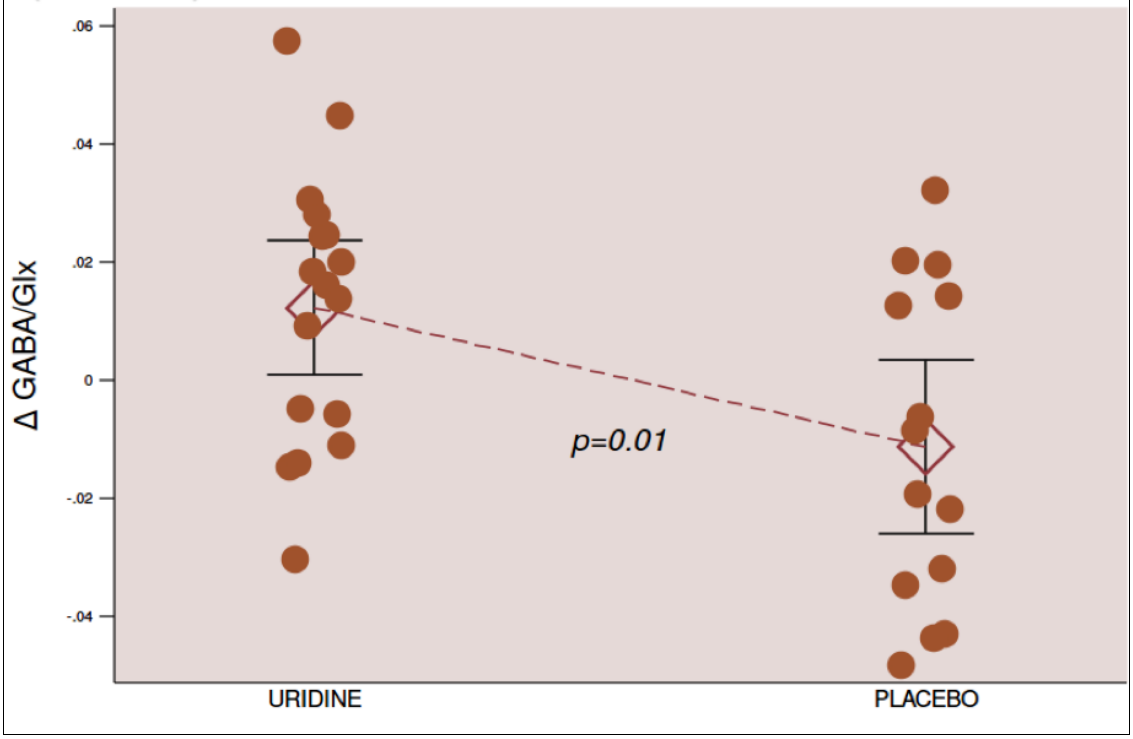
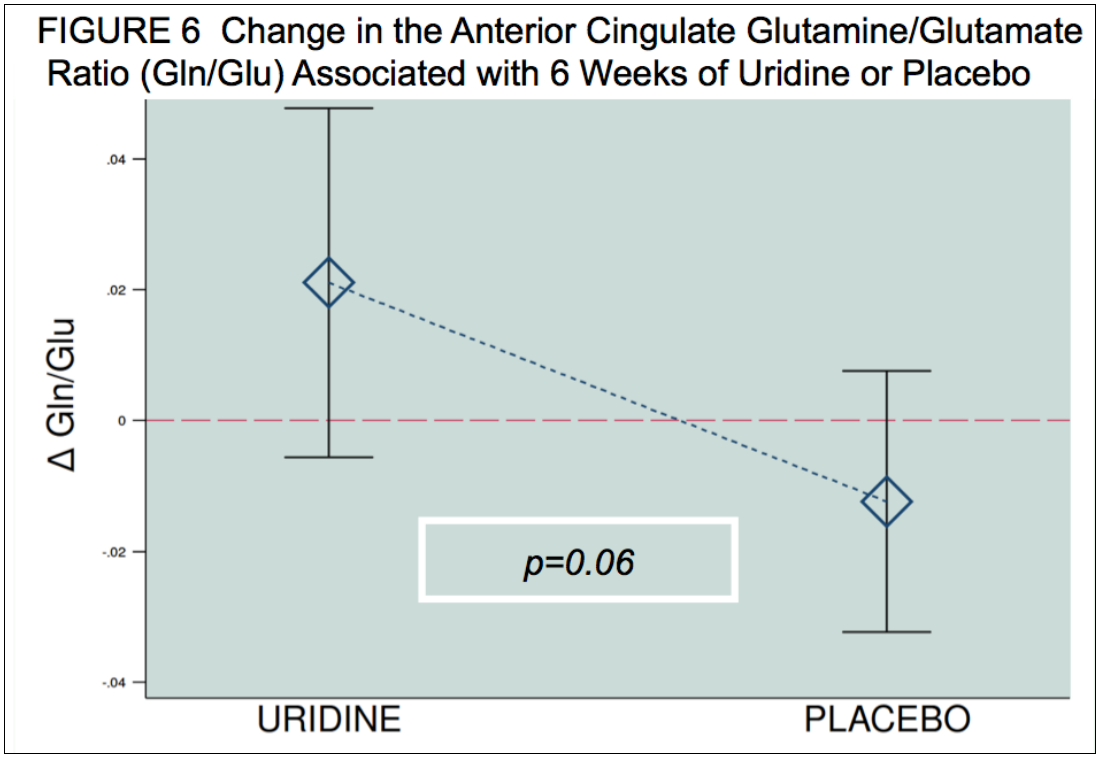


Figure 6 displays the change in another ratio of ^{1}H-MRS metabolites, the Glutamine/Glutamate Ratio (Gln/Glu). Recalling that ketamine increases Gln,[10] as well as a 'proxy' measure of Gln,[22] our results are consistent with uridine having a similar effect, albeit reduced Glu could also account for the increase in the Gln/Glu ratio that we observed.



To eliminate the inherent ambiguity of expressing ^{1}H-MRS results as the ratio between two metabolites, we have developed and validated novel methods (described below) that allow individual measures of GABA, Gln and Glu despite their overlapping resonances. The proposed study thus has the potential to provide new information regarding the *in vivo* differences in neurochemistry between suicidal and non-suicidal veterans.

Preliminary Study #3: New Imaging Methods Applied to a 3-Tesla High-Field MRI Scanner Enable Valid and Reliable Individual Measures of GABA, Glutamine and Glutamate.

Rationale: Low spectral resolution and severe metabolite peak overlap are associated with ^{1}H-MRS data acquired from human brain using clinical MR systems. The objective of this study was to interrogate the magnitude and variability of the between-metabolite correlation coefficients as calculated in human brain using 2-dimensional *J*-resolved ^{1}H-MRS and "ProFit" analysis.

Methods: Spectra were acquired from the anterior cingulate cortex (ACC) and parietal-occipital cortex (POC) of n=10 healthy adult volunteers at a magnetic field strength of 3 Tesla.

Results: Favorable between-metabolite correlation coefficients (<20%) were observed for a range of ^{1}H-MRS metabolites (**Table 2**). Most ACC and POC metabolites showed acceptable intra- and inter-subject CV values of <15% and <20%, respectively. The group mean correlation coefficient existing between ACC GABA and Gln was calculated as 1.45% ± 1.0%; between ACC Gln and Glu it was -18% ± 4.1%.

Table 2 Mean Metabolite ACC Concentrations and Coefficients of Variation for ^{1}H-MRS *ProFit* Method Test-Retest Reliability Study

Metabolite	Metabolite/Water (Mean ± SD; x10^{-5})	Intra-Subject CV (%)	Inter-Subject CV (%)	CRLB (% ± SD)
NAA	10.3 ± 1.2	7.0	10	0.5 ± 0.1
GABA	1.3 ± 0.4	15	24	5.7 ± 1.3
Glutamine	1.8 ± 0.3	9.9	16	5.9 ± 1.3
Glutamate	8.6 ± 1.2	4.5	14	1.4 ± 0.2
Myo-Inositol	5.4 ± 0.6	3.6	11	1.8 ± 0.2
Lactate	0.6 ± 0.2	12	30	7.4 ± 1.3

ACC, anterior cingulate cortex; NAA, N-acetyl aspartate; GABA, gamma-amino butyric acid; SD, standard deviation; CV, Coefficient of Variation (defined as 100 x (standard deviation / mean)); CRLB, Cramer-Rao Lower Bounds

Conclusions: The observed signal discrimination makes these techniques suitable for investigating ACC GABA, Gln and Glu in a variety of brain-based disorders.

B2 Rationale for the Study

Suicidal Behavior is an Urgent Concern for the Veterans Administration.

The high risk of suicide in veterans who receive care in the Veterans Health Administration (VHA) has persisted, despite efforts to improve mental health services and programs aimed at suicide prevention. Suicide is more common among VHA patients than in the general population. For example suicide is the ninth leading cause of death in the U.S. population, but ranks first and second, respectively, among female and male veterans. In fact compared to non-veterans, the number of veteran suicides is approximately 60% higher than expected (Standardized Mortality Ratio (SMR) = 1.63).[23] This burden falls disproportionately on female veterans, whose rate of suicide is nearly 600% higher than expected (SMR = 5.89).[23] Despite this, in a systematic review of n=91

suicide prevention publications, none were focused on veteran populations. In part because of this, veteran-specific reviews have concluded there is a gap in randomized controlled trials of suicide-related interventions.[24] To bring needed innovation to treatment development for veterans, recommendations include using suicide-related outcomes, examining rarely-studied treatments and testing interventions that can be delivered 'upstream' of an actual suicide attempt or death. Taken together, the rate of suicide among veterans and the lack of evidence-based treatments for suicidal patients in VHA point to a critical unmet need for veteran-focused clinical research in this area. In addition to controlled trials of efficacy and effectiveness, attention is now focused on the discovery objectively-measureable 'biomarkers' of suicide.[25] Therefore we propose a 4-week, double-blind, placebo-controlled clinical trial of uridine that features translational brain imaging at baseline, and following treatment with either uridine or placebo.

There is a Critical Unmet Need for Rapid-Acting Treatments for Veterans with Suicidal Ideation.

At this time, only clozapine is approved the U.S. Food and Drug Administration (FDA) to reduce suicidal behavior. This indication is for patients with schizophrenia; however 94% of attempted or completed suicides reported by VHA Suicide Prevention Coordinators (SPCs) occur in veterans with a primary or secondary diagnosis of depression or bipolar disorder. Clearly, research in non-schizophrenic veterans with SI is needed.

A series of recent studies have investigated the timing and content of the final VHA appointment attended by veterans who died by suicide. Among the key findings was among veterans with a depression diagnosis, 23% of suicide completers saw a VHA provider within 7 days of their suicide, and 51% had been seen within 30 days.[3] Another important result was that roughly half of final VHA contacts took place in primary care, as opposed to the mental health service.[3] In the primary care setting, 18-48% of veteran suicide decedents were assessed for suicidal thoughts,[26] a minority of which (38%) had endorsed SI when asked. The investigators concluded that areas for improvement include the management of SI in primary care, increased referrals to mental health, and attention to mental health issues, in other VHA settings.[3] Thus a rapid-acting treatment for SI that could be delivered in the primary care setting, could constitute an important opportunity for suicide reduction in VHA. Even with a goal as important as suicide reduction, it may not be possible to offer manualized psychotherapy and/or I.V. ketamine infusions, in every primary care clinic in VHA. However just as oral medication for type II diabetes is available in 100% of those clinics, so too could a proven treatment for SI.

We believe that an orally-administered, rapid-acting treatment for SI would help to address several of the issues identified by this research. Within the 7- and 30-day windows identified by Smith et al.,[3] lies the opportunity to intervene in veterans with SI. Whereas other promising somatic SI treatments, such as lithium or repetitive transcranial stimulation (rTMS), would be administered by the mental health service, a safe and effective rapid-acting SI treatment could be started by any VHA prescriber, including emergency department physicians--perhaps serving as a 'bridge' to a referral to mental health. Given that easy access to care is a protective factor against suicide, an intervention available VHA system-wide could have a significant impact.

The absence of FDA-approved medications for SI is a recognized public health issue. The problem is exacerbated by the fact that patients with SI or prior suicide attempts are often excluded from FDA registration trials, on scientific and ethical grounds. At the

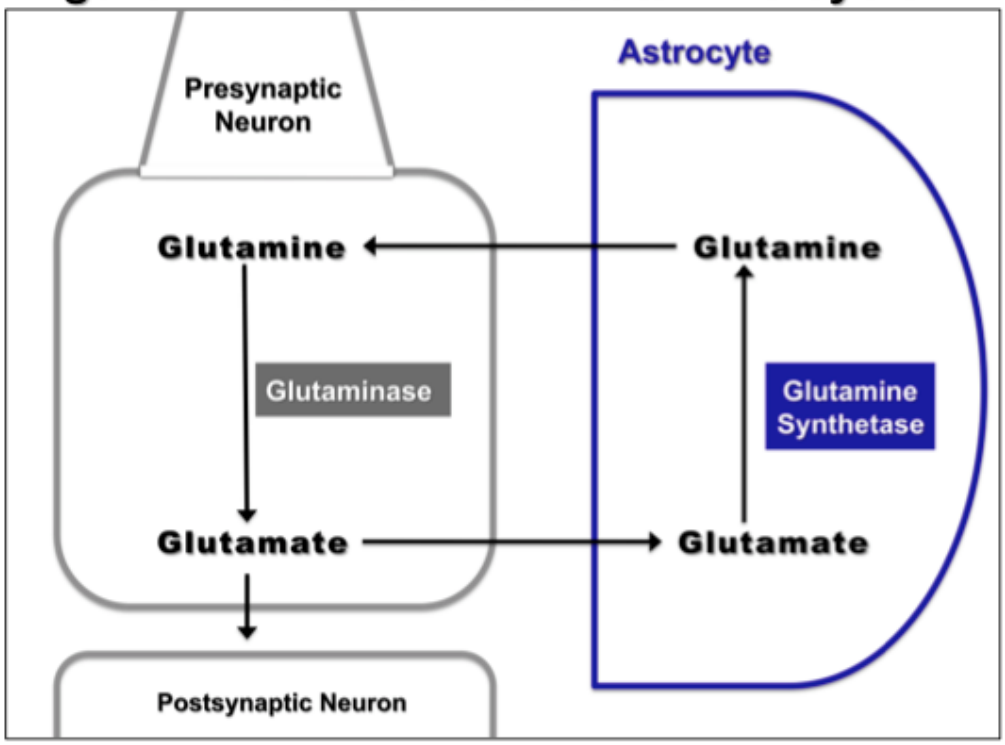
same time, the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) has designated “suicidal behavior disorder” as a condition for further study (p. 801), and the National Institute of Mental Health (NIMH) has adopted experimental medicine studies of “target engagement” as a model for clinical trials. The proposed pilot study will evaluate uridine’s effect on SI at the critical 7-day and 30-day intervals identified through outcomes research, and serve as a companion to the Better Resiliency Among Veterans and Non-Veterans With Omega-3's (BRAVO) study, which employs imaging but focuses on chronic, as opposed to rapid-onset, treatment effects.[27]

Neuroimaging of the Anterior Cingulate Cortex (ACC) is a Powerful Tool for use in the Development of Novel Mental Health Treatments.

In psychotropic drug development, neuroimaging has progressed to the point where it is used in a variety of ways: to validate biomarkers of disease and treatment response, to define a dose-response relationship, or to demonstrate “target engagement” with a clinically-relevant brain mechanism. The concept of “target engagement” refers to expanding the focus of clinical research from simple tests of efficacy, e.g. scores on the Hamilton Rating Scale for Depression, to include objective measures of disease mechanism, or the mechanism-of-action of the intervention. Recognizing the inherent complexity of psychiatric disorders, the NIMH has expanded the definition of “target engagement” to include a treatment’s hypothesized mechanism-of-action and its ability to modify a disease, behavior or functional outcome. One way to conceptualize this is that interventions are used as probes of disease mechanisms, as well as tests of efficacy. This allows negative clinical trial results to be informative: if “target engagement” is not associated with clinical improvement, the target is abandoned, and resources can be devoted to the discovery of new treatment targets.

Recent discoveries at the intersection of mood disorders and SI highlight the importance of the glutamatergic system in suicide. The system includes the amino acid neurotransmitters glutamate (Glu), glutamine (Gln) and gamma-Aminobutyric acid (GABA). As the only method for measuring these neurochemicals *in vivo*, proton-1 magnetic resonance spectroscopy (^{1}H-MRS) is uniquely suited for studying SI. In a recent review of the role of GABA and Gln in the mechanism-of-action of ketamine, Lener et al. noted that innovation in MRS imaging techniques that improves our ability to individually measure cerebral GABA, Glu and Gln are needed to advance the field, through the use of biomarkers to create biologically homogenous subgroups, in order to deploy targeted interventions.[28] It is therefore notable that at our site, ^{1}H-MRS methods have been recently developed allow us to individually quantitate Glu, Gln and GABA,[29] which is unreliable without specialized methods. ^{1}H-MRS has been used to study the effects of lithium and ketamine,[9] two drugs believed to have anti-suicidal properties.

Figure 7 The Glutamate-Glutamine Cycle



Glutamatergic neurons account for >80% of synapses in the cerebral cortex. Synaptic Glu is taken up by astrocytes and converted to Gln, and studies combining spectroscopy with positron emission tomography (PET) have shown that this "Glutamate-Glutamine Cycle" (**Figure 7**) is the major metabolic pathway in the central nervous system (CNS), consuming ~85% of glucose delivered to brain.[30] After neuronal release into the synaptic cleft, Glu is taken up by astrocytes, where it is converted to Gln by glutamine synthetase. Gln is then shuttled into neurons, where it is converted back to Glu by glutaminase. ^{1}H-MRS Glu and Gln are believed to represent glutamatergic function because: 1) Glu does not cross blood-brain barrier, and must be synthesized in CNS; 2) The Glu and Gln content of macromolecules including proteins does not contribute to ^{1}H-MRS signals; and 3) The Glu -> Gln flux is directly coupled to neuronal activity, and displays a 1:1 stoichiometry with glucose oxidation. Rapid conversion of Glu to Gln is a neuroprotective measure, intended to prevent hyperactivation of Glu receptors, glutamate-mediated excitotoxicity, and cell death via apoptosis. Gln from astrocytes is the precursor for GABA synthesis in neurons, and GABA->Gln flux comprises 23% of total (Glu plus GABA) neurotransmitter cycling and 18% of total neuronal tricarboxylic acid cycle flux.[31] Thus the contribution of GABAergic neurons and inhibition to cortical energy metabolism has broad implications, and dysfunction of the amino acid neurotransmitter cycling has predictable effects on GABA concentrations.

Since the seminal work of Drevets et al. published in *Nature*,[32] the anterior cingulate cortex (ACC) has often been the focus of neuroimaging studies of mood-related symptoms. Because of its role in processing both emotion and cognition, we have selected the ACC as the region-of-interest for the imaging in this proposal. In addition,

PubMed lists n=74 publications for the search parameters “suicide or suicidal AND anterior cingulate.” Finally, in addition to two systematic reviews implicating the ACC in suicidal behavior,[33, 34] there is one study of veterans with, and without, a history of SI, that found significant differences in the ACC.[35]

Ketamine is the Prototypical Rapid-Acting Anti-Suicidal Drug. However, Ketamine May Be Unsafe for Veterans.

Beginning with the original report of the drug’s rapid-acting antidepressant activity, enthusiasm for ketamine built to the point that NIMH Director Dr. Thomas Insel wrote of ketamine, “The doom and gloom surrounding medication development, at least for depression, seems to be rapidly resolving.”[36] In 2010 came the first report of ketamine as a rapid-acting treatment for suicidal thoughts,[1] and there are now several reports highlighting ketamine’s effect on SI, with a rapid onset ranging from 40 minutes to 10 days.[1] Although experts have pointed to the need for more research prior to ketamine use in clinical settings, the drug is being sold online and by compounding pharmacies, and for-profit ketamine clinics have opened around the United States.

There are a number of drawbacks associated with using ketamine to treat SI in depression. First is the evidence for ketamine’s short duration-of-action, with SI returning in as soon as 1-2 days.[37] Next, ketamine is a derivative of phencyclidine, which gives it dissociative and psychotomimetic properties. Ketamine has also been shown to have both mu opioid,[38] and stimulant-like effects[39] -- and it is not in dispute that ketamine is a drug of abuse.[6] A striking similarity between the biological and clinical properties of ketamine and alcohol has also been noted. In terms of feasibility within VHA as a treatment for acute SI, potential problems include reports of ketamine-associated mania,[40] tolerance and addiction,[41] and dysphoria,[7] all in mental health patients receiving treatment (as opposed to ketamine abusers). Most alarming are the reports of new-onset SI in response to ketamine,[7] and completed suicide while under the influence of ketamine.[42] Furthermore, a survey of military anesthesia clinicians found significant concerns regarding delirium, when ketamine is administered to the following patient groups: combat veterans, soldiers with traumatic brain injury (TBI), or patients with posttraumatic stress disorder (PTSD).[8] For use within VHA, then, alternatives to ketamine may be required. And finally, all research involving veterans must be ethical. In addition to the many unanswered scientific questions regarding ketamine, concerns have been raised regarding the potential for breaches of moral, ethical and medico-legal principles, when ketamine is administered off-label in psychiatry.[43]

The Brain Imaging Technique Magnetic Resonance Spectroscopy (MRS) Can be Utilized to Reveal the *In Vivo* Effects of Ketamine on Human Brain Chemistry.

^{1}H-MRS has been used to investigate ketamine’s effects on brain chemistry, in humans and animal models. For example, ketamine reduces depression-like behavior and increases ACC GABA in chronically-stressed rats.[44] Ketamine also increases Gln, in rat ACC and prefontal cortex.[45] Another study combined ^{1}H-MRS with carbon-13 MRS, and found that sub-anesthetic ketamine significantly increased prefrontal GABA and Gln.[46]

Human ^{1}H-MRS studies have reported similar findings. Rowland et al. studied healthy volunteers, and found that ketamine significantly increases ACC Gln.[10] A study of depressed patients reported that ketamine significantly increased prefrontal GABA above baseline values.[9] And finally, Salvadore and colleagues reported that lower prefrontal Glx/Glu, a proxy measure for Gln, predicts a greater response to ketamine.[22]

Taken together, animal and human studies suggest that ketamine enhances GABA and Gln in brain.

Converging Scientific Evidence Implicates the Neurotransmitters GABA and Glutamine in Suicidal Behavior.
The neurobiology of suicidal behavior has not been fully elucidated, but evidence is accumulating for several brain systems. These include the glutamatergic system, which includes the neurotransmitters Glu, Gln and GABA. The imaging in this proposal focuses on GABA and Gln, because they can be measured using ^{1}H-MRS. Other likely contributors include: cortisol,[47] the glucocorticoid stress response and hypothalamic-pituitary-adrenal (HPA) axis; interleukin 6 (IL-6)[47] and the immune system inflammatory response; and brain-derived neurotrophic factor (BDNF),[47] a neurotrophic growth factor. Although these cannot be measured *in vivo*, evidence will be presented below, that uridine and its nucleotides alter cortisol, IL-6 and BDNF -- in the opposite direction of their pro-suicide associations -- thus providing further support for uridine.

GABA: The Glutamate-Glutamine Cycle described above is a major regulator of GABA metabolism, and modulates GABA neurotransmission by regulating $GABA_A$ subunit composition. The literature supports GABA-related alterations in suicide at the neurochemical, genetic, cellular, localization and functional levels.[48] For instance in suicide decedent brains, GABA receptor subunit genes are “globally altered.” Recently it was reported that low $GABA_A$ expression levels are associated with suicide. There is also evidence for epigenetic hypermethylation of the $GABA_A$ promoter region, suggesting a potential mechanism for this.

Interestingly biomarkers of SI in the peripheral circulation have also been studied, among male veterans of Operation Enduring Freedom and Iraqi Freedom (OEF/OIF). The investigators were unable to measure GABA or Gln, but did find elevations of spinal cord and retina transmitter glycine, in the serum of veterans with SI.[49]

Importantly for this proposal, uridine is a known modulator of GABA receptors, and when administered to mice, uridine increases GABA concentrations by 96% after 80 minutes.[50] This caused the investigators to speculate that in addition in increasing GABA, uridine may impact the dopaminergic system possibly via agonistic effects at $GABA_A$ receptors.[50] Thus uridine may have a ‘dual’ mechanism underlying its GABAergic effects: 1) Increasing the neurotransmitter level; and 2) Biological activity at the $GABA_A$ receptor. This would explain why uridine is believed to be involved in sleep, and has shown promise as an antiepileptic.

Glutamine (Gln): The literature on Gln in suicide is small but intriguing. For example Gln levels are low in suicide victim brains.[51] Post-mortem work also provides what may be a crucial insight: glutamine synthetase, the enzyme that catalyzes Gln synthesis, is differentially expressed in suicide decedents with vs. without depression.[52] Suicide research has a built-in confound, because ~90% of suicide victims have a mental illness. The recent findings related to Gln and suicide may help investigators begin to parse the differences between the risk for mood and other psychiatric disorders, and the risk for suicide itself.

Uridine is a Safe, Hypothesis-Driven Strategy for Engaging Suicide-Related Neural

Targets in the Brain – Without the Toxicity and Risks Associated with Ketamine and Lithium.

The purinergic system appeared early in evolution, using adenosine triphosphate (ATP) and uridine triphosphate and diphosphate (UTP; UDP) as transmitters.[53] The system is involved in modulating behavioral motivation and reward, as well as neuro-regeneration. Uridine receptors are expressed on all primary CNS cell types including neurons, astrocytes, microglia and endothelium. In mammalian brain, uridine nucleotides serve as native agonists at four specific receptors: P2Y2, P2Y4, P2Y6 and P2Y14.[11]

Uridine Pharmacokinetics: Uridine is orally bioavailable, is actively transported across the blood-brain barrier (BBB), and taken up by neurons and astrocytes via proteins of the SLC29 family. The salvage pathway then converts uridine sequentially to UMP, UDP and UTP. Human trials have shown that oral administration of uridine or a precursor increases plasma uridine concentration: this is critical, because mammalian brain has limited capacity to synthesize uridine, and thus depends on uptake from the circulation.[54] Brain uptake of circulating uridine was first demonstrated in the 1970s. This was followed by evidence that uridine transport across the BBB begins within 15 seconds of administration. Tritiated uridine was then used to trace uridine's path from blood to cerebrospinal fluid (CSF), then to the extracellular space in brain, followed by entry into cells, and ultimately phosphorylation. It was next demonstrated that administration of a uridine source by gavage increases brain concentrations of both uridine and UTP, within 30 minutes. In the experiment utilizing the closest relative to man, uptake of tritiated uridine into monkey choroid plexus, and then to multiple brain regions, was confirmed following intravenous injection.[55]

Uridine Metabolism Pathways: P2Y4 and N-methyl-D-aspartate receptor (NMDA) receptors co-localize precisely in fluorescence studies, and immunoprecipitation shows that P2Y4 is present within NMDA receptor immune complexes. Approximately half of ACC neurons express P2Y4, with 100% co-expressing the NMDA receptor. P2Y4 receptors are also present on astrocytes, though there are no extant expression studies of the ACC. With regard to functional data, similar to the NMDA receptor antagonist ketamine, in rat cortex uridine decreases NMDA-induced $^{45}Ca^{2+}$ uptake into synaptosomes, in a concentration-dependent manner.[56] Using recombinant NMDA receptors, it was further demonstrated that UTP reduces Glu-evoked electrical current, suggesting a model of competitive antagonism at the Glu binding site of the NMDA receptor. In addition to NMDA receptor blockade, sub-anesthetic doses of ketamine paradoxically increase Glu release and glutamatergic transmission. Likewise, UTP activation of P2Y4 receptors stimulates Glu release from astrocytes.[57] Direct astrocyte-to-neuron signaling ("gliotransmission") was reported in *Nature* and *Science* in 1994.[58, 59] Thus astrocytes can modulate synaptic transmission by releasing Glu via exocytosis. This pathway is regulated by UTP's activation of P2Y receptors. Notably, it is thought that *increased* glutamate activation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors, in combination with *decreased* NMDA activity -- i.e. the NMDA/AMPA balance -- is the key to ketamine's rapid effect on mood and suicidality. In light of this, it is noteworthy that uridine acts at NMDA receptors, *decreasing* the calcium flux of NMDA activation and *increasing* the current produced by AMPA stimulation.[56]

Uridine Mechanisms-of-Action that are Relevant to Suicidal Behavior: This proposal focuses on GABA, because in addition to being strongly implicated in suicide,[48] GABA is measurable *in vivo* using ^{1}H-MRS. However there are a multitude of other,

interrelated brain mechanisms associated with suicidality that uridine has also been shown to impact. A significant amount of evidence has accumulated for the stress HPA axis, and cortisol activity (corticosterone in rodents). There is replicated evidence that baseline cortisol levels are low in suicide attempters, and that HPA axis activity is blunted in suicide attempters, when compared to patients with similar clinical characteristics who have never attempted suicide.[60] Meanwhile, adrenocortical cells increase both production,[61] and secretion,[62] of cortisol in response to UTP. The mechanism for this is a non-activating K^+ current (Iac), that sets the resting membrane potential. ATP stimulates cortisol secretion, by inhibiting the Iac; UTP and UDP also inhibit Iac, by up to 81.4%, thereby mediating cortisol secretion.[61] Two other drugs with anti-suicidal effects -- ketamine and lithium -- also increase cortisol levels.

Table 1 Brain Mechanisms Common to Uridine*, Ketamine and Lithium

DISEASE	EFFECT	URIDINE *	KETAMINE	LITHIUM
Suicide	Engages Glutamatergic Neurotransmitter System	*Preliminary Data*	10, 63	64, 65
Suicide	Increased $GABA_A$ Receptor Activity	50	66	67
Suicide	Increased Cortisol or Corticosterone	68	69	70
Suicide	Increased Brain-Derived Neurotropic Factor	19	18	71
Suicide	Decreased Interleukin 6	72	73	74
Suicide	Decreased GSK3β Function	75	76	77
Suicide	Increased Synapse Formation	78	12	79
Suicide	Increased Long-Term Potentiation	80	81	82

***** Includes Uridine Precursors, and Uridine Nucleotides such as Uridine Triphosphate (UTP)

Ketamine's rapid antidepressant effect is dependent on mTOR activation.[12] mTOR activation leads to *de novo* pyrimidine synthesis,[13, 14] and the endogenous, circulating human pyrimidine = uridine.[83]

Abbreviations: GABA, gamma-aminobutyric acid; GSK3β, glycogen synthase kinase 3 beta

BDNF has also been strongly associated with suicide. As published in *Nature* and elsewhere, response to the prototypical rapid anti-suicidal drug ketamine is dependent on BDNF synthesis and release.[84] Here again, uridine has activity: the orally-administered uridine prodrug PN401 increases cortical BDNF by 11%.[19]

Recent work has focused on the immune system and inflammation in suicide. Specifically, serum and CSF measures of IL-6 are higher in suicide attempters vs. non-attempters.[85] Uridine, in the form of UTP and UDP, inhibits IL-6 release.[72] Similarly ketamine and lithium, have both been shown to decrease IL-6.

Finally, along with the HPA axis, perhaps the most evidence has accumulated for abnormalities of the serotonergic system in suicide. Inflammation may lead to serotonin deficiency via consumption of tryptophan, the serotonin precursor. Consistent with this,

selective serotonin reuptake inhibitor (SSRI) drugs are associated with a decreased risk of suicide. It is well-established that the onset of clinical benefit from an SSRI occurs 2-3 weeks after drug initiation, at the earliest. It is of specific relevance for this proposal, then, that chronic administration of the SSRI paroxetine for 24 days induces "profound" alterations in pyrimidine metabolism, with increased ratios in the high-to-low energy uridine nucleotides, i.e. UTP/UDP/UMP,[86] which the investigators stated suggests their involvement in the "delayed therapeutic treatment effect" of SSRIs.[86]

Summary of the Scientific Rationale for this Study.
Novel, rapid-acting treatments for veterans with SI are urgently needed. A promising lead in this arena is presented by uridine. As shown in **Table 1** above, the literature reflects a startlingly diverse overlap in effects relevant to SI shared by ketamine, lithium and uridine. What accounts for the commonality in effects and directionality shared by uridine, ketamine and lithium? The answer may lie in the mechanistic target of rapamycin (mTOR) pathway, on which the behavioral effects of ketamine are dependent.[12] Two *Science* articles reported that mTOR activates *de novo* pyrimidine synthesis.[13, 14] This is relevant to our proposal, as uridine is central to *de novo* biosynthesis. In fact, the authors of one paper found that rapamycin disrupted their experiment, but that it was rescued by application of uridine, in order to bypass pyrimidine biosynthesis.[14, 87] The fact that mTOR stimulates pyrimidine synthesis implies that ketamine up-regulates uridine as a downstream effect. This hypothesis was confirmed by Weckmann et al., who showed that pyrimidine metabolism is enriched and uridine concentration increases, 2 hours after ketamine injection.[88]

Experts recommend that with respect to the neurobiology of suicide, studies that acquire neuroimaging before and after interventions are instrumental for understanding the mechanisms, and treatment response, in suicidality. Furthermore, the imaging method for this proposal, ^{1}H-MRS, has been called "a pathway to diagnosis, novel therapeutics, and personalized medicine."[89] Ketamine's anti-suicidal effect appears to be independent of its antidepressant activity,[90] which offers the prospect that the proposed study will enable us to gather data regarding uridine's effect on SI, and simultaneously investigate ACC GABA as a biomarker of SI.

C Study Objectives

C1 Primary Aim 1

Aim 1. ***To Demonstrate that Uridine Decreases Suicidal Ideation in Veterans***. We hypothesize that 4 weeks of uridine 2000 mg daily will decrease the probability and severity of suicidal ideation, compared with placebo.

C2 Secondary Aims 2 and 3

Aim 2. ***To Measure Rapid Changes in Brain GABA, in Uridine-Treated Veterans with Suicidal Ideation***. The hypothesis is that GABA levels, measured with magnetic resonance spectroscopy, will show a greater increase after 1 week in uridine-treated vs. placebo-treated veterans with suicidal ideation.

Aim 3. ***To Examine the Duration/Durability of Uridine Treatment Response, in Veterans with Suicidal Ideation***. The hypothesis is that veterans with suicidal ideation randomized to uridine will demonstrate a durable clinical response over 4 weeks, in addition to acceptable patient compliance, satisfaction and engagement.

C3 Rationale for the Selection of Outcome Measures

The clinical outcome measures for this study were selected following a review of the suicidal ideation clinical trials literature. For example, the Columbia-Suicide Severity Rating Scale (C-SSRS) and Beck Scale for Suicide Ideation (SSI) were both administered, in nearly every study of suicidal ideation that has resulted in a peer-reviewed publication to date. Therefore, selection of the C-SSRS means that this study's results will be interpretable in the context of the extant literature. In addition, the C-SSRS has been widely adopted for use by both the VA mental health service, and by the active duty Armed Forces. Furthermore it is well-known that the U.S. Food and Drug Administration (FDA) recommends that the C-SSRS be administered in clinical trials of psychiatric drugs, antiepileptic drugs, and drugs with central nervous system activity [http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm225130.pdf]. All the clinical rating scales utilized in this study are standardized and validated, and their psychometric properties have been tested and published.

The magnetic resonance spectroscopy imaging, i.e. "translational neuroimaging," brain chemistry outcome measures of "target engagement," were selected based the Preliminary Studies conducted in our lab, and the converging scientific evidence that the amino acid neurotransmitters GABA, Glutamate and Glutamine may together play a role in suicidal behavior. Under the *National Institute of Mental Health* (NIMH) *Strategic Plan* [https://www.nimh.nih.gov/about/strategic-planning-reports/highlights/highlight-what-is-a-target.shtml], clinical trials must now utilize the "experimental medicine" format, in which the treatment intervention's "target" is specified; the term "target" refers to the hypothesized mechanism-of-action and it's ability to modify disease, behavior or functional outcomes.

In the case of this study, we hypothesize that Uridine will rapidly reduce suicidal ideation in veterans, and that this clinical effect will correlate with a concomitant increase, i.e. "target engagement" with, concentrations of GABA in the anterior cingulate cortex.

D Investigational Agent Uridine

D1 Uridine: An Endogenous Human Pyrimidine Nucleoside

Uridine is the circulating pyrimidine in human beings, and is required for normal brain function.[11] The purinergic system appeared early in evolution, and uses adenosine triphosphate (ATP) and uridine triphosphate and diphosphate (UTP; UDP) as transmitters.[53] The system is involved in modulating behavioral motivation and reward, as well as neuroregeneration. Uridine receptors are expressed on all primary cell types within the central nervous system; this includes neurons, astrocytes, microglia and endothelium. In mammalian brain, uridine nucleotides are the native agonist at four specific purinergic receptors: P2Y2, P2Y4, P2Y6 and P2Y14.[11]

In terms of safety versus potential toxicity, it is well-established that uridine is a key constituent in human mother's breast milk.[91-94] Furthermore, uridine is compounded as an ingredient into commercial infant formula products.[95]

Previously published studies of uridine, and uridine precursor drugs, have not provided evidence of any medically-significant morbidity or mortality, associated with uridine administration. In fact two uridine precursor drugs have recently been approved for sale

by prescription in the U.S.[96, 97] Other uridine precursors are available as dietary supplements, and are sold "over-the-counter" (OTC) in the U.S., i.e. without a prescription,[98, 99] as is uridine itself (https://binscience.com/brain-health-cognitive-support/choline-supreme; https://www.absorbyourhealth.com/product/uridine/).

D2 Uridine: Preclinical Data

Preclinical data is outlined in the *Uridine Investigator's Brochure*, which is included below in Section K3.

D3 Uridine: Clinical Data

Clinical data is presented above in Section B. Additional clinical data are presented in the *Uridine Investigator's Brochure*, which is included below in Section K3.

D4 Uridine: Dosing Rationale and Risk/Benefit

The proposed study is a single-center study, targeting a volunteer sample of veterans aged 18-65 with SI, to contrast the degree of change in C-SSRS and ACC GABA obtained between 2 treatment strategies: uridine 2000 mg daily or matching placebo. The dose in our previous adolescent uridine studies was 1000 mg daily; this was well-tolerated and no significant toxicity was observed in laboratory blood and urine studies, 12-lead electrocardiograms, or magnetic resonance imaging of the brain. In addition, there were no unresolved treatment-emergent adverse events reported by participants. In addition, the *Uridine Investigator's Brochure* reports that in human studies, doses up to 4000 mg – i.e. twice the dose administered in this study – have not been associated with dose-limiting adverse events or medical harm.

E Study Design

E1 Overview / Design Summary

This protocol is 4-week, randomized, double-blind, placebo-controlled clinical trial of uridine 2000 mg daily for Veterans with suicidal ideation.

Translational neuroimaging has been integrated into the study design. Magnetic resonance spectroscopy brain scans will be performed at baseline ("pre-treatment"), and then repeated after 7 days of treatment with uridine or placebo ("post-treatment"). The imaging will measure whether change in suicidal thoughts is associated with "target engagement," i.e. altered brain chemistry metabolites, including GABA.

As noted above, a systematic review found the reduction in suicidal thoughts in ketamine treatment responders lasts an average of ~3 days.[1] Thus the study is of more than adequate length (4 weeks), for comparison with ketamine's duration-of-action.

E2 Subject Selection and Withdrawal

2.a Inclusion and Exclusion Criteria

Inclusion Criteria

Inclusion Criterion	Rationale
Must be able to Provide Informed Consent	Good Clinical Practice
Must be a Veteran of the U.S. Armed Forces	Population of Interest
Columbia-Suicide Severity Rating Scale Indicates Current Suicidal Ideation	Disorder of Interest
Beck Scale for Suicide Ideation Score $\geq$ 4	Indicator of Significant Suicidal Ideation
History of $\geq$ 1 Suicide Attempt or Hospitalization to Prevent Suicide in Past 12 Months or Functionally Impairing Suicidal Ideation Not Due to a DSM Axis II Diagnosis, in Past 12 Months	Minimizes Placebo Response; Justifies Treatment
Females and Males Ages 18-65 Inclusive	Matches Neuroimaging Outcome Domains
Willing and Able to Identify an Alternative Contact, e.g. Family Member or Friend	Safeguard of Participant Safety

Exclusion Criteria

Exclusion Criterion	Rationale
Schizophrenia or Other Psychotic Disorder	Requires Different Treatment
Active Substance Use Disorder Requiring Stabilization (N.B. Does Not Include Nicotine).	Requires Different Treatment
Unstable Medical Condition(s)	Requires Medical Treatment Before Valid Psychiatric Assessment Can Be Performed
Pregnancy or Breastfeeding	Avoid Risk to the Unborn/Breastfed Child
Contraindication to MRI, e.g. Metallic Implant or Claustrophobic Anxiety	Standard Procedure for Imaging Research
Concurrent Enrollment in Another Clinical Trial	Standard Procedure for Clinical Research
Significant Risk of Protocol Non-Adherence (e.g. lives >50 miles from the hospital, and has no automobile or alternate transportation).	Unethical to Impose Research Burden on Participants Likely to Drop Out or Be Withdrawn

2.b Ethical Considerations

Participants will be withdrawn by the principal investigator, at any point in time when it is deemed no longer in the participant's best interests to continue in the study. This includes protocol non-adherence, as it is not possible to ascertain participant safety if the participant does not present for their scheduled study appointments, and does not respond to phone calls and emails from research personnel.

The patient population the study targets for enrollment, i.e. veterans with suicidal ideation, is a high-risk group. Thus it is anticipated that serious adverse events (SAEs) may occur during the course of the study. These will be handled according the policies

and regulations of the U.S. Veterans Health Administration, the U.S. Food and Drug Administration, and University of Utah Institutional Review Board.

2.c Subject Recruitment Plans and Consent Process

Participants will be recruited through clinician referrals, and IRB-approved advertising. The Salt Lake City VA Medical Center has a suicide prevention office, an inpatient psychiatry unit and a large network of multifaceted mental health outpatient programs. Recruitment efforts will target each of these resources, on an ongoing basis.

Veterans will provide both verbal and written informed consent, prior to the performance of any study-related procedures. They will also be told that informed consent is an ongoing 'process', as opposed to a one-time 'event' – and that they are welcome to revisit any aspect of it at any time. They will also be informed that research participation is 100% voluntary, which means that they are free to withdraw consent for further study participation at any time, for any reason – and in addition they are not required to offer a reason, should they choose to withdraw consent.

The Salt Lake City VISN 19 Rocky Mountain MIRECC has been serving as site for the VA Cooperative Studies Program Study 590 (CSP #590; Lithium for the Prevention of Recurrent Suicide Attempts; PI Dr. Ira Katz). As a result of this experience, the MIRECC has developed a system of identifying and screening veterans at the Salt Lake City station who have a significant history of suicidal behavior. Many potential CSP #590 participants refuse that study when approached – due to a prior history of receiving Lithium treatment, or because of recommendations from peers to avoid Lithium. We anticipate that veterans with suicidal ideation who refuse CSP #590 may be willing to consider enrolling in the uridine study as an alternative.

2.d Randomization Method and Blinding

A randomized, double-blind, placebo-controlled format will be used. The VA Salt Lake City Research Pharmacy will receive and package the active uridine capsules and matching placebo capsules. No study personnel who have participant contact will participate in this. The study drug will be stored in, and dispensed by, the VA Salt Lake City Research Pharmacy.

Block randomization will be performed in Stata (StataCorp LLC, College Station, Texas), by research personnel who have no contact with participants, and no contact with the VA Salt Lake City pharmacy. Based on the Stata output, a randomization log will be created which will be used to assign consecutively-enrolled participants to one of two treatment arms (e.g. 'A' or 'B') representing uridine or placebo, for the clinical trial.

2.e Risks, Benefits and Alternatives

Benefits: This is an IND study, so there are no guaranteed benefits to participants in this study.

Alternatives: At present the only medication with FDA approval for suicide prevention is clozapine, for patients with schizophrenia. However, veterans with schizophrenia are not eligible to enroll in this study; therefore, there are currently no FDA-approved alternative treatments available to the potential participants for this study.

The Potential Risks of Participation Include:

- During the intake and assessment interview, participants may become emotionally upset when asked about their psychiatric history including suicide attempts, or past experience of physical and/or sexual abuse, or traumatic experiences during military service and/or civilian life.

- Participants may experience discomfort or swelling when blood is drawn for laboratory testing. Rarely, mild infection can result from these blood draws.

- It is possible that a participant's illness could worsen during the study. This could be unrelated, or related, to the study. Veterans with suicidal ideation are at-risk for depressive symptoms, self-injurious behaviors, substance use disorders, worsening suicidal ideation, suicide attempts and psychiatric hospitalization as part of their condition. Study psychiatrists are available 24 hours per day, 365 days per year, including weekends and holidays. If a participant's illness worsens to the point that he or she presents an imminent suicidal or homicidal danger to themselves or to others, they will be hospitalized. Participants who are hospitalized will be withdrawn from the study.

- It is possible that treatment with uridine will not be effective for veterans with suicidal ideation, and that study participation will therefore delay the start of an effective treatment. If there were an FDA-approved drug to treat suicidal ideation other than Clozapine for persons with schizophrenia, study personnel would inform veterans of that fact during this study's informed consent process. However, veterans with schizophrenia are not eligible to enroll in the study because the presence of a psychotic disorder is one of the Exclusion Criteria.

- The researchers will take precautions to safeguard participant confidentiality, but it is possible that a breach of confidentiality could occur.

- Participants may experience gastrointestinal discomfort as a result of taking study medication. Therefore, we recommend taking the study medication with food to reduce possible stomach discomfort.

- In a research study of advanced cancer patients,[100] continuous intravenous (IV) infusions of uridine were administered at doses of 1.0 and 2.5 g/m^2/hour (or 1809 mg/hour and 4524 mg/hour, for a reference person 170 cm tall and weighing 70 kg, i.e. 5 feet 8 inches and 155 pounds). In the same study, intermittent uridine IV infusions were given to advanced cancer patients, at doses of 1.0-3.0 g/m^2/hour (1809 mg to 5429 mg/hour), for 72 hours.[100] In the intermittent group IV uridine for 3 hours alternated with 3-hour infusion-free periods. The total uridine in the intermittent treatment patients was between 21708 mg and 65148 mg, over a 72-hour period. The advanced cancer patients' blood levels of uracil were markedly elevated during the uridine infusions, and remained elevated during the 3-hour infusion-free periods. This cancer researchers concluded that this indicated, "a rapid and saturated catabolism of uridine to uracil."[100]

  In humans, ribonucleic acid (RNA) is a molecule that participates in the coding, regulation and expression of genes. RNA is made up of four nucleotide bases, including adenine, cytosine, guanine and uracil. In animal research, rats fed a

diet of 3% uracil for 15 weeks developed bladder stones.[101] The bladder stones were made of uracil, which does not dissolve well in water or urine. When the uracil 3% diet was stopped after 15 weeks, the researchers found the uracil stones gradually dissolve and disappear.[101] However the uracil 3% was continued for a total of 30 weeks in 5 rats. In these animals, transitional cell cancer of the bladder was found in 1 of the 5 rats.[101] The bladder tissue of rats is sensitive to mechanical irritation, and foreign bodies are known to cause tumors in rodents; this lead the researchers to comment that the hyperplasias (including 1 bladder cancer) and papillomas observed in the rats "were probably not due to a carcinogenic effect of uracil itself."[101] However, uracil could not be ruled out as the direct cause of the cancer found in 1 out of the 5 rats that were fed uracil 3% for 30 weeks. Therefore, the scientists' final conclusion about their research was: "It is unknown whether this (cancerous) transformation was due simply to the mechanical stimuli of the uracil stones or to some other unknown factors."[101]

2.f Early Withdrawal of Study Participants

Participants will be withdrawn automatically, if they experience a serious adverse event and are hospitalized. In addition, the principal investigator retains the right to withdraw participants from the study, if in the PI's judgment it is no longer in the participant's best interests to continue, e.g. if additional treatment(s) that are outside of the study protocol are clinically indicated, and it is unethical to withhold such treatment(s).

Because this protocol is not approved for "Research With Prisoners," if a participant is incarcerated during the study, they will be withdrawn.

The principal investigator may also withdraw participants, if they are unable or unwilling to be adherent to the protocol. This is because protocol non-adherence renders a participant's research data invalid and unusable. When this occurs, and therefore nothing of scientific value can be gained from an individual's further participation, it is unethical to impose further research burden(s) on the participant. In cases where the principal investigator arrives at this conclusion vis-à-vis non-adherence and continued participation, it is their ethical responsibility to withdraw the participant.

2.g When and How to Withdraw Participants

Participants will be informed they are being withdrawn from the study during a face-to-face meeting with the principal investigator. Clinical assessments will be collected that mirror the rating scales administered at the Week 4 visit. In addition, safety labs will be performed to document any treatment-emergent laboratory abnormalities.

Participants in clinical trials are sometimes "Lost To Follow-Up," meaning they stop coming to their appointments and no longer return phone calls and emails from the study staff. Sometimes this occurs because the participant has physically relocated, or the subject simply decides to no longer interact with the research team. If this occurs, staff will send a letter to the participant's last known mailing address via U.S. Mail, requesting that the participant return for a final visit, for clinical and laboratory testing.

2.h Data Collection and Follow-up for Withdrawn Participants

The principal investigator may withdraw participants for reasons including adverse effects, safety concerns or protocol violations. In addition, participants may elect to

discontinue study participation at any time. The research team will ask the participant to return the unused portion of the study medication, and the facility's treating mental health providers will assume responsibility for further patient care.

Participants who prematurely discontinue treatment due to an adverse effect or other event will be asked to participate in a clinical assessment approximately 4 weeks after the date of their last dose of study drug, to complete safety assessments. In addition, if indicated the principal investigator will repeat laboratory testing at this visit, to identify any treatment-emergent abnormalities.

E3 Study Drug

3.a Description

Uridine is a naturally-occurring pyrimidine nucleoside, that is essential for the synthesis of RNA and biomembranes. It is also a critical element in the regulation of cellular energetics and post-translational modifications; and in the synthesis of membrane ion channels, receptors, intracellular glycoproteins and glycolipids, excretion of drugs, steroids and bilirubin. Uridine is present in human breast milk,[91] and is compounded into commercial infant formulas.[102]

3.b Treatment Regimens

Uridine and matching pill placebo will be administered for 4 weeks at a fixed dose of 1000 mg twice daily by mouth, i.e. a total daily dose of 2000mg/24 hours.

3.c Method for Assigning Participants to Treatment Groups

As described above, a randomization log will be generated using the statistical software Stata (Release 15; StataCorp, College Station, TX). The VA Salt Lake City Research Pharmacy will utilize this log to assign consecutively enrolled participants to one of the two treatment arms (i.e. uridine or placebo).

3.d Preparation and Administration of Study Drug

The oral uridine study drug, and matching pill placebo, will be prepared for the study by Natural Pharmacia International, Inc. (NPI; Burlington, MA).

Dr. Perry Renshaw is Medical Director of the VISN 19 Rocky Mountain MIRECC, and one of the investigators on this study's research team. Dr. Renshaw is also the holder and sponsor of Investigational New Drug Application (IND) #74,122 for human studies of uridine; the IND was issued to Dr. Renshaw by the U.S. Food and Drug Administration. Dr. Renshaw has conducted several previous clinical trials of uridine, and for each of these studies the U.S. Food and Drug Administration granted its approval for NPI to serve as the manufacturer and provider of the investigational drug uridine.

3.e Treatment Adherence Monitoring

Adherence to treatment will be monitored. Participants will be asked to bring their study drug bottle with them to appointments, and pill counts will be monitored with the percentage of pills taken as-directed to be estimated. In addition to this, verbal inquiry and discussion of adherence will occur at study visits.

In addition, during the time between the 'pre-treatment' and 'post-treatment' brain scans, i.e. during the first 7 days of randomized, placebo-controlled treatment, participants will receive daily reminders to take their study drug from research staff. These will occur via the participant's preference of a combination of confidential methods, such as the following: email messages that do not contain Protected Health Information (PHI); and/or cellular phone text messaging. Email reminders can be automated using the RedCap software application package. Based upon individual preference, standard telephone calls will also be available, as a reminder method to support adherence with investigational drug.

3.f Concomitant Treatments

Concomitant medications, psychotherapy participation and adjunctive psychosocial interventions will be recorded at baseline, and any changes to the participant's mental health treatment plan will be recorded during the study.

3.g Packaging of Study Drug

Uridine and matching pill placebo will be manufactured by Natural Pharmacia International, Inc. (Burlington, MA) and shipped to the VA Salt Lake City Research Pharmacy. There, investigational study drug will be packed in bottles containing a uridine or matching pill placebo, with all bottles labeled: "Uridine/Placebo."

3.h Blinding of Study Drug

The study has a double-blind design: the participants and all research staff who have contact with participants, will be blind to treatment assignment (uridine vs. placebo). The capsules of uridine and placebo will be identical in appearance and weight. The blind will be maintained at the VA Salt Lake City Research Pharmacy

3.i Receiving, Storage, Dispensing and Return of Study Drug

At VA Salt Lake City and the University of Utah, investigational new drug (IND) studies are conducted in accordance with the principles of Good Clinical Practice, and in compliance with the U.S. Food and Drug Administration's published guidance documents for investigational drug studies.

F Study Procedures

F1 Screening for Eligibility

Potential participants will undergo a telephone screening, which establishes basic eligibility, i.e. "Are you enrolled as a patient in the VA health care system?" or "Are you currently enrolled in another clinical trial or research study?" Potential participants who pass the telephone screen will be invited for a baseline / screening visit, at which Informed Consent and a structured diagnostic interview are conducted.

F2 Study Protocol Schedule of Visits

The protocol is a 4-week randomized placebo-controlled trial, followed by a final safety visit that takes place 1 week after participants have discontinued study drug.

Uridine Randomized Controlled Trial: Schedule of Procedures

WEEK #	- 1	0	1	2	3	4	5
Informed Consent	☑						
SCID-5-RV Diagnostic Interview	☑						
PhenX Demographic Common Data Elements	☑						
Medical History; Physical Examination	☑						
Serum Laboratory Tests; Urinalysis; Urine Drug Screen	☑						☑
Pregnancy Test (females)		☑	☑				
Magnetic Resonance Spectroscopy Brain Scan		☑	☑				
Adverse Events; Concomitant Medications	☑	☑	☑	☑	☑	☑	☑
** C-SSRS, SSI, SBQ-R, INQ, ACSS, FNRS, BPAQ, RFLI, MADRS, HAM-A, BIS-II	☑	☑	☑	☑	☑	☑	☑
Patient Satisfaction; Patient Engagement	☑	☑	☑	☑	☑	☑	☑
Safety Planning Intervention	☑	☑	☑	☑	☑	☑	☑

** Note: The assessment measures that are included in the study protocol, and will be administered to participants, are described in detail in **Section F9** below, entitled "*Study Outcome Measurements and Ascertainment*."

F3 Baseline Screening Visit

Baseline-Screening Visit: Written and verbal consent are obtained prior to any study procedures. The following assessments are administered: Structured Clinical Interview for DSM-5, Research Version (SCID-V-RV); Beck Scale for Suicide Ideation (SSI); Columbia-Suicide Severity Rating Scale (C-SSRS); Suicidal Behaviors Questionnaire-Revised (SBQ-R); Interpersonal Needs Questionnaire (INQ); Acquired Capability for Suicide Scale (ACSS); Frustrative Nonreward Responsiveness Subscale (FNRS); Buss-Perry Aggression Questionnaire (BPAQ); Reasons for Living Inventory (RFLI); Montgomery-Asberg Depression Rating Scale (MADRS); Hamilton Rating Scale for Anxiety (HAM-A); Barratt Impulsiveness Scale, Revised Form (BIS-II). A medical history and physical exam are performed. Laboratory tests include a complete blood count, comprehensive metabolic panel, urinalysis and urine drug screen.

F4 Week 0 and Week 1: Neuroimaging Visits.

Week 0 and Week 1 - MRS Brain Scanning Visits: The format is the same for the Week 0 (pre-treatment) and Week 1 (post-treatment) scan visits. Neuroimaging with Magnetic Resonance Spectroscopy is performed, to measure any 'rapid' alterations in brain chemistry associated with 1 week of treatment with uridine vs. placebo. Clinical assessments are also collected.

F5 Weeks 1, 2, 3 and 4: Randomized Treatment Visits.

Week 1, 2, 3, 4 – Treatment Visits: At these visits participants' clinical status is assessed with the aforementioned rating scales, adverse events and concomitant medications are recorded, and the 24-hour safety plan is reviewed.

F6 Week 5: Post-Treatment Follow-Up Safety Visit.

Investigational drug treatment with uridine or placebo will be discontinued at the Week 4 visit, in compliance with the directions of the FDA. The protocol's final visit will occur at Week 5, when participants have been off study drug for one week. Clinical and safety assessments will be performed, participants will be thanked for their participation, and plans will be coordinated for participants to follow-up with their mental health treatment team. As a safety measure, study psychiatrists will remain available to study participants on a 24/7/365 basis until the participant has an appointment with a member of their VA mental health treatment team. Participants who are not enrolled with the VA Mental Health Service will be referred for an intake appointment.

F7 Neuroimaging Visits (Week 0 and Week 1): Proton-1 Magnetic Resonance Spectroscopy (^{1}H-MRS) Scans.

Proton-1 Magnetic Resonance Spectroscopy (^{1}H-MRS) Brain Scans**:** Scans will be acquired on a Siemens 3-Tesla whole-body MRI system (Siemens AG, Erlangen, Germany). Participants may discontinue scanning at any time if they experience discomfort, e.g. claustrophobic anxiety.

Anatomic MRI: Imaging to rule out structural abnormalities is obtained at the baseline scan, and board-certified Radiologists review all study scans. Scanning is performed at the University of Utah Neuropsychiatric Institute. The MR anatomic session allows localization of the Region of Interest (ROI) grid.

Two-Dimensional (2D) *J*-Resolved Proton MR Spectroscopy and Prior Knowledge Fitting (*ProFit*) Data Acquisition: A circularly polarized body coil and a manufacturer-supplied 12-channel phased-array head coil are used for radiofrequency (RF) transmission and signal reception, respectively. Participants are positioned supine with foam pads used to fixate the head within the coil housing. Three orthogonal low-resolution proton-weighted gradient echo (repetition time/echo time [TR/TE] = 20/5 ms; field-of-view (FOV) = 280 x 280 mm; matrix size = 192 x 144; 8 mm slice thickness) localizer images are obtained to confirm optimal head positioning.

Subsequently, static magnetic field (B_0) shimming is performed over the whole head FOV using a standard phase map method. Three-dimensional (3D) high-contrast and high-resolution T_1-weighted, magnetization-prepared, rapid gradient echo (MP-RAGE; TR/TE/TI = 2000/3.53/1100 ms; FOV = 256 x 256 x 224 mm; isotropic 1 mm in-plane resolution) MR images are then acquired and used to facilitate accurate MRS voxel positioning and for post-hoc within-MRS voxel tissue-type segmentation. The MRS anterior cingulate voxel measures 25 x 25 x 30 mm^3 for and is obliqued along the sagittal plane and positioned to cover predominantly gray matter. Within-voxel B_0 shimming is achieved using a manufacturer-supplied automated phase map procedure in combination with interactive manual shimming, until a full-width at half-maximum

(FWHM) of ≤ 11 Hz is observed for the real component of the ACC unsuppressed water signal.

A standard PRESS sequence was modified to enable 2D *J*-resolved ^{1}H-MRS measurements, where the first PRESS TE period (TE1) is fixed at 12 ms and the second TE period (TE2) is progressively incremented to sample the second (*J*) dimension. Spatial localization is achieved using a Hanning-filtered RF pulse of 2.6 ms duration (bandwidth (BW) = 5 kHz) followed by two identical optimized RF pulses of 7.0 ms duration (BW = 1 kHz) for slice-selective refocusing. The 2D *J*-resolved ^{1}H-MRS parameters are as follows: TR/TE = 2400/31-229 ms; ΔTE = 2 ms; 4 signal averages per TE step with online averaging; 2D spectral width = 2000 x 500 Hz; 2D matrix size = 2048 x 100).

The spectral data are obtained using a maximum-echo sampling scheme whereby the analogue-to-digital converter (ADC) on-time was fixed for all 100 TE steps. Outer-volume suppression (OVS) is achieved using six saturation bands positioned at least 1.5-cm away from the MRS voxel faces; band saturation is achieved using hyperbolic secant adiabatic full passage RF pulses. A three-pulse water elimination through T_1-effects (WET) scheme is interleaved with the OVS module for global water suppression. In addition, water unsuppressed 2D ^{1}H-MRS data are acquired from the voxel with 2 signal averages recorded for each TE step. The RF transmitter carrier frequency is set to 3.0 and 4.7 ppm for water suppressed and unsuppressed data, respectively.

Tissue Segmentation: Skull stripping and whole brain tissue-type segmentation is performed on MP-RAGE images using the BET and FAST tools provided with the FMRIB software library. In-house MATLAB (version R2010b, The MathWorks, Natick, MA) functions are used to extract the 3D volume corresponding to the positioned MRS voxel to obtain the within-voxel gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) tissue content for each patient. The GM percentage is calculated as the ratio to total brain matter.

Spectral Processing and Quantification: The 12-channel receive-only head coil is operated in the 4-cluster mode with each cluster comprised of 3 coil elements. Before channel recombination, eddy current distortions initially are accounted for using a previously reported time-domain method, where each TE step from a given 2D ^{1}H-MRS dataset is corrected using the corresponding cluster-specific water unsuppressed recorded at the same TE. Coil cluster-specific signal weighting coefficients are determined on an individual basis using the real component of the phased unsuppressed water signal data. A weighting coefficient for a given cluster is calculated as the maximum amplitude of the corresponding water spectrum, divided by the sum of the maximum amplitudes for all four-coil clusters. The weighting coefficients determined for each patient are applied to all water suppressed and unsuppressed FID data before signal recombination. The eddy current corrected and signal weighted time domain data from all four clusters are recombined on a TE-by-TE basis to afford a 2D matrix, characterized by 100 TE steps and 2048 complex points. The residual water signal is removed from each row of water suppressed 2D matrices using a Hankel singular value decomposition (HSVD) routine written in MATLAB. Finally, the 2D matrix is reformatted to produce the individual file types required for *ProFit* read-In. The *ProFit* algorithm is applied identically to all ^{1}H-MRS data using the supplied 2D basis set generated without considering the effects of spatial localization. Before the 2D fast Fourier transformation

(FFT), the raw 2D matrix is zero-filled to 200 points along the indirectly detected (*J*)-dimension.

The basis set is comprised of nineteen ^{1}H-MRS metabolites including Glutamate (Glu), Glutamine (Gln), GABA, Creatine (Cre), N-acetyl aspartate (NAA), and Lactate (Lac). The Cre methylene (CH_2) and methyl (CH_3) protons are fitted separately. For considering Cramer-Rao Lower Bound (CRLB) on the individual metabolite concentrations, the *ProFit* software first constructs the Fisher information matrix, and all metabolite CRLB values are calculated by the *ProFit* software with CRLB values <20% included in the data set. The software was further modified to calculate signal-to-noise (SNR) ratios based on the NAA CH_3 resonance at 2.0 ppm. 2D spectral regions-of-interest (ROI) are defined between 9.0 and 10.0 ppm and 1.75 and 2.25 ppm, which correspond to noise and NAA ROIs, respectively. All points along the *J*-dimension are used for reconstructing the 2D ROIs. The SNR is defined as the maximum absolute peak height calculated for the NAA ROI divided by the standard deviation of the real part of the noise ROI. The estimated metabolite 2D peak areas are normalized to the short TE = 31 ms unsuppressed water signal, which is calculated after fitting a Voigt lineshape to the real component of the phased frequency-domain unsuppressed water data. The nonlinear least-squares "lsqnonlin" function provided with the MATLAB Optimization Toolbox™ is used to fit the water data, with the initial estimate for signal amplitude being patient-specific, and based on the maximum peak amplitude. An initial estimate of 8 Hz is used for signal linewidth (LW) with the lower and upper bounds set to 1 and 20 Hz, respectively. The resulting metabolite/water ratios are corrected for within-voxel CSF-fraction determined using the relevant segmented MRI data.

F8 Safety and Adverse Events

8.a Safety Monitoring

Adverse events are recorded at baseline (i.e. prior to administration of investigational drug), and at every subsequent study visit. The National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE) will be used to classify events by organ system, with events further classified as either ‘Related’ or ‘Unrelated’ to study participation.

8.b Medical and Safety Monitoring

Investigators and the Research Team. The VISN 19 Rocky Mountain MIRECC Medical Director, Co-Director, principal investigator, co-investigators, and research coordinators will work as a team to monitor the study. The research team has worked closely together at the University of Utah since 2008, and has conducted clinical research with a variety of high-risk populations, including: veterans with traumatic brain injury and/or posttraumatic stress disorder and/or suicidal ideation and/or substance use disorders; adults and adolescents with treatment-resistant depression; adults and adolescents with bipolar disorder; and major psychotic-spectrum disorders such as schizophrenia. In conducting research with these challenging and complex patient populations, we have built a reservoir of experience to draw upon, and we have never been investigated, or sanctioned for unsafe conduct of human subjects research by any regulatory body or agency with oversight of our human studies.

Independent Expert Monitoring. For reasons outlined in the paragraph above, there is no scientific rationale for independent medical experts to monitor this study. The research team has conducted two prior clinical trials of uridine at the University of Utah, and for neither study did the U.S. Food and Drug Administration and University of Utah Institutional Review Board recommend that an Independent Medical Expert be employed as a medical monitor.

Independently-Chartered Data Monitoring Committee (DMC). An independent Data Monitoring Committee (DMC) will be chartered to monitor the study. The DMC consists of clinicians with experience treating patients with suicidal ideation, and scientists with experience in suicide research. The Committee will be vested with the authority to halt the study, if it concludes based on the yearly DMC Report that the study's risks to participants have become unacceptable, and the PI cannot agree with the Committee on how to revise the study so it can proceed safely.

8.c Definitions of Adverse Events

An "adverse event" is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not the occurrence is considered to be drug-related (21 CFR 312.32(a)).

The National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE) will be utilized to classify adverse events by organ system, with events further classified as "Related" or "Unrelated" to study participation and/or underlying illness.

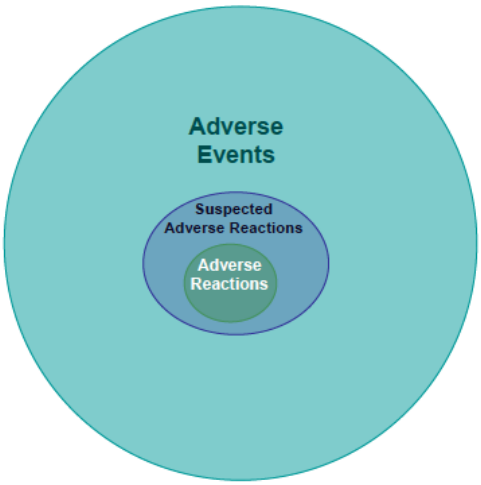
8.d Classification of Adverse Events

The investigators will follow and adhere to the U.S. Food and Drug Administration document, entitled: "Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies" (December 2012).

i Relationship to Study Participation

An adverse drug reaction will be classified as "related" to study participation, in the event a causal relationship with investigational drug is suspected or confirmed.

The diagram below was published by the U.S. Food and Drug Administration, to depict the relationship between adverse events, suspected adverse drug reactions, and adverse reactions:



ii Severity of Adverse Events

Adverse events will be classified as 'Mild', 'Moderate' or 'Severe'.

A separate category is reserved for occurrences classified as Serious Adverse Events (SAEs). An adverse event or suspected adverse drug reaction is considered "serious" if, in the view of the investigator or the sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect (21 CFR 312.32(a)).

iii Expected and Unexpected Adverse Events

"Expected" adverse events are those listed in the drug's Package Insert or the Investigator's Brochure, as well as the adverse events that are known to be associated with other medications in the same class of drugs.

An "Unexpected" adverse event is defined as an adverse event reported by a study participant, that is not listed in any of the above documentation.

8.e Data Collection Procedures for Adverse Events

At each participant contact, staff will inquire about adverse events and record them on the Case Report Form for entry into the study's RedCap database.

8.f Reporting Procedures

Reportable adverse events will be documented for submission to FDA using Forms 3500A and 1571.

8.g Mandatory Safety Reporting Periods

Initial Reporting: Unexpected serious suspected adverse reactions and observations from animal studies suggesting significant risk to human subjects will be reported to FDA no later than within 15 calendar days following receipt of the information. Unexpected

fatal or life-threatening suspected adverse reactions will reported no later than 7 calendar days following initial receipt of the information.

Follow-up Reporting: Information pertaining to a previously-submitted safety report will be submitted to FDA no later than 15 calendar days after the information is received. The type of report (initial or follow-up) will be indicated, and identified using the specific type of report:

- "IND Safety Report" for 15-day reports; or
- "7-day IND Safety Report" for unexpected fatal or life-threatening suspected adverse reaction reports; or
- "Follow-up IND Safety Report" for follow-up information.

F9 Study Outcome Measurements and Ascertainment

The Structured Clinical Interview for DSM-5–Research Version (SCID-5-RV).[103] The Structured Clinical Interview for DSM-5 (SCID-5-RV) is a semi-structured interview guide for making DSM-5 diagnoses, in the context of a research study. It is administered by a clinician or trained mental health professional who is familiar with the DSM-5 classification and diagnostic criteria for mental disorders.

PhenX – Mental Health Research Core Measures.[104] Investigators are encouraged to include the PhenX Core measures in VHA-funded mental health research. The research team will therefore include "Tier 1" PhenX Core Mental Health Measures in the study protocol.

The Beck Scale for Suicide Ideation (SSI).[105] The SSI is a 19-item self-report instrument, with each item scored 0-2, for a maximum total score of 38. A score of $\geq$ 4 has been reported to confer an increased risk of eventual death by suicide.[106] Therefore as an indicator of clinically-significant suicidal ideation, this will serve as one of the study's inclusion criteria.

The Columbia-Suicide Severity Rating Scale (C-SSRS).[107] The C-SSRS is a suicidal ideation rating scale created by researchers at Columbia University to evaluate suicidality in patients ages 12 years and up. It rates an individual's degree of suicidal ideation on a scale, ranging from "wish to be dead" to "active suicidal ideation with specific plan and intent." The scale identifies behaviors which may be indicative of an individual's intent to commit suicide. An individual exhibiting even a single behavior identified by the C-SSRS is thought to be 8 to 10 times more likely to commit suicide.

The Suicidal Behaviors Questionnaire-Revised (SBQ-R).[108] The SBQ-R is a brief self-report measure of past suicidal behaviors. The questionnaire assesses four domains: previous suicide attempts, frequency of suicidal ideation, previous suicidal communication, and subjective likelihood of future suicide attempt.

The Interpersonal Needs Questionnaire (INQ).[109] This measure was designed to measure participants' current beliefs about the extent to which they feel connected to others (i.e., Belongingness), and the extent to which they feel like a burden on the people in their lives (i.e., Burdensomeness). These, along with the Acquired Capacity for Suicide, are some of the key concepts of the Interpersonal Theory of Suicide,[110, 111]

which is a prevalent model of suicidal behavior in clinical psychology.

The Acquired Capability for Suicide Scale (ACSS).[112] The ACSS is a 5-item measure designed to assess one's fearlessness about, and ability to enact, lethal self-injury. These, along with Burdensomeness and Thwarted Belongingness, are some of the key concepts of the Interpersonal Theory of Suicide,[110] which is a prominent model of suicidal risk and behavior in clinical psychology.

The Buss-Perry Aggression Questionnaire (BPAQ).[113] Within the National Institute of Mental Health's (NIMH's) Research Domain Criteria (RDoC) initiative, "Frustrative Nonreward" is designated as a "Negative Valence System," that can be measured via self-report with the BPAQ.[114] To make this study's results interpretable in the context of RDoC, the BPAQ will therefore be administered.

The Frustrative Nonreward Responsiveness Subscale (FNRS).[115] The FNRS is a self-report subscale to be used with the BIS/BAS Scales, the well-established measure originally developed by Carver and White.[116] The FNRS measures lowered approach motivation, following nonreward. Within the NIMH RDoC framework, this "Frustrative Nonreward" is designated as a "Negative Valence System," that can be measured using the FNRS.[117] Inclusion of the FNRS will therefore support the relevance of the study's findings, in the context of RDoC.

The Reasons for Living Inventory (RFLI).[118] The RFLI includes 48 self-report items that measure respondent attitudes about living versus attempting or committing suicide, and the reasons that underlie them. The RFLI includes subscales for survival and coping beliefs, responsibility to family members, child-related concerns, fear of suicide, fear of social disapproval and moral objections. Each item is rated on a Likert scale ranging from 1 ("not at all important") to 6 ("extremely important"). Ratings on the individual items are added together to give scores for the individual subscales, and to obtain an overall score for the entire inventory; higher scores reflect greater reasons for living. The RFLI is a brief, low-burden, and widely-used questionnaire with good internal reliability, test-retest reliability and concurrent validity. Importantly, the RFLI also captures changes in beliefs (e.g., reductions in suicidal behavior).

The Montgomery-Asberg Depression Rating Scale (MADRS).[120] The MADRS is a ten-item diagnostic questionnaire used to measure the severity of depressive episodes in patients with mood disorders. It was designed to be sensitive to the changes brought on by antidepressant medication treatment. The MADRS suicide item has been shown to have a correlation of $r > .80$ with the first five items of the Beck SSI.[121]

The Hamilton Rating Scale for Anxiety (HAM-A).[122] The HAM-A was one of the first rating scales developed to measure the severity of anxiety symptoms, and remains widely-used in both research and clinical settings. The scale consists of 14 items, each defined by a series of symptoms, and is designed to measure both psychic anxiety (i.e. mental agitation and psychological distress) and somatic anxiety (i.e. physical complaints and symptoms related to anxiety). The HAM-A is a frequent outcome measure in clinical trials of both medication and psychotherapy.

The Barratt Impulsiveness Scale, Revised Form (BIS-II).[123] The BIS-II is a questionnaire designed to assess the personality and behavioral construct of

impulsiveness. It is the most widely-used measure of impulsive personality traits, and has helped to advance our understanding of the relationship between impulsivity other clinical phenomena, including suicidal behavior. The BIS-II includes 30 items that are scored to yield six first-order factors (attention, motor, self-control, cognitive complexity, perseverance and cognitive instability impulsiveness), and three second-order factors (attentional, motor and non-planning impulsiveness).

Safety Planning Intervention (SPI).[124] The SPI has been identified as a best practice by the Suicide Prevention Resource Center/American Foundation for Suicide Prevention Best Practices Registry for Suicide Prevention (www.sprc.org), which can be administered as a stand-alone intervention. The SPI consists of a written, prioritized list of coping strategies and sources of support that patients can use to alleviate a suicidal crisis. The basic components of the SPI include (a) recognizing warning signs of an impending suicidal crisis; (b) employing internal coping strategies; (c) utilizing social contacts and social settings as a means of distraction from suicidal thoughts; (d) utilizing family members or friends to help resolve the crisis; (e) contacting mental health professionals or agencies; and (f) restricting access to lethal means.

G Statistical Plan

G1 Sample Size Determination and Power

Sample Size Calculations. Each Approach section and Specific Aim maintains an adequate estimated required sample size. The minimum required sample size satisfying our study hypotheses is n=72 veterans with SI, or n=36 each in the uridine and placebo groups. We anticipate ~20% attrition due to dropouts and unusable scans due to motion artifact in the scanner. A total enrollment of n=90 supports our research goals.

G2 Interim Monitoring and Early Stopping

Interim data analyses will be performed by staff who have no contact with research participants, for inclusion in the annual DMC Report. Because a major portion of Year 1 of the study will be devoted to administrative and research start-up tasks, we anticipate enrollment, and thus the total sample size, will be modest at the conclusion of Year 1. Therefore, interim analyses will be included in the annual DMC Report for Years 2, 3, and 4. The analysis will be performed using a modified Kaplan-Meier approach,[125] with pre-specified Peto-Haybittle monitoring boundaries.[126, 127] Use of statistically conservative Peto-Haybittle stopping rules (i.e. $p<0.001$) for an investigational drug's benefit and futility, avoids prematurely terminating a clinical trial when there is insufficient scientific evidence to do so.[128, 129]

The study's independently-chartered DMC will use the interim analysis, together with the DMC Annual Report's comprehensive summary of unanticipated, related and serious adverse events, in its determination of whether to halt the study on ethical and/or scientific grounds.

G3 Analysis Plan and Statistical Methods

Specific Aim 1. **To Demonstrate that Uridine Decreases Suicidal Ideation in Veterans**: We hypothesize that 4 weeks of uridine 2000 mg daily will decrease the probability and severity of suicidal ideation, compared with placebo.

This hypothesis evaluates the efficacy of uridine in rapidly decreasing the probability of "wish to be dead" in veterans with SI. Based on our C-SSRS preliminary data (Figure 4), the change in binary probability is estimated to be 0.42 and 0.11 for uridine and placebo, respectively. The autocorrelation is assumed to be 0.65. Consequently, when longitudinal logistic regression analysis (Generalized Estimating Equation) is used for n=2 repeated measures of "Wish to be Dead," the required sample size is calculated to be a total of n=72 (n=36 per treatment group), at an alpha level of 0.01 and 80+% power with an independent working correlation.[130]

We will also score the C-SSRS quantitatively after the PhenX recommendations and the publication of Sahlem et al,[131] with whom we have corresponded. Analyses of a continuous variable (C-SSRS scoring range 0-25) are likely to have superior power to analyses of dichotomous variable (e.g. presence vs. absence of SI). Thus, we believe our estimated sample size is 'conservative', and that power is more than adequate.

Specific Aim 2. **To Measure Rapid Changes in Brain GABA, in Uridine-Treated Veterans with Suicidal Ideation**: The hypothesis is that brain GABA levels, measured with magnetic resonance spectroscopy, will show a greater increase after 1 week, in uridine-treated vs. placebo-treated veterans with suicidal ideation.

This hypothesis tests whether uridine will alter the inhibition/excitation ratio and increase GABA, the major inhibitory neurotransmitter in brain. The *Preliminary Data* from our uridine study of adolescent bipolar depression suggests that an increase in GABA/Glx is associated with uridine administration (**Figure 5**). The effect size for △GABA/Glx was approximately 11% of the mean, with a standard deviation of 16%. Therefore, if we set the anticipated minimum detectable effect size (MDES)[132] for the proposed study at 11%, a total sample size of n=72 achieves 88% power to detect the change in GABA/Glx ratio associated with uridine treatment (two repeated measures, and a conservative intra-class correlation of ρ=0.65).

Specific Aim 3. **To Examine the Durability of Uridine Treatment Response, in Veterans with Suicidal Ideation:** The hypothesis is that veterans whose suicidal ideation responds to treatment with uridine will demonstrate a durable clinical response over 4 weeks, in addition to acceptable patient compliance, satisfaction and engagement.

We believe it is worth noting our previous experience with conducting a 6-month uridine extension trial. In our placebo-controlled uridine trial of bipolar depression, a total of n=37 participants were randomized. Of those, n=28 completed 6 weeks of randomized treatment and 2 brain scans, and were subsequently offered the 6-month extension phase of the protocol. Of these n=18 (64.3%) *completed all six months of open-label uridine*. We are therefore optimistic that for veterans whose suicidal ideation responds to uridine, we may be able to demonstrate a more 'durable' anti-suicidal effect, than the average 3-day duration of reduced SI with ketamine infusions, that was reported by a systematic review of the peer-reviewed ketamine literature.[1]

Milestones and Indicators of Uridine Feasibility as a Rapid-Acting Treatment for Veterans with Suicidal Ideation in a 4-week Randomized Placebo-Controlled Trial:

- Evaluate screening feasibility. *Indicator = Number screened per month. Goal = 2-3/month.*
- Evaluate recruitment feasibility. *Indicator = Number enrolled per month. Goal = 1-2/month.*
- Evaluate retention feasibility. *Indicator = Retention rate for each treatment group (uridine and placebo).*
- Evaluate randomization feasibility. *Indicator = Proportion eligible who enroll. Goal ≥ 90%.*
- Evaluate assessment feasibility. *Indicator = Fealty to the PhenX Measures and C-SSRS; duration of visit.*
- Evaluate treatment adherence. *Indicator = Adherence rates for each treatment group (uridine and placebo).*
- Evaluate implementation feasibility. *Indicator = Participant satisfaction.*
- Evaluate data collection, management and sharing feasibility. *Indicator = No missing data in key variables for analysis, except for missed study visits or failed brain scan attempts (e.g. due to claustrophobic anxiety, or subject movement in the scanner during the MRI). No unauthorized release of Protected Health Information. Data is securely archived and transferrable to digital media for data sharing.*

G4 Missing Outcome Data

Missing data has become an important topic in contemporary clinical trials design and analysis. Publications on the prevention, and statistical management, of missing data have been published recently by the *National Research Council*,[133] the *New England Journal of Medicine*,[134] *Biological Psychiatry*,[135] and the *American Journal of Psychiatry*.[136] We have reviewed this and other literature on this subject, and will follow published expert recommendations.

Missing data are defined as values that are not available, and that would be meaningful for analysis if they had been observed and recorded. The assumption that statistical methods can compensate for missing data is not always justified; therefore aspects of trial design, and conduct, that limit the likelihood of missing data are an important consideration in human subjects research.

The missing data article in the *New England Journal of Medicine* (Table 1; page 1356)[134] lists eight key “ideas” for limiting missing data, in the design of a clinical trial. The protocol for this study adopts four of these eight recommendations:

- Target a population that is not adequately served by current treatments and hence has an incentive to remain in the study.

- Utilize add-on designs, in which a study treatment is added to an existing treatment, typically with a different mechanism-of-action known to be effective in previous studies. (This study does not feature a medication “washout,” and

veterans will have uridine or placebo added to their existing medications.)

- Shorten the follow-up period for the primary outcome. (The primary neuroimaging endpoint is collected following 1 week of treatment, and the primary clinical endpoint data is collected after 4 weeks of treatment.)

- Avoid outcome measures that are likely to lead to substantial missing data. (Almost every study conducted by our research team at the University of Utah has an imaging component. Our experience with magnetic resonance spectroscopy dates back 29 years, to *in vivo* measurement of lithium in human brain.[137] The cumulative experience allows us to minimize the amount of missing data in our current research studies.)

Four statistical methods for handling missing data, that are often utilized in published reports of longitudinal treatment trials, include the following:

1) Complete-Case Analysis: analyzing only participants with no missing data;
2) Single Imputation Methods: these include use of the last observation carried forward, or baseline observation carried forward, for analysis;
3) Estimating-Equation Methods; and
4) Methods Based on a specified Statistical Model.

Complete-case analysis and single imputation methods were strongly discouraged by a panel of statistical experts convened by the *National Research Council*.[133] By contrast, weighted estimating equations and multiple-imputation models offer an important advantage: they are capable of incorporating auxiliary information about the missing data into the analysis, and also provide standard errors and p-values that take missing data uncertainty into account, including it the analyses. For example, pattern mixture models can incorporate the pattern of missing data in the study's dataset into the model.[138] Another approach is shared parameter modeling, in which the assumptions with regard to missingness are incorporated into the model.[139] To assess the robustness of the study's statistical results, sensitivity analyses will be performed using a variety of assumptions; if the "treatment effect" associated with uridine is maintained for a range of clinically-plausible offset assumptions, then the reductions in suicidal ideation and/or the increases in brain GABA concentrations will be considered to be robust. An issue we could encounter, is that the statistical methods for handling missing data can be reliant on relatively large sample sizes.[140] If our dataset is not sufficiently large for reliable and valid utilization of the methods advocated by the *National Research Council*, we will explore other options and consult with other neuroimaging laboratories.

The principal investigator and research coordinator will monitor the Case Report Forms (CRFs) for missing data. When missing data is noted, the CRF is flagged. The principal investigator and study coordinator will review forms with missing or ambiguous data on an ongoing basis. If possible, participants will be contacted in an attempt to collect the missing data. If the data cannot be captured, it will be coded in the study's database as "missing." The quantity, and rate of missing data will be included in the annual report to the study's Data Monitoring Committee.

H Data Handling and Record Keeping

H1 Confidentiality and Security

Only research staff will have access to participants' Protected Health Information (PHI); this information will be stored in locked cabinets, and on password-protected computers inside locked offices. The hard drives of all computers used for the study are encrypted by the University of Utah's Information Technology department. Data analyses will be performed in the aggregate, and no participant's PHI will be featured in scientific presentations or publications.

H2 Training

All study personnel are required to complete research ethics training through the Collaborative Institutional Training Initiative [CITI; www.citiprogram.org].

H3 Case Report Forms and Source Documents

The study's Case Report Forms will contain participants' initials, and a unique study identification number. CRF binders are stored in locked cabinets, in locked offices, and on a floor that requires badge identification to access. All documentation and reports transmitted as required, e.g. to the Institutional Review Board, U.S. Food and Drug Administration, or the centralized VA Data Monitoring Committee will be HIPAA compliant.

H4 Records Retention

In terms of "records retention," the research team will comply with all aspects of the "Guidance on VA Research Records and the Impact of the Federal Records Act."

I Study Monitoring, Auditing, and Inspecting

I1 Study Monitoring Plan

The principal investigator (PI) is responsible for monitoring the quality and completeness of study data. In addition, all VA merit review grant clinical trials are required to be monitored by the VA's national, centralized Clinical Science Research & Development Data Monitoring Committee (DMC). In compliance with that VA requirement, this study will be monitored by the DMC.

Purpose and Responsibilities of the National Veterans Administration Centralized Data Monitoring Committee (DMC). The DMC is primarily responsible for safeguarding the interests of study participants, assessing the safety and efficacy of trial interventions, and monitoring the progress of the study. The DMC will conduct reviews of this clinical trial, and monitor its recruitment. The DMC will serve as an independent advisory group to the Veteran Administration's Director of Clinical Science Research & Development (CSR&D), and is required to provide recommendations about starting, continuing, and stopping the study.

Approximately every 4 months, the DMC will receive data from the research team, and will evaluate the following:

- Review the study protocol, pertinent accessory documents, and plans for data and safety monitoring
- Review methodology used to help maintain the confidentiality of the study data and the results of monitoring by reviewing procedures put in place by investigators to ensure the privacy of study participants
- Monitor study design, procedures, and events to maximize study safety and minimize risks to study participants
- Evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment and retention, participant risk versus benefit, performance of the study site(s), and other factors that may affect study outcome
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study
- Review serious adverse event (SAE) documentation and safety reports and make recommendations regarding protection of the safety of the study participants
- Report to the Director, CSR&D, and the Principal Investigator (PI) on issues concerning study safety progress
- Evaluate and report to the Director, CSR&D, and the study PI on any perceived problems with study conduct, enrollment, sample size, and/or data collection
- Provide to the Director, CSR&D, and the PI a recommendation regarding continuation conditionally or unconditionally, probation, termination or other modifications of the study based on the cumulative experience including the observed benefit or adverse effects of the treatment under study; as well as data observations that indicate the likelihood of definitively addressing the goals of the study.

The VA DMC is responsible for identifying mechanisms for the completion of various tasks that will impact the safety and efficacy of study procedures and overall conduct of the study.

The study team will furnish the VA Centralized DMC Office with the following reports, at a minimum of 30 days, before each meeting or conference call in order to allow the DMC members adequate time to review and prepare for the meeting at hand. Meeting materials will include the following research reports and data:

- Adverse Events (AE) Data
- Serious Adverse Events (SAE) Data
- Unexpected Problems (UP) Data
- Graphed Enrollment Data (Actual versus Projected)
- Recruitment and Retention Data
- Statistical Analysis of Study Progression.

The DMC will review the above information at DMC meetings depending on the type of review being conducted to ensure proper conduct of the study. The DMC may also apply other metrics in making its assessments such as targeted enrollment versus actual enrollment.

Proper records will be collected at each DMC meeting to ensure that there is a physical record of any and all decisions and recommendations. The required documentation for DMC meetings for the study includes the following:

- Identification of DMC members available for the meeting
- Identification of DMC administrative personnel available for the meeting
- Identification of excused members
- Notification of changes in membership and next scheduled DMC meeting
- Review of study data
- Review of study summary report
- Committee recommendations and approvals.

The VA CSR&D Program Staff will be responsible for the preparation of committee minutes that address the above. Minutes must then be reviewed for accuracy in collaboration with the VA DMC Administrator and VA DMC Manager and then endorsed by the DMC Chairperson before being submitted to Director of CSR&D for the VA, for final approval. Meeting minutes, endorsed by the DMC's Chairperson, must be submitted to the Director, CSR&D within a timely manner, typically 10 working days following a convened DMC meeting.

The Director, CSR&D, will review the meeting minutes, request additional information and then endorse the minutes. A copy of these minutes, reflecting the committee's review, and the Director, CSR&D's, requests or recommendations, is then sent to the PI and the PI's VA facility Associate Chief of Staff for Research (ACOS/R).

Once DMC minutes have been endorsed by the Director, CSR&D, they are considered final and archived with other VA DMC documentation. Investigators receive copies of signed and approved minutes from the DMC for open sessions only while the study is active.

The finalized DMC meeting minutes from the national centralized VA DMC, will be submitted to the University of Utah Institutional Review Board annually.

Tracking and Monitoring of Data as Required by the University of Utah Institutional Review Board. The following is a summary of the data that will be collected and reported to the Institutional Review Board (IRB), when the study's IRB application undergoes its annual review.

I. Proposed amendments to the study protocol, and the IRB's decision to approve or not to approve them.

II. An accounting of the total number, and the rate, of Adverse Events reported to the research team by study participants, presented by treatment condition (Investigational Drug vs. Healthy Control) and by event category:
    a. Serious Adverse Events and their outcomes
    b. Unanticipated Adverse Events
    c. Adverse Events related to the research
    d. Adverse Events unrelated to the research

III. A description of Unanticipated Problems that have occurred, and their outcomes:
    a. Unexpected, research-related adverse events
    b. Breaches of participant confidentiality or privacy that involved real or potential risks, such as unauthorized use or disclosure of participants' protected health information (PHI)
    c. New information about the effect on health or safety, or any life-threatening problem or death caused by, or associated with, the study investigational drug
    d. New information indicating a change to the risks or benefits of the research that may be different than initially presented to the IRB
    e. Publication(s) showing that the risks or potential benefits of the research may be different than initially presented to the IRB
    f. A change in FDA labeling, or withdrawal from the market of the investigational drug (uridine) used in the research protocol
    g. Incarceration of a participant, because the study is not permitted to enroll prisoners
    h. Complaints from participants or others involved in the research that indicate unexpected risks to participants, and their outcomes

IV. A list of any warning or determination letters regarding the investigational drug (uridine) issued by a funding agency or regulatory body, including the Office of Human Research Protections (OHRP), the Department of Health and Human Services (DHHS), or the Food and Drug Administration (FDA):
    a. Warning Letters
    b. Notice of Initiation of Disqualification Proceedings and Opportunity to Explain Letter (NIDPOE)
    c. Notice of Opportunity for Hearing (NOOH)
    d. Notice of Disqualification
    e. Consent Agreements
    f. Clinical Hold Letters

V. A list of Protocol Deviations that occurred, and their outcomes:

a. Deviations that were intended to eliminate an apparent or immediate hazard to a research participant
b. Deviations that caused harm to participants or others, or placed them at increased risk of harm – including physical, psychological, economic, or social harm
c. Episodes of Serious Noncompliance, meaning an act or omission to act that resulted in increased physical, psychological, safety, or privacy risk that compromised the rights and welfare of participants
d. Episodes of Continued Noncompliance, meaning a pattern of repeated actions or omissions to act suggesting the future likelihood of reoccurrence, and that indicated a deficiency in the ability or willingness of the research team to comply with the Code of Federal Regulations (CFR) or the policy, requirements, and determinations of the IRB governing human participant research at the University of Utah

VI. A list of Breaches of Privacy that occurred, their outcomes, and recommendations for added protections to reduce the likelihood of reoccurrence

VII. A list of Breaches of Confidentiality that occurred, their outcomes, and recommendations for added protections to reduce the likelihood of reoccurrence

VIII. An assessment of the study's Data Quality and Integrity:
a. An evaluation of participant Recruitment and Retention, including the reasons given for early withdrawal in participants who did not complete the protocol
b. An evaluation of participants' adherence to the study protocol:
i. Adherence to the study medication regimen
ii. Missed clinic visits
iii. Completion rate of procedures, such as MRI scans and laboratory studies
iv. Recommendations to increase protocol adherence, if applicable
c. An evaluation of the completeness and quality of the key data elements needed to characterize the participants and their primary and secondary outcome measures
d. An evaluation of the research team's efforts at data capture and management, including the quantity of Missing Data and recommendations to reduce the rate of Missing Data, if applicable
e. An evaluation of data entry and accuracy, and recommendations for improvement, if applicable

The integrity of the study's data will be protected and monitored through: a) training for research team members; b) research staff meetings; c) regular review of case report forms (i.e. paper data), and regular review of electronic data (i.e. RedCap database).

Adverse Events Monitoring. At each research visit, the investigator will record information regarding any adverse events that have occurred by specifically questioning and, when appropriate, by examination. All adverse events occurring during the study period will be documented. The investigator will follow the clinical course of each adverse event until resolution, stabilization, or until it has been determined that the research procedures or interventions were not the cause of the adverse event. Serious adverse events that are ongoing at the end of the study period will be followed to determine a final outcome. The PI will determine whether an unexpected adverse event is related or unrelated to the investigational drug, or to a research study procedure. An adverse event is "related to the research" if in the opinion of the PI, it was more likely than not related to the investigational agent or intervention. If unanticipated problems (UPs) or unanticipated adverse events (UAEs) occur, they will be promptly reported to the FDA in accordance with published guidelines.

Tracking of Participants and Data. Using a password-protected computer with access limited to study personnel, the research team will use an encrypted computers to record the research data in RedCap. Participant screening, eligibility status, and consent for participation status will be recorded in the Participant Screening Database.

As participants enroll in the study, a record in the Participant Tracking Database will be created with entries for each visit containing each piece of data captured. The date of the visit will be entered into the database. Missed visits will be recorded, along with the reason. Within each visit, a variable will be coded for each data point expected for that evaluation, containing either the data a code for "missing data." If a participant withdraws from the study prior to completing the protocol, the date and reason for early termination will be recorded in the Participant Tracking Database. The study's recruitment, retention, protocol adherence, and protocol completion data will be monitored on an ongoing basis, and the research team will identify and address problem areas that arise.

Data Confidentiality and Security. Study CRFs will be designed to contain participants' initials and a unique study identification number. All documentation and reports transmitted to the IRB, FDA, and VA DMC will be HIPAA compliant. Only the study staff will have access to participants' Protected Health Information (PHI), and this information will be stored in locked cabinets in a locked office, and on encrypted, password-protected computers inside locked offices. The hard drives of all desktop, laptop and notebook computers used for the study are encrypted by the University of Utah's and/or Salt Lake City VA Medical Center's Information Technology departments. Data analyses will be performed in the aggregate, and no participant's PHI will be featured in scientific presentations or publications.

I2 Auditing and Inspections

The study will undergo an Investigational New Drug (IND) pre-review at the University of Utah. Once the study begins, the research team will comply with all inspection and auditing requests and directives from the U.S. Department of Veterans Affairs, the U.S. Food and Drug Administration and the University of Utah Institutional Review Board.

J Study Administration

J1 Organization and Participating Centers

In one sense, this clinical trial is considered a multi-site study, because the study's research data will be collected at two sites: 1) the Salt Lake City VA Medical Center; and 2) the University of Utah School of Medicine, where the research-dedicated MRI scanner is located. However, the Salt Lake City VA and University of Utah are affiliated with one another, and the investigators for this study are appointed to the medical staff of both institutions.

J2 Funding Source and Conflicts of Interest

The funding source is a Merit Review grant (1-I01-CX001611) from the Veterans Health Administration. Conflicts of interest at the University of Utah are identified and managed through the Conflicts of Interest Office, in the Office of the Vice-President for Research.

J3 Committees

This study is a single-site, small-scale, translational neuroimaging trial. It is not anticipated that there will be a need for standing committees.

J4 Participant Stipends or Payments

Participant Compensation for Time and Travel. Veteran participants will be offered compensation for time and travel for their initial informed consent, evaluation and screening visit. The compensation is $100 for each of two brain MRI imaging visits (Week 0 and Week 1), $25 for the randomized treatment non-MRI study visits (Weeks 2, 3 and 4). The compensation is $75 for the final Week 5 visit, which includes clinical assessments and safety laboratory testing including blood and urine sample collection.

If you are traveling more than 50 miles round trip per study visit, you will be given an additional $20 for each completed research visit. Therefore, your total compensation for the study will be $590.

J5 Study Timetable

Study Activity	Months 0-6	Months 6-12	Months 12-18	Months 18-24	Months 24-30	Months 30-36	Months 36-42	Months 42-48	Months 48-54	Months 54-60
VA, FDA, IRB Approvals										
Participant Recruitment										
Clinical Trial Treatment										
Data Collection & Storage										
Data Analysis										
Presentation, Publication										
Dissemination of Findings										

K Attachments

K1 Targeted/Planned Enrollment Table

After accounting attrition and subject movement artifact in the MRI scanner that renders data unusable, we plan to enroll a sample of n=90 veterans, in order to collect a complete clinical and imaging datasets for n=72 participants, all at the Salt Lake City Veterans Affairs Medical Center. Participants will be adults of both genders, and of any race and ethnicity. Because of the potential for brain chemistry to change with advancing age, we plan to enroll participants between the age of 18 and 65 years. To our knowledge, there are no known race or ethnic differences in clinical response, or adverse events, associated with uridine. Therefore, the proposed study is designed to include all eligible minority participants, who are estimated to comprise 10% of the total sample. The Targeted/Planned Enrollment Table below is based upon demographics for Utah taken from the 2010 United States Census, which showed that the Salt Lake City metropolitan area has a population that is greater than 80% white. African-American, Asian and Native American persons combined to make up 5.8% of the local population. Similarly in 2013, it was estimated that approximately 10% of veterans in the VA Salt Lake City catchment area were female.

Targeted/Planned Enrollment Table

Study Title: Clinical and Imaging Biomarker Trial of Uridine for Veterans with Suicidal Ideation
Total Planned Enrollment: 90

TARGETED/PLANNED ENROLLMENT: Number of Participants			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	8	9
Not Hispanic or Latino	8	73	81
Ethnic Category: Total of All Participants *	9	81	90
Racial Categories			
American Indian/Alaska Native	1	2	3
Asian	1	2	3
Native Hawaiian or Other Pacific Islander	1	2	3
Black or African American	1	2	3
White	5	73	78
Racial Categories: Total of All Participants *	9	81	90

K2 Questionnaires, Surveys and Rating Scales

Inclusion of copies of the clinical assessment measures and questionnaires that will be administered to veteran participants in this protocol (e.g. SCID-5-RV, C-SSRS, SSI, SBQ-R, INQ, ACSS, FNRS, BPAQ, MADRS, PhenX) would increase the length of this protocol by >200 pages.

Therefore, copies of all the instruments are readily available from the research team, but have not been compiled within this document. However, they are described above in Section F9, which includes references for each instrument or questionnaire.

(The remainder of this page is intentionally left blank.)

K3 Uridine Investigator's Brochure and Bibliography

Uridine (RG2417)

INVESTIGATOR'S BROCHURE

Edition No: 7
Release Date: May 20, 2009
Replaces Previous Edition: 6 (February 27, 2008)

This Investigator's Brochure is provided to you as a principal investigator, potential investigator, or consultant for review by you, your staff, and Institutional Review Board/Ethics Committee. The information contained in this document is privileged and confidential, and except to the extent necessary to obtain informed consent, must not be disclosed unless such disclosure is required by federal or state law or regulations. Persons to whom the information is disclosed in confidence must be informed that the information is confidential and must not be disclosed by them.

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ACRONYMS AND ABBREVIATIONS

AUC 0-∝
bid Area under the curve To be taken twice a day
CGI-S Clinical Global Impressions Scale, Severity Scale
cGMP Current Good Manufacturing Practices
CHO Chinese hamster ovary
Cmax ClT CNS Maximum concentration Clearance
Central Nervous System
CO2 CSF Carbon Dioxide Cerebrospinal Fluid
DNA Deoxyribonucleic acid
ECG Electrocardiogram
FOB Functional observation battery
g Gram
g/d Grams/day
GABA Gamma aminobutyric acid
GAF Global assessment of functioning
GI Gastrointestinal
H2O HAM-A Water
Hamilton Anxiety Scale
HPLC High performance liquid chromatography
hr Hour
IND Investigational New Drug
IP Intraperitoneal
IV Intravenous
kg Kilogram
L Liter
µg Microgram
µM Micromolar
µmol Micromole
MADRS Montgomery and Asberg Depression Rating Scale
MCC Microcrystalline Cellulose
mg Milligram
mL Milliliter
mm Millimeter
m2 Square meter
MRS Magnetic Resonance Spectroscopy
Na+ Sodium
ND Not determined
NMT Not more than
NLT Not less than
NOAEL No observed adverse effect level PK Pharmacokinetics
PO Per oral
PRPP Phosphoribosyl pyrophosphate
Q-LES-Q Quality of Life Enjoyment & Satisfaction Questionnaire RNA Ribonucleic acid
RSD Relative standard deviation
RT Retention Time
T1/2 Half-life
TAU Triacetyluridine
Tmax Time at maximum concentration
UMP Uridine monophosphate
UMPS uridine monophosphate synthetase USP United State Pharmacopoeia
Vd Apparent volume of distribution
YMRS Young Mania Rating Scale
5-FU Fluorouracil
ω3FA Omega-3 fatty acid

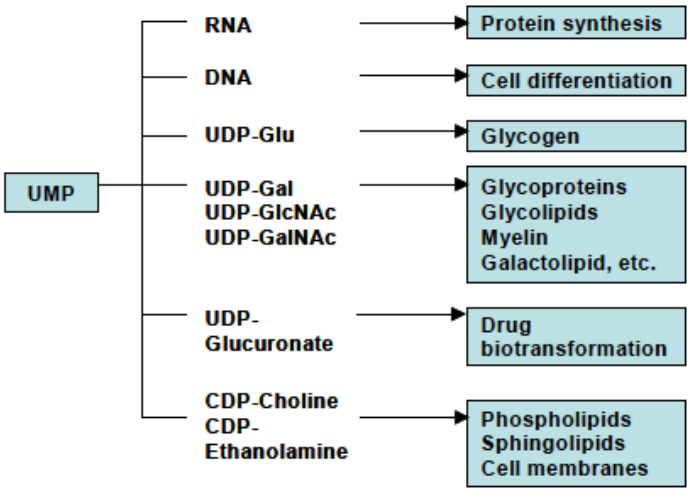
1 INTRODUCTION

RG2417 is a solid oral formulation of uridine, a naturally occurring pyrimidine nucleoside. Uridine is essential for the synthesis of RNA and biomembranes. It is also a critical element in the regulation of cellular energetics and post-translational modifications; synthesis of membrane ion channels, receptors, intracellular glycoproteins and glycolipids, excretion of drugs, steroids and bilirubin. RG2417 is being developed as a treatment for suicidal ideation, bipolar disorder, anxiety, epilepsy and attention-deficit hyperactivity disorder.

1.1 Background

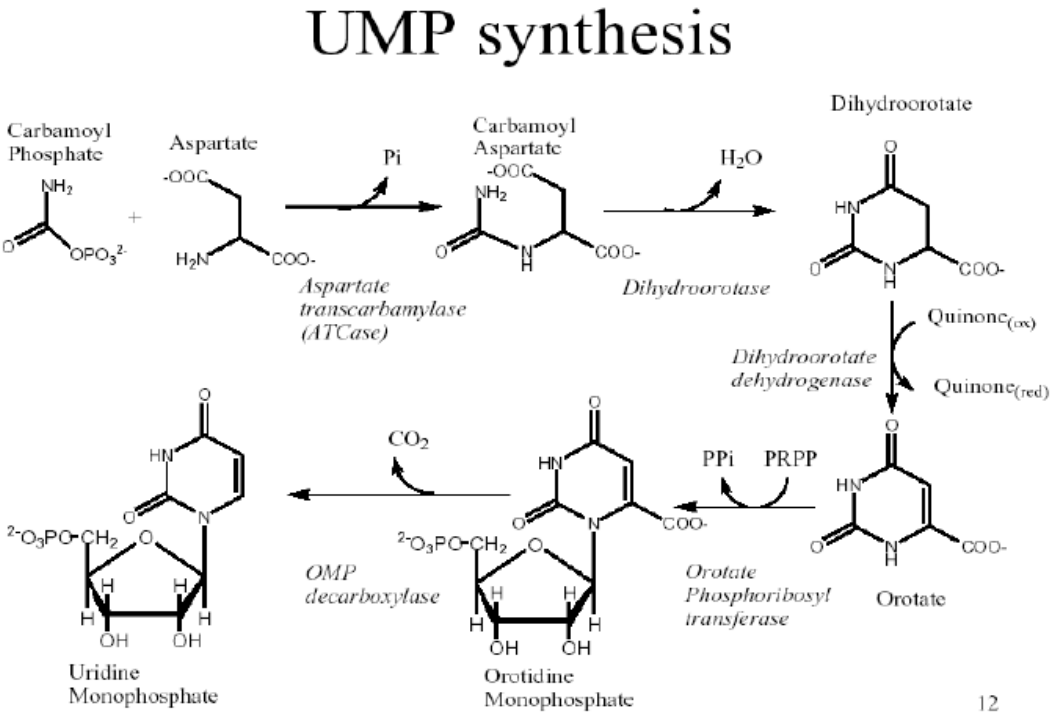
Uridine is a naturally occurring pyrimidine nucleoside with numerous physiological, metabolic and structural functions. It plays a role in such diverse pathways as synthesis of biomolecules, regulation of cellar energetics and excretion of drugs (Figure 1).

Figure 1: Biochemistry of Uridine



Uridine is synthesized from aspartate through a multistep pathway involving the dehydrogenation of dihydroorotate to orotic acid. The de novo synthetic pathway described in Figure 2 occurs primarily in the liver. However, uridine can also be obtained from endogenous salvage pathways following degradation of RNA, DNA or nucleotides and from dietary intake. Uridine is taken up from the gut or from circulation via facilitated diffusion or specific uridine transporters. After absorption from the gut, uridine is converted to UMP and enters cellular metabolism. Uridine concentrations in plasma, bone marrow and CSF are tightly regulated and range from 2 to 8 µM, depending on species [1, 2]. Over 90% of endogenous circulating uridine is cleared in a single pass through the liver by the metabolic activity of uridine phosphorylase and is replaced in a highly regulated manner by “new uridine” formed from de novo synthesis [1]. Because of the presence of active transporters in a variety of tissues, concentrations within cells are much higher than plasma levels. Uridine synthesis is coupled to oxidative phosphorylation in the mitochondria via dihydroorotatedehydrogenase. It is hypothesized that disorders that disrupt mitochondrial function, such as oxidative stress, lead to a decrease in cellular pyrimidine synthesis. Supplementation in these disorders is likely to benefit pyrimidine homeostasis and oxidative phosphorylation, and the many downstream pathways dependent on these functions.

Figure 2: Biosynthesis



1.2 Clinical Uses of Uridine and Uridine Prodrugs

The pharmacology of uridine has been extensively studied and a number of uridine formulations and prodrugs have been used in a wide variety of clinical indications including uridine deficient states, autism, diabetic neuropathy, rescue regimens for cancer chemotherapy and antiretroviral therapy, mitochondrial disease, HIV-associated lipoatrophy and bipolar depression (Table 1).

Table 1: Previous Human Experience with Uridine

Ref.	Disease	Study Design	n	Dose	Route	Safety	Comments
Van Groeningen, 1991	Normal volunteers	Open-label, single dose	6	0.3-12 g/m^2	Oral	0.3 – 8 g/m^2: no side effects ≥10 g/m^2: abd. cramps & diarrhea	Side effects w/ plasma uridine levels 60 – 80 μM
Webster, 1995	Hereditary orotic aciduria	Open-label; case reports	14	25-300 mg/kg/d; life-long admin.	Oral	5 patients treated > 20 years. No AEs reported. Two women treated during 6 pregnancies; 5/6 infants normal; one born with aneuploidy derived from familial balanced chromosomal translocation. One male patient fathered a normal infant.	Rapid adsorption from gut, salvage of uridine to make nucleotides bypassing the metabolic block, UMP production increases. Resolution of symptoms of hereditary orotic aciduria
Salerno, 2000	Adenyl-succinate lyase deficiency	Case report	1	50 mg/kg/d x 2 months	Oral	"Tolerated…without complaints…"	Increase intracellular PRPP
Van Groeningen, 1991	Cancer	Ph. 1, single dose Ph 1, mult. dose	3 6	3.5–10 g/m^2 5, 10 g/m^2 q6h x 3d	Oral Oral	≥10 g/m^2: abd. cramps & diarrhea 5 g/m^2 q6h x 3d: no side effects 10 g/m^2 q6h x 3d: diarrhea	Side effects w/ plasma uridine levels 60 – 80 μM
Page, 2001	Hyper-uricosuric autism	Case report	1	50 – 500 mg/kg/d x >2 years	Oral	No adverse drug effects.	Improvement in autistic symptoms
Page, 1997	Pervasive development delay	Open-label & double-blind, placebo-controlled	4	1 g/kg/d for over 1 year	Oral	Intermittent diarrhea and poor weight gain in 1 patient @ 0.5 g/kg/d; dose decreased but subsequently increased to 1 g/kg/d.	Purine 5'-nucleotidase activity & nucleotide synthesis increases.Improvement in autistic symptoms
Gallai, 1992	Diabetic peripheral neuropathy	Double-blind, placebo-controlled	40	900 mg/d x 180 d.	Oral	No side effects reported.	Increases lipid metabolism Improvement on EMG
Leyva, 1984	Cancer	Ph 1, PK, open-label w/ 5-FU	7	1-12 g/m^2	IV	Shivering at 10 - 12 g/m^2	Plasma uridine to 2000 μM Decreases side-effects of 5-fluorouracil
Van Groeningen, 1986	Cancer	Ph 1, PK, open-label w/ 5-FU	7	1-3 g/m^2/h	IV	Fever, phlebitis	Plasma uridine to 1000 μM

Uridine is well tolerated by humans. The maximum tolerated dose was 10 to 12 g/m2 (~ 250 mg/kg) for a single oral dose of uridine in solution and 5 g/m2/dose for a multiple- dose regimen. Since uridine is relatively poorly bioavailable a number of "prodrugs" have also been studied. These range from classical prodrugs such as acetylated uridine to molecules such as cytidine which is metabolized to uridine in vivo.

1.2.1 Oral Uridine

Oral uridine has been used in numerous indications for durations of over 20 years. The most common side effects seen are diarrhea and nausea.

Toxicity and pharmacokinetics of oral uridine in solution were studied in single-dose administrations escalating from 0.3 to 12 g/m2 and as multiple-dose administrations every six hours for three days at doses of 5 and 10 g/m2 in six healthy volunteer/subjects and in nine patients with metastatic colorectal cancer [3]. The maximum tolerated dose was 10 to 12 g/m2 (~250 mg/kg) for a single dose of uridine and 5 g/m2/dose for the multiple- dose regimen. Diarrhea was the dose-limiting toxic effect. For single-dose oral uridine in solution given at 8 -12 g/m2, peak plasma concentrations were 60 to 80 μM or 10-20 fold higher than basal levels [3]. Absorption from the gastrointestinal tract was saturated at dose levels of 8 g/m2, since higher doses did not result in increased peak levels. The mean residence time was approximately four hours and the apparent volume of distribution was 11.5 L/kg. Plasma uridine clearance had a mean value of 41 mL/kg/min and appeared to be independent of the dose of uridine. The bioavailability was low, ranging from 5.8% to 20% depending on dose [3].

Long-term exposure of 20 years or more in patients with hereditary orotic aciduria, an inborn error of metabolism that produces a primary deficiency in uridine biosynthesis, has shown uridine to be well tolerated at doses of up to 300 mg/kg/day. Several of these patients received uridine during their reproductive years and had normal progeny [4]. The report included two women who between them had six pregnancies, and one man who fathered a single child. Six of the seven infants were normal. The seventh was born with several congenital anomalies ultimately shown to be due to aneuploidy due to a familial balanced chromosomal translocation found in the mother and several other family members.

Uridine exhibited mild-to moderate side-effects of diarrhea and nausea when used in the above mentioned indications of colorectal cancer [3] and hereditary orotic aciduria [4], as well as in the six healthy volunteer/subjects. These gastrointestinal side effects may have been due, in part, to local irritation as very large doses of formulations with poor bioavailability (<10%) were used.

In a clinical form of Autism Spectrum Disorder (ASD), patients were found to excrete uric acid at a level that was greater than two standard deviations above the normal mean, with de novo purine synthesis elevated approximately four-fold over normal control subjects [5, 6]. Page et al. [7] report that one of these hyperuricosuric autistic patients showed dramatic improvement with oral dosing of uridine increased from 50 to 500 mg/kg/day. Side effects included intermittent diarrhea and poor weight gain in one subject.

In a case report studying adenyl succinate lyase deficiency [8] uridine was administered orally at 50 mg/kg/day over two months to a single patient. The patient reportedly tolerated the dosing without complaints, and the investigator reported an increase in intracellular phosphoribosylpyrophosphate (PRPP). Gallai et al. [9] treated patients with diabetic peripheral neuropathy with oral uridine in a double-blind, placebo-controlled study. Forty patients received

900 mg/day for over six months. Subjects reported no side effects and investigators observed an increase in lipid metabolism and an improvement on electromyography (EMG).

Uridine is also used as supplemental therapy in patients with uridine monophosphate synthetase (UMPS) deficiency. This disorder results in a deficiency of endogenous uridine and is characterized by symptoms such as failure to thrive, megaloblastic anemia, language and motor delays and immune deficiency. Following administration of uridine, most abnormalities were reversed. In these cases, patients were administered up to
300 mg/kg/day divided into 3-5 doses. The requirement for supplemental uridine however, is life-long [4].

Uridine supplementation using natural dietary extract has been used in the treatment of mitochondrial toxicity associated with nucleoside analogue reverse transcriptase inhibitors (NRTIs) [10]. The activity of uridine to counteract HIV associated lipoatrophy was demonstrated in 20 patients on stable antiretroviral therapy. Treatment over a 3 month period was associated with a significant change in body morphometry without elevating cholesterol, decreasing lean body mass or fasting insulin levels [11].

1.2.2 Intravenous Uridine

IV uridine has been used to "rescue" normal cells in cancer patients undergoing certain types of chemotherapy at doses that have resulted in sustained elevations in plasma uridine, sometimes exceeding 2,000 μM [12, 13]. The main side effects seen with IV uridine are phlebitis at the site of the infusion and transient shivering and fever. The absence of gastrointestinal side effects at these doses suggests that the diarrhea noted with high doses of oral uridine may be due to local irritation by the oral formulation used in those studies and reflect the formulation's reduced bioavailability.

1.2.3 Uridine Prodrugs

Due to the low bioavailability of uridine in solution, other "prodrugs" of uridine have been investigated including triacetyl uridine (2', 3', 5'-tri-O-acetyluridine; TAU) and cytidine. TAU is rapidly converted to uridine before entry into the bloodstream by the action of blood and tissue esterase activity. Pharmacokinetic studies of TAU in rats and dogs demonstrated that its bioavailability was increased 6-7 fold compared to free uridine at the same dosage. The bioavailability of uridine in man is higher than in animals.

TAU has been used in open label studies of children with neurological or renal manifestations of mitochondrial dysfunction [14-16]. Doses of 2 g/m2 were administered to the children three times daily for up to 2 years. Significant symptomatic improvement was noted in the accompanying renal tubular acidosis [14-16]. In children with a pre- existing seizure disorder, there was a brief increase in seizure frequency followed by a decline to below baseline levels [17]. No other adverse events were reported. The theoretical argument for treating with exogenous uridine is to compensate for uridine synthesis through elevation of cellular uridine levels. In vitro, cells impaired by mitochondrial depletion, using ethidium bromide, regain proliferative capacity when supplemented with uridine and pyruvate. Additionally, fibroblasts derived from patients with mitochondrial disease are dependent on uridine supplementation for survival [18].

TAU has also been used to "rescue" normal cells during certain types of cancer chemotherapy [13, 19]. The uridine competes with anti-cancer uridine analogs, such as 5-FU, thus partially

sparing non-cancerous tissues [13, 19]. In the chemotherapy studies, patient received 6 g of TAU four times a day with no adverse effects [19, 20]. Kelsen et al. [19] sought to determine the appropriate dose of TAU to use as rescue therapy. They investigated doses of 3.3 g, 6.6 g, and 9.9 g, given as a liquid suspension, every six hours for ten doses after a bolus infusion of 5-FU. The 5-FU and subsequent TAU was dosed weekly for six weeks followed by a two-week rest period. Uridine bioavailability was about 7%, the elimination half-life was 2-4 hours, and the volume of distribution was 0.634 L/kg. In the setting of 5-FU toxicity, therapeutic blood levels of uridine were 50- 250 μM. Peak levels were obtained 2-3 hours after an oral dose.

1.3 Bipolar disorder

Bipolar disorder is a chronic illness associated with substantial morbidity and mortality, ranking worldwide behind only unipolar depression and alcohol abuse among psychiatric illnesses for related disabilities [21, 22]. The lifetime financial burden of bipolar disorder in the United States is about $625,000 per patient, depending on treatment refractoriness and chronicity of symptoms [23]. Although lithium and anticonvulsants such as valproic acid have substantially improved the prognosis of bipolar disorder, many individuals are unable to tolerate treatment-related side-effects. Incomplete clinical response, relapse and recurrence remain common clinical problems [24-27]. The depressive phase of bipolar disorder is particularly challenging to treat and is understudied in comparison to bipolar mania [28]. One of the most vexing challenges in the treatment of bipolar depression has been the induction of mania in patients receiving treatment for depression with standard antidepressants [29].

Although the exact mechanism of action of uridine in the treatment of bipolar depression is not known, several lines of evidence suggest a possible role.

First, bipolar disorder appears to be characterized by the decreased expression of genes coding for mitochondrial proteins responsible for oxidative phosphorylation [30]. Studies of brain high-energy phosphate metabolism with magnetic resonance spectroscopy (MRS) and genetic studies have demonstrated mitochondrial dysfunction in subjects with unipolar and bipolar disorders [31-33]. Because of this dysfunction, mitochondrial oxidative phosphorylation is compromised in favor of glycolysis. Uridine may alleviate mitochondrial dysfunction by increasing the synthesis of uridine high- energy phosphates.

Second, bipolar disorder is related to concomitant increases in CNS lactate and glutamate [34], and provision of exogenous uridine has been shown to decrease lactate production during periods of cerebral ischemia [35]. Bipolar patients not taking medications have approximately 20% higher levels of brain lactate on proton MRS compared to healthy volunteers, and brain lactate levels correlate with the severity of depression [34]. Hence, exogenous uridine is hypothesized to impact the pathophysiology of bipolar disorder by decreasing brain lactate levels.

Third, gamma aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the mammalian brain, and impairment of its function is thought to play an important role in mood disorders [24-26]. Clinical trials have determined that certain GABA-mimetics have antidepressant properties [29, 30, 33]. Uridine inhibits in vitro GABA binding in the frontal cortex, hippocampus and thalamus, thus inhibiting its reuptake. Taken together with the observation that 14 day pyrimidine administration increases GABAergic activity, it is hypothesized that oral uridine will increase GABAergic activity [32, 36, 37].

Fourth, uridine works synergistically with omega-3 fatty acid (ω3FA) in rat models of depression [38]. It is hypothesized that uridine increases membrane synthesis, thus facilitating the incorporation of ω3FAs into neuronal membranes and increasing phospholipid membrane fluidity [39, 40].

Finally, uridine and uridine prodrugs have had antidepressant effects on rats and in humans. Uridine exhibited antidepressant-like effects in the rat forced swim test [38], and TAU and cytidine showed modest effects in patients with bipolar depression without inducing mania [41]. There are also preliminary clinical reports that uridine has anxiolytic and antidepressant effects [42].

1.3.1 Uridine Treatment in Bipolar Depression

Researchers have directly examined the effects of TAU on bipolar depression. TAU was well-tolerated and patients showed modest improvements in mood without the induction of mania (Stoll, personal communication) [41].

Nineteen patients were studied in a 6-week, dose-escalation study with TAU. Patients started at 6 g/day and, depending on response and tolerability, the dose was increased by an additional 6 g/day every two weeks to a maximum of 18 g/day. This TAU dosing corresponds to 4 g/day to 12 g/day of uridine. Montgomery-Asberg Depression Rating Scale (MADRS) scores decreased modestly for both unipolar and bipolar subjects over the course of the 6-week study, suggesting a decrease in depression. Depressive symptoms for the bipolar subjects were the lowest at week 3 of the study, whereas depressive symptoms for the unipolar depressed subjects were lowest at the end of the study. Mania symptoms improved for bipolar subjects as indicated by a decrease in Young Mania Rating Scale (YMRS) scores. Modest improvement in the Clinical Global Impression of Severity (CGI-S) in the bipolar patients was seen during the first 4 weeks of treatment with TAU. Both bipolar and depressed subjects also showed modest improvements in the Global Assessment of Functioning (GAF) and the Hamilton Anxiety Scale (HAM-A) when comparing baseline to week six. Side effects included mild self- limited hypomania, dizziness, stomach cramps, nausea, decreased appetite, increase in constipation, diarrhea, muscle cramps, headaches, fatigue, sleep problems, increased flatulence, decreased libido, and visual disturbance (including some loss of depth perception) and eye pain. Neither of the latter two events was related to study drug, according to an examining ophthalmologist. One patient did leave the study because of a mild worsening of hypomania that was present at baseline (Stoll, personal communication).

A study in bipolar I disorder with RG2417 has also been completed and details of this study are in Section 4.1.2.

1.4 Uridine (a.k.a. RG2417)

1.4.1 Non-clinical studies

The toxicology of elevated blood and tissue levels of uridine can be difficult to assess in animals without an efficient means of oral drug delivery due to species-specific, high levels of uridine phosphorylase in the gut (the main catabolic enzyme of uridine). The prodrug triacetyl uridine (TAU) provides a means of assessing the potential toxic consequence of elevated uridine on physiology since it is readily absorbed by passive diffusion and converted to uridine before circulation in the blood. Studies in rats and dogs have been performed to investigate the toxicity

of uridine through dosing with TAU. The NOAEL in rats dosed twice daily with TAU for 13 weeks was 990 mg/kg/day uridine (equivalent to TAU at 1500 mg/kg/day). The NOAEL in dogs dosed twice daily with TAU for 13 weeks was also 990 mg/kg/day uridine (equivalent to TAU at 1500. mg/kg/day).

1.4.2 Clinical studies

Previously, uridine/RG2417 was studied in two Repligen sponsored clinical trials (Table 2). The studies included a Phase I healthy volunteer study to compare the safety and pharmacokinetics of RG2417 administered at 1, 2 or 4 g as tablets or as an oral solution. The other study was a Phase II study to assess the safety and tolerability of RG2417 administered at 2 or 4 g/day for six weeks in the treatment of bipolar I disorder.

Table 2: RG2417 Summary of Clinical Experience

Study # of patients (Rx/placebo)	Indication	Age	Design*	Total exposures (exposure range/patient)	Dose/Route	Safety results
RG2417-02 13 (crossover)	Healthy Volunteers	24-53	DB-PC-CO (3 doses of RG2417 and 1 dose of placebo)	49 TOTAL 8 (1/subject) 8 (1/subject) 9 (1/subject) 8 (1/subject) 8 (1/subject) 8 (1/subject)	1, 2 or 4 g solution PO 1, 2 or 4 g tablet PO	Upper abdominal pain Headache
RG2417-01 84 (40/44)	Bipolar Disorder	21-61	DB-PC-Dose escalation	2856 TOTAL 18 patients 1 g bid for 1-6 weeks 15 patients ↑ to 2 g bid after 1 week 6 patient ↑ to 2 g bid after 2 weeks 1 patient ↑ to 2 g bid after 4 weeks	1 g PO bid, 6 wks 2 g PO bid, 6 wks	Nausea Dry Mouth Dyspepsia Fatigue

* DB = Double blind
PC = Placebo controlled
CO = Cross over

The most common adverse events reported in these studies were headache, fatigue and gastrointestinal events such as dry mouth, nausea, abdominal pain and dyspepsia. There was only one serious adverse event report that was possibly related to uridine/RG2417. This subject, with a co-morbid hypertension experienced a worsening of this pre-existing condition and required in-patient monitoring. There were no other ischemic or cardiopulmonary sequelae of this event.

In general, uridine/RG2417 is safe and well tolerated when dosed at up to 4 g/day for 6 weeks.

Currently, a Phase IIb clinical trial to further assess the safety and efficacy of uridine/RG2417 in eight weeks of oral dosing in bipolar I depression is being conducted under protocol RG2417-03, entitled, “A Phase II Randomized, Double-Blind, Placebo-Controlled, Flexible-Dose Study to

Assess the Safety, Tolerability and Efficacy of RG2417 (Uridine) in the Treatment of Bipolar I Depression."

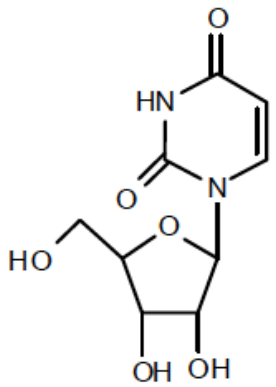
This study is a randomized, eight-week, double blind, placebo-controlled, Phase II study to assess the safety and efficacy of uridine/RG2417 in bipolar I depression. One hundred and fifty (150) subjects of both sexes will be enrolled at up to thirty (30) sites and will be randomized on a 1:1 basis to receive either placebo or uridine/RG2417.

2 PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES

2.1 Active Constituent of the Uridine/RG2417 Formulation

The active constituent of RG2417 is uridine (Uracil-1-β-D-Ribofuranoside), which has the formula C9H12N2O6 and a molecular weight of 244 g/mole. The structure of RG2417 is shown in Figure 3.

Figure 3: Structure of RG2417



RG2417 (Uridine)

2.2 Pharmaceutical Properties

Uridine is a naturally occurring pyrimidine nucleoside with numerous physiological and metabolic functions. The biological pathways uridine participates in range from cell membrane synthesis to excretion of compounds to cellar energetics (see Figure 1). In addition, uridine synthesis is coupled to oxidative phosphorylation in mitochondria via dihydroorotate dehydrogenase.

2.3 Manufacturing

Uridine study drug and matching pill placebo are manufactured by Natural Pharmacia International, Inc. (NPI; Burlington, MA), which is a Good Manufacturing Practice facility.

2.4 Physical Properties

Uridine/RG2417 tablets are uncoated, oval, and white to off-white in color, with dimensions of 0.64 x 0.23 inches. They have an average weight of 750 mg. When used, matching placebo is visually identical to RG2417 tablets. The specifications for uridine/RG2417 and placebo tablets are shown in Table 3 and Table 4, respectively.

2.4.1 Specifications for Uridine/RG2417 Tablets: Table 3 Uridine Specifications

Table 3: RG2417 Drug Product Specifications

Test	Specification
Appearance of tablet	Off white intact tablet
Identity	RT ± 2% of uridine std, spectrum matches reference standard
Weight variation	85-115% of label claim, RSD ≤ 6.0%
Potency	90-110% of label claim
Purity	main peak > 95.0%, no single impurity > 1.0%
Dissolution	NLT 80% in 45 minutes
Moisture	Report Result

NLT-Not Less than
NMT-Not more than

2.4.2 Specifications for Placebo Tablets Table 4: Placebo Specifications

Table 4: Placebo Specifications

Test	Specification
Appearance of tablet	Off white intact tablet
Disintegration	NMT 15 minutes in simulated gastric fluid
Absence of API	No peak > 0.5% by peak area in the sample preparation corresponding to the retention time of RG2417 in the standard preparation
Moisture	Report result

NMT-Not more than

2.5 Formulation

2.5.1 Uridine/RG2417 Formulation

Uridine/RG2417 tablets weigh 750 mg and contain 500 mg of RG2417. Tablets have the composition listed in Table 5. Tablets are designed for immediate release of RG2417.

Table 5: Formulation of 500 mg RG2417 Tablets

Material	% w/w
RG2417	66.7
Croscarmellose	3.0
Microcrystalline Cellulose	10.0
Maltodextrin	8.0
Lactose	10.9
Magnesium Stearate	1.25
Silicon Dioxide	0.2

2.5.2 Placebo Formulation

Placebo tablets weigh 750 mg and have the composition listed in Table 6.

Table 6: Formulation of Placebo Tablets

Material	% w/w
Croscarmellose	2.0
Microcrystalline Cellulose	10.0
Lactose	87.6
Magnesium Stearate	0.5

2.6 Storage and Handling

Uridine/RG2417 and placebo tablets should be stored at room temperature, between 20°C and 30°C.

2.7 Intended Route of Administration:

Uridine/RG2417 is intended for daily oral dosing.

3 NON-CLINICAL STUDIES

Uridine is found in circulation in humans at approximately 2-5 µM. Uridine-containing metabolites are formed from either exogenous or endogenous sources of uridine. The absorption and metabolism of exogenous uridine are influenced by the location and level of the enzyme uridine phosphorylase. Many animals, including rodents, contain high levels of this enzyme compared to man, especially in the gut. This makes pharmacology and toxicology assessments relevant to man more difficult. To circumvent this problem, prodrugs of uridine, such as triacetyluridine (TAU), that are converted to uridine prior to entering the circulation can be used and provide a means of obtaining high blood and tissue levels of uridine in rats and dogs.

3.1 Pharmacology

Two factors that affect the bioavailability of oral doses of uridine are the mechanism of drug absorption from the gut and the process of drug catabolism. Uridine uptake is mediated by equilibrative and concentrative nucleoside transporters. The metabolism of uridine is controlled

by uridine phosphorylase. The extent to which these systems are constant across species will determine if animal pharmacology is predictive of drug exposure in humans.

The most relevant nucleoside transporter for uridine uptake is the CNT1 receptor which functions in a Na+ dependent manner [43, 44]. The receptor distribution is highest in stomach and duodenum, decreasing through the jejunum and ileum [45]. The relative receptor density across different species is not well defined. An active transport system that is regionally restricted to the upper gastrointestinal tract would suggest that uridine uptake might be saturable and limited to early times after ingestion. Pilot studies in pigs showed decreased absorption efficiency with enteric-coated solid dose forms of uridine (data on file).

While the brain takes up uridine and converts the nucleoside to phosphorylated forms [46], the homeostasis of circulating uridine appears to take place principally in the liver. Catabolism of uridine in the liver is controlled by uridine phosphorylase, the enzyme that converts uridine to uracil [2]. Catabolism proceeds through dihydrouracil dehydrogenase, dihydropyrimidinase, and ureidopropionase ending at ß-alanine [47]. Uridine phosphorylase is present in liver non-parenchymal cells while the running enzymes are present in hepatocytes.

Uridine phosphorylase is present in rodents at high levels in the GI tract as well as the liver. Non-rodent animals have lower GI levels but have relatively abundant enzyme in liver non-parenchymal cells. Humans appear to possess relatively low levels of uridine phosphorylase in the GI tract, which is the postulated mechanism of higher bioavailability. Conversely, dosing of uridine in rodents, dogs or minipigs leads to high levels of uracil because passage through the GI epithelium and liver in these animals converts uridine to the base and results in poor and variable availability of the nucleoside.

3.1.1 Testing for Anticonvulsant and Neuroprotective Activity of Uridine

Anticonvulsants have been used to treat aspects of bipolar I disorder (valproate, carbamazepine, lamotrigine). Since such an activity may be relevant for use in bipolar depression, RG2417 was screened in one electrical and two chemical induced seizure models. Uridine/RG2417 was dosed at 30-300 mg/kg IP 1-4 hours before seizure induction. Studies were conducted using both rats and mice (4/group). RG2417 did not alter seizure severity in any of the three models.

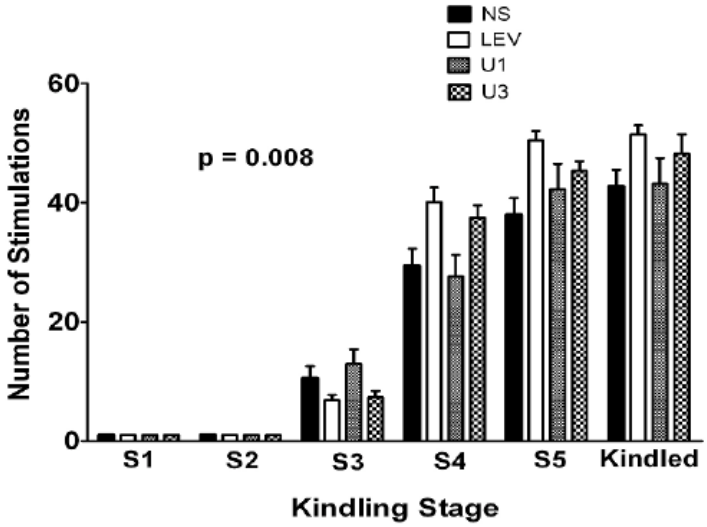
Uridine/RG2417 was also tested in two models of epileptogenesis, recovery from status epilepticus and hippocampal kindling [48]. In the recovery model, status epilepticus was induced with lithium-pilocarpine. After termination of seizures, rats were dosed with uridine/RG2417 four times per day at 150 mg/kg IV for 14 days (6/group). At that time, animals were tested in the Morris water maze, then sacrificed and selected hippocampal histopathology sections scored for neuronal cell loss. Uridine/RG2417-treated animals were significantly improved in finding the water maze platform. There was no statistically significant improvement in histology scores.

Uridine/RG2417 was also tested in a rapid kindling model. Rats were kindled via repeated hippocampal stimulation (8/group, 12 stimulations/day over 4 days). The number of stimulations required to achieve seizures of defined severity with or without treatment indicates effectiveness in reducing the rate of kindling. RG2417 treatment resulted in an increase in the number of stimulations to reach the more severe stage 4 and 5 seizures ($p < 0.05$, t test). These results are interpreted as demonstrating a modest neuroprotective effect [48].

To further study the possible antiepileptogenic properties of uridine, we compared the rate of kindling with uridine or levetiracetam when kindling stimulation was done just once per day. We administered uridine either once or three times daily (12/group, 200 mg/kg per dose), and used normal saline (NS) and levetiracetam (LEV) as negative and positive controls [49].

There were significant differences between the saline group and the rats given U3 ($F(1) = 4.617$, $p = 0.043$) and LEV ($F(1) = 15.22$, $p < 0.001$). There was also no difference in the LEV and U3 groups ($F(1) = 3.515$, $p = 0.063$) [49]. Figure 4 shows that both levetiracetam and uridine (given three times daily) slow the kindling rate and that levetiracetam and thrice daily uridine injections are equivalent in slowing the kindling rate.

Figure 4: Effect of RG2417 in a Daily Kindling Model

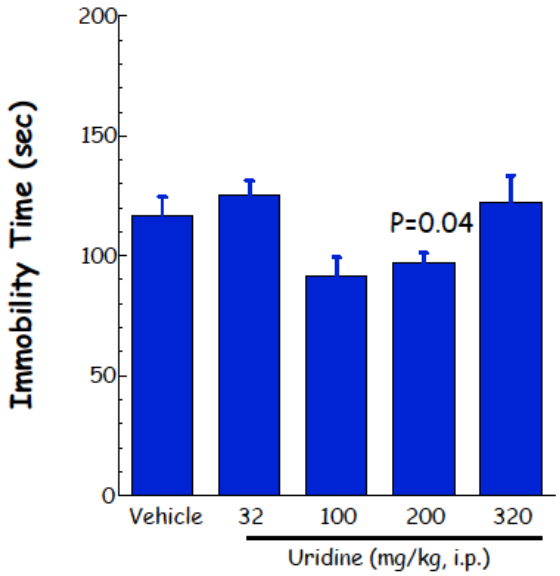


3.1.2 Activity of Uridine in the Rodent Forced Swim Test

An animal model of antidepressant effects of drugs often used when studying depression in man is the rodent forced swim test [50]. The procedure measures the behavior of immobility in an inescapable cylinder of water. Compounds with antidepressant activity in man reduce the immobility time in this model. This model has been used to show that treatment with cytidine, a pyrimidine rapidly converted to uridine in blood, has an antidepressant effect in rats [38]. The positive effect of uridine is potentiated in rats by combined treatment with omega 3 fatty acids [51].

This result has been reproduced in mice as well. Animals were injected with uridine IP three times over a 24 hour period prior to testing in the cylinder of water. Figure 5 shows the antidepressant effect of uridine dosing in BALB/c mice as reflected by a decrease in total immobility time.

Figure 5: Effect of RG2417 in the Forced Swim Model Performed in BALB/c Mice



3.2 Safety Pharmacology

3.2.1 Neurobehavioral evaluation of TAU in male rats

The purpose of this study was to assess the potential acute neurotoxicity of uridine in adult male rats after oral dosing with TAU. This study consisted of four groups of animals each containing eight adult male rats. Animals were dosed once by oral gavage at either 0 mg/kg (placebo), 300 mg/kg, 1000 mg/kg or 2000 mg/kg of TAU. Functional observational battery tests were performed pre-dose and at 1 and 24 hours post-dose. Categories evaluated were activity/arousal, neuromuscular, sensorimotor, autonomic and physiological. Each animal was scored on a standard 12-point Functional Observation Battery (FOB).

No neurobehavioral changes were observed in any rats administered TAU at doses up to 2000 mg/kg. The NOAEL for this study was 1320 mg/kg uridine (equivalent to 2000 mg/kg TAU).

3.2.2 Evaluation of repeat oral dosing of TAU on cardiovascular function

An evaluation of cardiac function in beagle dogs was done in conjunction with a twenty eight-day repeat dose toxicology study (HLS 02-3010).

Electrocardiographic data were collected pre-dosing, on day twenty-eight and at the end of the recovery period. Data was analyzed for anomalies in ECG, including measures of QT interval. No anomalies were identified in the ECG-derived measures of cardiac performance. This study concludes that the no observed effect level for formulated TAU on cardiac function is 1320 mg/kg/day uridine (equivalent to 2000 mg/kg TAU).

3.3 Toxicology

The toxicology of elevated blood and tissue levels of uridine can be difficult to assess in rats and dogs without an efficient means of oral drug delivery due to species-specific, high levels of

uridine phosphorylase (EC 2.4.2.3) in the gut. The prodrug triacetyl uridine (TAU) provides a means of assessing the potential toxic consequence of elevated uridine on animal physiology since it is readily absorbed by passive diffusion and converted to uridine before circulation in blood. Even at high oral doses of TAU and after repeated dosing for 28 days it is not possible to detect TAU in the bloodstream while uridine levels are elevated more than 1000-fold. The bioavailability (efficiency of absorption) of uridine after uridine dosing in rats and dogs is 7-16% but with TAU is 40- 70%. Thus TAU provides a means of achieving higher blood levels of uridine in rats and dogs for toxicology assessments.

A number of studies have been performed to investigate the toxicity of uridine through prodrug dosing (TAU) in animals. The TAU used for these studies was ≥99.0% triacetyluridine. These studies are summarized in Table 7.

Table 7: In Vivo Triacetyl-Uridine Toxicology Study Summary

Species	Study #	Animals/ Dose Group	Dose[a] (mg/kg/day)	Duration of Dosing	NOAEL[b] mg/kg/day	Comments
Rat	HLS RSZ-002	5M/5F	1320	1 day	1,320	No effect at highest dose
Rat	HLS RSZ-004	4M/4F	0, 200, 660, 1320	bid, 7 day	660	No effect at highest dose
Rat	HLS RSZ-005	10M/10F	0, 200, 660, 1320	bid, 28 days	660	Microscopic findings in kidney and thyroid
Rat	HLS 08-2056	50M/50F	0, 198, 495, 990	bid, 13 weeks	990	No effect at highest dose
Dog	HLS 02-3010	5M/5F	0, 200, 660, 1320	bid, 28 days	660	40% decrease in platelets; microscopic findings in mesenteric lymph nodes and thyroid
Dog	HLS 08-3323	16M/16F	0, 198, 495, 990	bid, 13 weeks	990	No effect at highest dose

a – Dose values are given as amount of uridine; 0.66 mg uridine/mg TAU (approximately 2 mg uridine/3 mg TAU)
b – No observed adverse effect level

3.3.1 Single Dose Toxicology Study in Rats (HLS RSZ-002)

The purpose of this study was to assess the acute oral toxicity of uridine (dosed as TAU) to the rat. Ten rats (five male and five female) received a single oral gavage dose of TAU formulated in 0.75% hydroxy propyl methycellulose at a dose level of 2000 mg/kg bodyweight (1320 mg/kg uridine equivalents). Animals were observed for clinical response to treatment and body weight over fourteen days after which they were euthanized and subjected to a macroscopic examination.

Clinical signs of reaction to treatment were confined to increased salivation in two females. There were no abnormalities seen during the macroscopic examination.

The results of this study indicate the no observed adverse effect level (NOAEL) oral dose to rats is considered to be greater than 1,320 mg/kg uridine (equivalent to > 2000 mg/kg TAU).

3.3.2 7-Day Repeat Dose Study of TAU in Rats (HLS RSZ-004)

The purpose of this study was to assess the toxicity of uridine when administered twice daily by oral gavage as TAU to CD rats for a period of seven consecutive days. The study consisted of one control and three treated groups. Each study group, consisting of four male and four female rats, was administered TAU by oral gavage at 0 mg/kg/day (placebo control), 300 mg/kg/day (low dose), 1000 mg/kg/day (mid dose) or 2000 mg/kg/day (high dose), equivalent to 200, 660, or 1320 mg/kg/day as uridine, respectively, for seven consecutive days. Animals were observed and monitored daily during the seven-day treatment period including food and water consumption, body weight, and observation after each dose. Upon termination, all rats were subjected to a macroscopic examination and organ weights were recorded.

There were no treatment related changes in body weights, food consumption or organ weights at any dose level. Two of the eight animals receiving 2000 mg/kg/day TAU showed pelvic dilation of the kidney. Salivation was seen after dosing in animals dosed at 2000 mg/kg/day and for one male and one female at 1000 mg/kg/day. In conclusion, oral administrations up to 1,320 mg/kg/day uridine (equivalent to 2000 mg/kg/day TAU) produced no significant toxic response and the NOAEL was 660 mg/kg/day.

3.3.3 28-Day Repeat Dose Toxicity Study of TAU in Rats (HLS RSZ-005)

The purpose of this study was to assess the toxicity of uridine when administered twice daily by oral gavage as TAU to CD rats for twenty-eight consecutive days and to assess the reversibility of any adverse effects of treatment following a four-week recovery period. The study consisted of one control and three treated groups. Each study group, consisting of ten male and ten female rats and designated as the Main Phase animals, was administered TAU by oral gavage at 0 mg/kg/day (placebo control), 300 mg/kg/day (low dose), 1000 mg/kg/day (mid dose) or 2000 mg/kg/day (high dose) equivalent to 200, 660, or 1320 mg/kg/day as uridine, respectively for twenty-eight consecutive days. An additional five male and five female rats designated as Recovery Phase animals received TAU either at 0 mg/kg/day (placebo control) or 2000 mg/kg/day for twenty-eight consecutive days. Upon completion of treatment, all Main Phase animals were euthanized while the Recovery Phase animals remained on the study for an additional four weeks (not dosed) after which they were euthanized.

There were no drug related body weight or food consumption changes noted. Elevations in serum lactate observed in control animals were decreased in the mid and high dose groups. As observed in the seven-day repeat dose study a high incidence of salivation associated with dosing was apparent in animals receiving 2000 mg/kg/day of TAU and a low incidence in animals receiving 1000 mg/kg/day. Examination of all urine samples indicated the presence of crystals at 2000 mg/kg/day TAU. The NOAEL was 660 mg/kg/day uridine (equivalent to 1000 mg/kg/day TAU). The NOAEL is based on microscopic findings in kidney and thyroid tissue.

3.3.4 13 Week Oral Toxicity Study in Rats Dosed with TAU with a 4-Week Recovery Period (HLS 08-2056)

The purpose of this study was to assess the toxicity of uridine when administered twice daily as TAU by oral gavage at dose levels 300, 750, or 1500 mg/kg/day to Sprague- Dawley CD rats for 13 weeks and to assess the reversibility of any adverse effects following a 4-week post-dose recovery period.

The study consisted of one control group (fifteen males and fifteen females 0 mg/kg/dose) and three treatment groups (ten males and ten females 300 mg/kg/day; ten males and ten females 750 mg/kg/day; and fifteen males and fifteen females 1500 mg/kg/day). TAU was administered by oral gavage twice daily for thirteen consecutive weeks. The four study groups were 0 mg/kg/day (placebo control), 300 mg/kg/day (Low Dose), 750 mg/kg/day (Mid Dose), or 1500 mg/kg/day (High Dose) equivalent to 0, 198, 495, or 990 mg/kg/day as uridine, respectively. At the end of the treatment period, up to ten animals/sex/group were euthanized and necropsied. At the end of a four week recovery period, five animals/sex in the 0 mg/kg/day and 1500 mg/kg/day groups were euthanized and necropsied. Satellite animals (nine/sex/group) were dosed twice daily, approximately eight hours apart with vehicle, 300, 750 or 1500 mg/kg/day TAU and blood was collected on Days 45 and 90 for toxicokinetic analysis.

Parameters evaluated during the study were: viability, clinical observations, ophthalmology, body weights, food consumption, clinical pathology (termination of dosing and end of recovery), organ weights, macroscopic observations and microscopic pathology.

There was a slight decrease in food consumption in males at 1500 mg/kg/day and increases in neutrophils at 750 and 1500 mg/kg/day and monocytes and eosinophils at 1500 mg/kg/day. In females, serum phosphorus was increased at 1500 mg/kg/day. These changes were minimal and not considered adverse effects. With the exception of phosphorus levels in females at 1500 mg/kg/day, these effects were no longer evident at the end of the recovery period. In conclusion, the NOAEL was 990 mg/kg/day uridine (equivalent to 1500 mg/kg/day TAU).

3.3.5 28 Day Repeated Dose Toxicity of TAU in Beagle Dogs (HLS 02-3010)

The purpose of this study was to assess the toxicity of uridine when administered twice daily as TAU by oral gavage to dogs at dose levels of 300, 1000 and 2000 mg/kg/day.

TAU for twenty-eight days and to assess the reversibility of any adverse effects following a twenty-eight-day recovery period.

The study consisted of one control (three male and three female) and three treated groups (five male and five female per group). TAU was administered by oral gavage twice daily for a period of four weeks. The four study groups consisted of 0 mg/kg/day (placebo control), 300 mg/kg/day (Low Dose), 1000 mg/kg/day (Mid Dose) or 2000 mg/kg/day (High Dose) equivalent to 200, 660, or 1320 mg/kg/dose as uridine, respectively. After treatment, terminal necropsy was performed on three animals/sex/group and after a 28- day recovery period the remaining two animal/sex/group were euthanized.

Parameters monitored during this study included clinical signs and measurements of body weight, food consumption and ECG. Complete clinical pathology was performed once prior to commencement of treatment, at the end of treatment and at the end of the recovery period. Microscopic analysis of control and high dose group animals was completed at the end of treatment and after a thirty-day recovery period. At termination, all dogs were subjected to a macroscopic examination and the weights of specific organs were recorded.

The administration of 2000 mg/kg/day of TAU was associated with a 40-50% decrease in mean platelet count after twenty-eight days. This finding was not evident following a twenty-eight-day recovery period and was not associated with any changes in prothrombin time and activated partial thromboplastin time. Also, at 2000 mg/kg/day of TAU, two out of six animals showed

minimal abnormal histopathology in mesenteric lymph nodes and thyroid tissue. There was no macroscopic or microscopic findings related to test article administration in animals examined after a thirty-day recovery period.

In conclusion, changes observed in animals were minimal and not considered adverse effects. Therefore, the no-observed-adverse-effect-level (NOAEL) for administration of TAU to beagle dogs for twenty-eight days was determined to be or 660 mg/kg/day uridine (equivalent to 1000 mg/kg/day TAU). This NOAEL is based on a 40-50% decrease in platelets and histopathologic findings in mesenteric lymph nodes and thyroid tissue after twenty-eight days of dosing.

3.3.6 13 Week Oral Toxicity Study in Dogs Dosed with TAU with a 4-Week Recovery Period (HLS 08-3323)

The purpose of this study was to assess the toxicity of uridine when administered twice daily as TAU by oral gavage at dose levels 300, 750, or 1500 mg/kg/day to beagle dogs for 13 weeks and to assess the reversibility of any adverse effects following a 4-week postdose period.

The study consisted of one control group (five males and five females 0 mg/kg/day) and three treatment groups (three males and three females 300 mg/kg/day; three males and three females 750 mg/kg/day; and five males and five females 1500 mg/kg/day). TAU was administered by oral gavage twice daily for thirteen consecutive weeks. The four study groups were 0 mg/kg/day (placebo control), 300 mg/kg/day (Low Dose), 750 mg/kg/day (Mid Dose), or 1500 mg/kg/day (High Dose) equivalent to 0, 198, 495, or 990 mg/kg/day as uridine, respectively. At the end of the treatment period, three animals/sex/group were euthanized and necropsied. At the end of a four week recovery period, two animals/sex in the 0 mg/kg/day and 1500 mg/kg/day groups were euthanized and necropsied.

Parameters evaluated during the study were: viability, clinical observations, ophthalmology, ECGs, body weights, food consumption, clinical pathology (pretest, termination of dosing, and end of recovery), toxicokinetics (Days 45 and 90), organ weights, macroscopic observations and microscopic pathology.

Clinical pathology findings indicative of slight dehydration were noted in males at 1500 mg/kg/day. Additionally in the male 1500 mg/kg/day group, hemoglobin, red blood cells, hematocrit, serum total protein and albumin were increased at the end of dosing. There were no unscheduled deaths during the study and no clinical signs or ophthalmology findings. There was no electrocardiographic evidence of test article action or effects on body weights and food consumption. There were not test article- related effects on organ weights or macroscopic and microscopic findings.

Although there was evidence from clinical pathology of slight dehydration in males at the 1500 mg/kg/day TAU level, there were no clinical signs, no effects on body weights or food consumption, and no microscopic findings. In conclusion, the NOAEL for was 990 mg/kg/day uridine (equivalent to 1500 mg/kg/day TAU).

3.3.7 Bacterial Mutagenicity Assay

The purpose of this study was to evaluate the mutagenic potential of RG2417 by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella and Escherichia coli in the presence and absence of metabolic activation.

The assay was performed in two phases using the plate incorporation method. The first phase was used to establish the dose range for confirmatory mutagenicity assay. The second phase was used to evaluate and confirm the mutagenic potential of RG2417.

The dose levels tested were 5.0, 1.58, 0.5, 0.16, 0.05, 0.016 mg per plate. In the initial assay no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Based on the initial findings, the maximum dose plated in the confirmatory assay was 5.0 mg per plate. In the confirmatory mutagenicity assay, no positive response was observed at levels of 5.0, 1.58 and 0.5 mg per plate. As in the initial assay, neither precipitate nor appreciable toxicity was observed. Under the conditions of this study, RG2417 was found to be negative in the Bacterial Reverse Mutation Assay up to the highest concentration tested (5.0 mg per plate).

3.3.8 In Vitro Mouse Lymphoma Assay

The purpose of this study was to evaluate the clastogenic or point mutation potential of RG2417 by measuring its ability to induce forward mutations at the thymidine kinase locus of mouse lymphoma LY5178Y cells.

The assay was performed in two phases, a dose range assay and a confirmatory assay. In the first phase, eight concentrations of RG2417 were used ranging from 5.0 to 0.0016 mg/ml. No toxicity was observed. In the second phase, RG2417 was used at concentrations of 5.0, 1.58, 0.5, and 0.16 mg/ml under both activating and non-activating conditions. For the non-activated test system exposures were for 4 and 28 hours while for the activated test system the exposure was for 4 hours. The clastogenic and mutagenic potential of RG2417 was evaluated by its ability to increase colonies on the restrictive agent trifluorothymidine. The mutant frequency of RG2417 was not more than two-fold increased above the corresponding control cultures. Based on the findings of this study, RG2417 is considered non-mutagenic.

3.4 Pharmacokinetics and Toxicokinetics in Animals

Blood levels of uridine are elevated after oral dosing with uridine or prodrugs of uridine. After oral dosing TAU is converted by ester hydrolysis to uridine during transit through or absorption from the gut. TAU is not detectable in blood at any time point at any dose used in these studies. Therefore, observations of elimination and metabolism of uridine after repeated drug exposure in animals using TAU aids in assessing the toxicokinetics of uridine.

The toxicokinetics of uridine after TAU dosing were assessed in rats and dogs by measuring plasma levels of uridine and uracil. The studies were designed to assess the rate of clearance of uridine at increasing dose levels of TAU and any change in the clearance rate of uridine after repeat administration of drug. The summary table for toxicokinetics in rats and dogs is shown in Table 6. Values obtained were not significantly different after 1 or 28 days of dosing and so only those values from the 28 days of dosing are shown. This data will be updated with toxicokinetic data from the 13 week studies of RG2417 in rats and dogs when the final reports are available.

Table 8: Toxicokinetics of Uridine after TAU Dosing

Study #	Species	Dose as Uridine (mg/kg)	Cmax µM	AUC µmol-hr/L	V_d L/kg
HLS RSZ-005	Rat	100 (TAU dose 150)	20.9	85	5.8
HLS RSZ-005	Rat	330 (TAU dose 500)	131	139	10.1
HLS RSZ-005	Rat	660 (TAU dose 1000)	197	231	9.1
HLS 02-3010	Dog	100 (TAU dose 150)	84	62	1.3
HLS 02-3010	Dog	330 (TAU dose 500)	430	574	1.4
HLS 02-3010	Dog	660 (TAU dose 1000)	780	1488	3.4

Basal levels in rats (~3 µM) have been subtracted. Dog basal uridine level is non-detectable.
The method has a lower limit of quantitation (LLOQ) of 0.8 µM for uridine.

An oral toxicology study in CD rats following TAU administration was conducted. A satellite group of animals was assigned for toxicokinetic sampling. A total of seventy- two rats were divided into four groups with nine females and nine males in each group. TAU was mixed with 1% carboxymethylcellulose, methylparaben (0.18%) and propylparaben (0.02 %), and homogenized to yield a suspension. Doses for groups 2, 3 and 4 were 150, 500 and 1000 mg/kg, respectively, twice a day. This is equivalent to 100, 330, and 660 mg/kg bid of uridine, respectively.

Blood was collected at 0, 10, 20, and 40 minutes, and 1, 2, 4, 8 and 24 hours after dosing on day 0 and day 27 and the plasma analyzed by HPLC analysis.

There was a dose dependent increase in blood uridine Cmax and AUC. There was no significant difference in the Cmax of uridine between Day 0 and Day 28. No significant difference in the absorption, distribution and metabolism of TAU was observed between males and females.

3.4.2 Absorption and elimination of uridine in dogs after 1 or 28 days of dosing with TAU (HLS 02-3010)

A 28-day oral toxicity study of TAU with a 28-day recovery period was conducted in dogs. Three treatment groups were given oral doses of TAU at 150, 500 and 1000 mg/kg, bid. This is equivalent to 100, 330, and 660 mg/kg bid of uridine, respectively. All animals were dosed at 5 ml/kg. Blood samples were collected at 0, 10, 20, 40, 60 and
90 minutes and 2, 3, 4, 8 and 24 hours after dosing on day 0 and day 27 and plasma was analyzed by HPLC analysis.

Plasma uridine Cmax and AUC0-∞ increased in a dose-dependent manner. There was no significant difference between Day 1 and Day 28 in terms of Cmax uridine. No significant difference was observed between males and females in terms of uridine absorption, distribution and metabolism.

Uridine half-life (T1/2) in dogs increased by 6-fold the dose 100 to 660 mg/kg dose (0.24 ± 0.03 hr to 1.55 ± 0.33 hr). This is possibly due to the saturation of uridine metabolism, namely, uridine conversion to uracil by uridine phosphorylase. This is supported by data indicating plasma

uracil increased linearly with dosing up to 330 mg/kg, and then reached an observed plateau in the range from 330 to 660 mg/kg of dose. Saturation of uridine phosphorylase was not observed under similar dosing in rats.

4 EFFECTS IN HUMANS

4.1 Previous Human Experience with Uridine/RG2417

Uridine/RG2417 has been studied in healthy volunteers and patients with bipolar I disorder. A tabular summary of these studies is in Appendix 7.2.

4.1.1 RG2417-02: Healthy Volunteers

RG2417 was administered to healthy human volunteers in a Phase I trial to compare the pharmacokinetics of RG2417 given as tablets or as an oral solution. Twelve subjects were given single doses of 1 g, 2 g and 4 g RG2417 and plasma concentrations were sampled at multiple times after the doses. PK variables in this study were maximum concentration (Cmax), time to Cmax (Tmax), AUC and T1/2.

Mean plasma uridine concentrations (Figure 6) and mean values for Cmax, AUC(0-t), and AUC(0-inf) (9) increased in a dose-related manner after oral administration of a single 1 g, 2 g, and 4 g dose of RG2417 as tablets or solution. Maximum plasma concentrations occurred at a median Tmax of 60 to 105 minutes and the T1/2 ranged from 175 to 274 minutes (2.9 to 4.6 hours). Neither Tmax nor T1/2 parameter appeared to be dependent on either dose or formulation (Table 7).

Figure 6: Mean Plasma Concentrations of Uridine after Oral Administration of Single 1 g, 2 g, and 4 g Doses of RG2417 as Tablets and a Solution to Healthy Volunteers

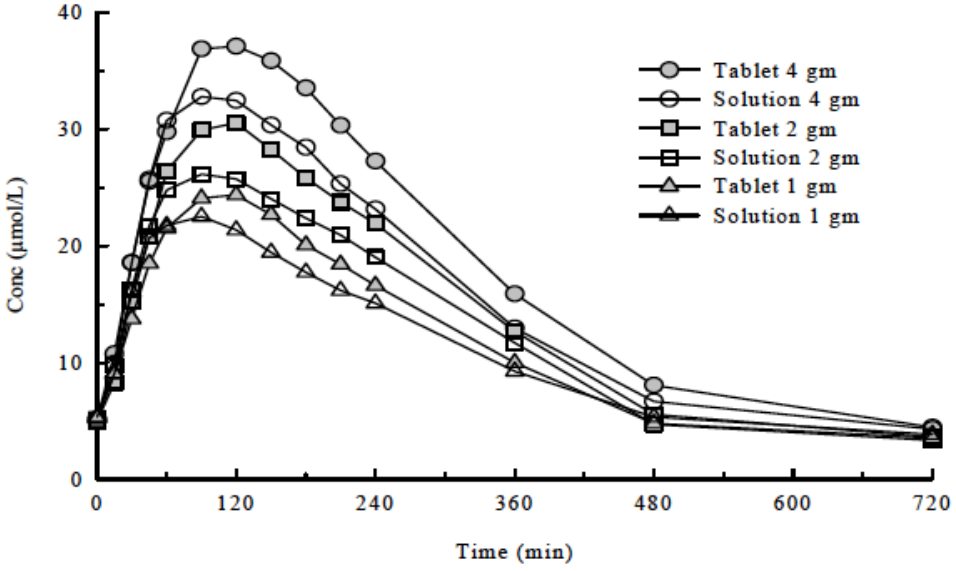


Table 9: Summary of Pharmacokinetic Parameters for RG2417-02 Uridine after Oral Administration of Single 1 g, 2 g and 4 g Doses of RG2417 as Tablets and a Solution to Healthy Volunteers

Parameter	Sol 1g	Tab 1g	Tab 2g	Sol 2g	Tab 4g	Sol 4g
C_{max} (µmol/L)	23.3 ± 5.3	26.5 ± 5.7[1]	32.3 ± 4.95	27.4 ± 5.4	39.1 ± 9.2	33.8 ± 12.50
T_{max} (min)	60.0	90.5	105	90.0	90.0	90.0
AUC (0-t) (min•µmol/L)	7788 ± 1650	8151 ± 998	10036 ± 1343	8952 ± 1989	12662 ± 3092	11006 ± 3688
AUC (∞) (min•µmol/L)	8914 ± 1871	9023 ± 1525	11149 ±1670	9655 ± 3228	14321 ± 3786	13613 ± 5270
$T_{1/2}$ (min)	274 ± 102	186 ± 37	177 ± 21	181 ±20	175 ± 18	176 ± 13

[1]Mean ± standard deviation except for T_{max} for which the median is reported.

The secondary objective of this study was to determine the safety of RG2417 in healthy volunteers. There were a total of 13 adverse events (AEs) in 5 subjects during this study summarized in Table 10. Adverse events includes any untoward medical occurrence in a subject administered RG2417 or placebo that does not necessarily have a causal relationship with treatment. The majority of AEs were mild in severity (11 AEs) with 1 AE judged by the Investigator to be severe (headache) and 1 AE judged by the Investigator to be moderate (headache). No AEs were reported to be life-threatening. Only 3 of the AEs (2 events of upper abdominal pain and 1 AE of headache) were judged by the Investigator to be possibly related to the study medication. Table 11 lists the adverse drug reaction of headache from RG2417. Adverse drug reactions include any unintended response(s) related to any dose of RG2417.

Table 10: RG2417-02 Summary of Adverse Events

Body System	Adverse Event[1]	Placebo	RG2417					
			Solution			Tablets		
			1 g	2 g	4 g	1 g	2 g	4 g
Nervous system disorders	**TOTAL**	**1**	**1**	**1**	**2**	**2**	**0**	**2**
	Headache	0	1	1	0	1	0	1
	Dizziness	0	0	0	1	1	0	1
	Lethargy	0	0	0	1	0	0	0
	Syncope	1	0	0	0	0	0	0
Gastrointestinal disorders	**TOTAL**	**1**	**0**	**1**	**0**	**0**	**0**	**0**
	Abdominal pain upper	1	0	1	0	0	0	0
General disorders and administration site conditions	**TOTAL**	**0**	**1**	**0**	**1**	**0**	**0**	**0**
	Influenza like illness	0	1	0	0	0	0	0
	Pain	0	0	0	1	0	0	0
Respiratory, thoracic, and mediastinal disorders	**TOTAL**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
	Rhinitis	0	0	0	0	0	0	1

[1]Listed are the number of subjects experiencing an adverse event. If a subject had multiple occurrences of an AE, the subject is presented only once in the respective subject category.

Table 11: RG2417-02 Severe Adverse Drug Reaction(s)

Body System	Adverse Event[1]	Severity
Nervous system disorders	Headache	Severe

[1]Listed are adverse events occurrences in at least one subject by severity. If more than one subject experienced an adverse event of the same severity, the adverse event is only listed once in the respective severity category.

There were no deaths, serious adverse events (SAEs) or other clinically-significant AEs reported during this study. No subjects were withdrawn from the study due to an AE.

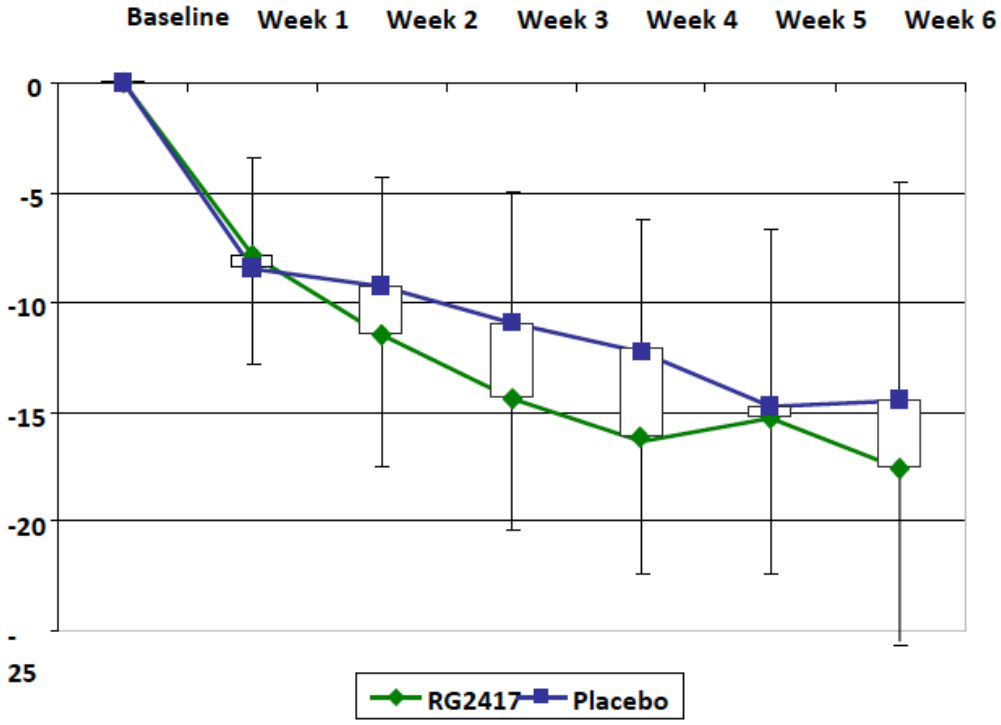
There were no laboratory differences of significance between the treatment groups. There were no clinically-significant vital sign or physical examination findings. The majority of ECG results were normal and any abnormal ECG results were not considered to be clinically significant.

Based on these findings, uridine/RG2417 via oral administration as a tablet or solution was safe and well-tolerated in healthy male volunteers when either 1 g, 2 g or 4 g doses were randomly given over 3 dosing sessions.

4.1.2 Uridine/RG2417-01: Bipolar I Depression

A clinical trial in bipolar I depression has been completed, protocol uridine/RG2417-01, entitled, "Dose-Escalating, Phase II Study to Assess the Safety and Tolerability of Uridine/RG2417 in the Treatment of Bipolar I Depression". This was a randomized, placebo-controlled parallel arm, double-blind multi-center study of the safety and efficacy of uridine/RG2417 as monotherapy in subjects with bipolar I disorder and a current depressive phase of longer than 30 days. After a 4 week period to taper off psychotropic medications, a total of 84 subjects were randomized (44 placebo, 40 RG2417) for weekly evaluations over a 6 week treatment period. Subjects started on placebo or RG2417 at 1 g p.o. bid, which could then be elevated to 2 g p.o. bid depending on tolerability and efficacy. The primary safety analysis was recording of adverse events, clinical and laboratory information, which was reviewed in two interim safety analyses by an independent Data Safety Monitoring Board (DSMB). Primary efficacy analysis was the treatment response, measured by the Montgomery-Asberg Depression Rating Scale (MADRS) and Clinical Global Impression of Bipolar Disorder (CGI-BP) compared to placebo.

Figure 7: Comparison of the Change from Baseline for Total MADRS Scores for RG2417 (Uridine) and Placebo Groups



In Figure 7, the average total MADRS score is plotted as the change from study day 1 (baseline) for the uridine/RG2417 and placebo groups. The true difference between groups is shown by the boxes at each evaluation time point. The solid boxes represent the magnitude of the uridine/RG2417 treatment benefit in comparison to placebo.

Uridine RG2417 shows treatment benefit from week 2 to the end of the study, however a strong placebo response is observed particularly at weeks 5 and 6. Uridine/RG2417 treatment resulted in an improvement in the MADRS assessments compared to placebo over the 6 week period (Figure 7), reaching statistical significance ($p = 0.011$; mixed effects trend to repeat measures).

The CGI-BP evaluation demonstrated an improvement ($p = 0.044$) for uridine/RG2417 treatment in comparison to placebo. Other clinical measurements included the Young Mania Rating Scale (YMRS) and the Hamilton Anxiety score (HAM-A), both of which showed no increase in the overall mania or anxiety of bipolar I patients on uridine/RG2417 treatment.

The safety analysis confirmed in this population that uridine/RG2417 is a safe and well-tolerated drug. Treatment of bipolar depression with standard antidepressant medications may result in an increase in treatment associated affective switch (TAAS) to mania or hypomania. In this study there were two hypomanic episodes, 1 from the treatment group and 1 from placebo. Although not all patients escalated to the high dose, all subjects taking uridine/RG2417 at 2 g p.o. bid were able to tolerate it at this level without any dose reductions. There were no clinically significant abnormalities of clinical laboratories, physical or vital signs, or urinalysis associated with uridine/RG2417 treatment.

In general, adverse events were about as common in the uridine/RG2417 group as placebo. Overall, 23 of the 40 (57.5%) subjects who received uridine/RG2417 experienced a total of 109 adverse events. Twenty nine of the 44 (65.9%) subjects in the placebo group experienced a total of 98 adverse events. There is no difference between placebo and uridine/RG2417 in the overall severity of AEs, but uridine/RG2417 is slightly more attributable in relationship to AEs. Table 12 summarizes the adverse event data from study RG2417-01.

Table 12: RG2417-01 Adverse Event Summary

Adverse Events		Placebo (n = 44)	RG2417 (n = 40)
TOTAL		29[1](98)[2]	23 (109)
Severity	Mild	9	5
	Moderate	15	13
	Severe	5	5
Relationship	Unrelated	8	2
	Unlikely	5	3
	Possible	13	13
	Probable	3	4
	Definite	0	1

[1]The number of subjects that experienced an adverse event.
[2]The total number of events are given in parentheses.

Table 13 provides a summary of adverse events from the uridine/RG2417 study. Adverse events include any untoward medical occurrence in a subject administered uridine/RG2417 or placebo that does not necessarily have a causal relationship with treatment. Gastrointestinal, nervous system disorders, and psychiatric disorders were commonly observed and consistent with published literature [3, 13]. Although AEs of the nervous system were relatively common (31% of total), there was little if any difference between RG2417 and placebo. There was no greater rate of somnolence and sedation in the uridine/RG2417 group compared to Placebo (3 events Placebo and 3 events RG2417). There were fewer observed adverse events for anxiety (3 Placebo and 1 Uridine/RG2417) and insomnia (5 Placebo and 4 Uridine/RG2417) in the uridine group compared to placebo.

Table 13: RG2417-01 Summary of Common Adverse Events

Body System	Adverse Event[1]	Placebo (n=44) N (%)	RG2417 (n=40) N (%)	All Combined (n=84) N (%)
Gastrointestinal	Dry Mouth	1 (2.3)	4 (10.0)	5 (6.0)
	Nausea	2 (4.5)	4 (10.0)	6 (7.1)
General Disorders and Administrative Site Conditions	Fatigue	1 (2.3)	4 (10.0)	5 (6.0)
Infections and Infestations	Upper Respiratory Tract Infection	3 (6.8)	2 (5.0)	5 (6.0)
	Urinary Tract Infection	2 (4.5)	4 (10.0)	6 (7.1)
Nervous System Disorders	Dizziness	2 (4.5)	2 (5.0)	4 (4.8)
	Headache	7 (15.9)	8 (20.0)	15 (17.9)
	Sedation/Somnolence	3 (6.8)	3 (7.5)	6 (7.2)
Psychiatric Disorders	Anxiety	3 (6.8)	1 (2.5)	4 (4.8)
	Insomnia	5 (11.4)	3 (7.5)	8 (9.5)

[1]Number of subjects with adverse events ≥ 5% of the total regardless of the relationship to study medication (RG2417 or Placebo)

Table 14 summarizes the most common adverse drug reactions. Adverse drug reactions include any unintended response(s) related to any dose of uridine/RG2417. Table 14 shows the number of patients with adverse events ≥ 5% of the total for which the rate in the treated group exceeds the rate for placebo from study RG2417-01. Specific reactions that were predominant in the uridine group included dry mouth (4 events in 4 subjects), fatigue (4 events in 4 subjects), and nausea (4 events in 4 subjects). The only AE that represented > 10% of total adverse events was headache (8 events Uridine/RG2417 and 7 events Placebo) which was similar between groups.

Table 14: RG2417-01 Common Adverse Drug Reactions

Body System	Adverse Reaction[1]	Placebo (n=44) N (%)	RG2417 (n=40) N (%)	All Combined (n=84) N (%)
Gastrointestinal	Dry Mouth	1 (2.3)	4 (10.0)	5 (6.0)
	Nausea	2 (4.5)	4 (10.0)	6 (7.1)
General	Fatigue	1 (2.3)	4 (10.0)	5 (6.0)
Nervous System	Headache	7 (15.9)	8 (20.0)	15 (17.5)

[1] Number of subjects with adverse reactions ≥ 5% of the total for which the rate in the treated group exceeds the rate for plabebo.

Table 15 shows less common adverse reactions (number of subjects with adverse reactions < 5% of the total are represented and for which the rate of the treated group exceeds the rate for placebo). Nervous system disorders, skin and subcutaneous tissue disorders, gastrointestinal disorders, and vascular disorders were highest among the less common adverse reactions. Hypotension (2 reactions, 1 considered serious) and clumsiness (2 reactions) occurred at the highest rates of the less common reactions.

Table 15: RG2417-01 Less Common Adverse Drug Reactions

Body System	Adverse Reaction[1]	Placebo (n=44) N (%)	RG2417 (n=40) N (%)	All Combined (n=84) N (%)
Gastrointestinal	Dyspepsia	0	1 (2.5)	1 (1.2)
	Haematochizia	0	1 (2.5)	1 (1.2)
Investigations	Weight Decreased	0	1 (2.5)	1 (1.2)
Musculoskeletal	Myalgia	0	1 (2.5)	3 (3.6)
Nervous System	Clumsiness	0	2 (5.0)	2 (2.4)
	Dysphasia	0	1 (2.5)	1 (1.2)
	Tension headache	0	1 (2.5)	1 (1.2)
Psychiatric	Bruxism	0	1 (2.5)	1 (1.2)
	Nightmare	0	1 (2.5)	1 (1.2)
Renal	Pollakiuria	0	1 (2.5)	1 (1.2)
Respiratory	Rhinorrhoea	0	1 (2.5)	1 (1.2)
Skin and Subcutaneous Tissue Disorders	Increased Tendency To Bruise	0	1 (2.5	1 (1.2)
	Pruritus	0	1 (2.5)	1 (1.2)
	Rash	0	1 (2.5)	1 (1.2)
Vascular Disorders	Hypertension	0	2 (5.0)	2 (2.4)
	Orthostatic hypertension	0	1 (2.5)	1 (1.2)

[1] Number of subjects with adverse reactions < 5% of the total are represented and for which the rate in the treated group exceeds the rate for placebo.

In reviewing adverse events associated with the bipolar population from study RG2417- 01, rates attributable to psychiatric etiology were slightly higher in the Placebo group (10 Placebo and 7 Uridine RG2417 total events). Bruxism and nightmare were the only psychiatric events considered related to uridine/RG2417 which occurred at a higher rate in uridine/RG2417 than

Placebo. There were 2 (2.4%) subjects with hypomania, 1 in each treatment group. Cardiac disorders were infrequent in both groups with only 1 (1.2%) palpitation event in the study, which was not considered related to uridine/RG2417.

Table 16: RG2417-01 Serious and/or Severe Adverse Drug Reactions

Body System	Adverse Reaction[1]	Severity	Serious
Nervous System Disorders	Migraine	Severe	No
	Headache	Severe	No
Psychiatric Disorders	Insomnia	Severe	No
Vascular Disorders	Hypertension	Moderate	Yes

[1] Adverse reactions in at least one subject by severity for which there is some basis to believe there is a causal relationship between the drug and the event. If more than one subject experienced an adverse reaction of the same severity, the reaction is only listed once in the respective severity category.

There were two serious adverse events observed during the study, one in the placebo group and one in the uridine/RG2417 group. The SAE in the placebo group was unrelated to treatment; the uridine SAE was possibly related to treatment (Table 16). The SAE in the uridine group was exacerbation of pre-existing hypertension requiring immediate monitoring and work-up. The subject in the uridine RG2417 group presented for weekly assessment and noted to have blood pressure of 180/100. The subject had pre-existing hypertension, and was taking diuretic medication. There were no complaints of dyspnea, shortness of breath or substernal chest pain. The subject was immediately referred to their primary care physician who placed the patient in a cardiac monitoring unit and ruled out an acute ischemic event. The work- up was negative and the subject was allowed to go home with the addition of an angiotensin-converting enzyme inhibitor (ACEI) medication. The SAE was considered to be possibly related to study drug. There have been no prior reports of SAEs with the phase I experience of uridine/RG2417, or in the published literature with uridine or uridine pro-drugs, so this SAE has no clinical context. The assurance that this SAE was an isolated event will require confirmation in further clinical studies.

Based on these safety findings, RG2417 as a monotherapy via oral administration up to 4 g was safe and well tolerated in Bipolar 1 depression.

4.1.3 Uridine/RG2417-03: Bipolar I Depression

A clinical trial to further assess the safety and efficacy of uridine/RG2417 in bipolar I depression, protocol RG2417-03, entitled, “A Phase II Randomized, Double-Blind, Placebo- Controlled, Flexible-Dose Study to Assess the Safety, Tolerability and Efficacy of RG2417 (Uridine) in the Treatment of Bipolar I Depression” is currently being conducted.

This study is a randomized, eight-week, double blind, placebo-controlled, phase II study to assess the safety and efficacy of RG2417 in bipolar I depression. One hundred and fifty (150) subjects of both sexes will be enrolled at up to thirty (30) sites and will be randomized on a 1:1 basis to receive either placebo or uridine/RG2417.

Use of psychotropic drugs other than symptomatic use of benzodiazepines and sedatives/hypnotics is not permitted during the study. Subjects will be tapered from all such medications for up to 28 days prior to Visit 2 (Day 0). This medication taper is necessary in order to measure the clinical outcomes of RG2417 as a monotherapy, therefore reducing the possibility of a false negative. The duration of the medication taper will be at the discretion of the

investigator. However, subjects must be off all psychotropic drugs (excluding fluoxetine) at least 24 hours prior to Visit 2 (Day 0); subjects must discontinue fluoxetine at least 2 weeks prior to Visit 2. During the treatment phase, symptomatic use of small doses of sedatives/hypnotics (equivalent to a total daily dose of 12.5 mg zolpidem) will be permitted, at the discretion of the investigators for no more than 19 days total as well as benzodiazepines (equivalent to a total daily dose of 2 mg lorazepam) for no more than 19 days total.

The clinical impact of treatment will be evaluated by physical examination, recording of adverse events, laboratory values, vital signs and clinical rating scales (Montgomery-Asberg Depression Rating Scale, Young Mania Rating Scale, Clinical Global Impression of Severity-Bipolar Disorder and the Columbia-Suicide Severity and Rating Scale).

4.2 Anticipated benefits and risks

It is anticipated that administration of uridine/RG2417 to patients with Bipolar Depression will result in an improvement in their clinical status, as measured by a decrease from baseline in their MADRS and Clinical Global Impression of Severity scores, without induction of mania, as measured by the YMRS. The primary risks associated with uridine/RG2417 are likely to be those seen with other formulations of uridine, including transient gastrointestinal discomfort, diarrhea, headache and hypertension.

4.3 Marketing Experience

There is no marketing experience with uridine/RG2417.

5 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

5.1 Investigational Product

Uridine/RG2417 is currently under investigation as a potential treatment for bipolar depression and suicidal ideation.

5.2 Drug Safety

Uridine/RG2417 does not have an effect on the neurobehavioral activity in rats. There is no alteration in cardiac function after single doses in minipigs or multiple doses in dogs. RG2417 does not appear to be genotoxic as measured by the Ames test and in vitro mammalian chromosomal aberration test. Repeat dosing for 28 days with the prodrug triacetyluridine (TAU) in rats showed microscopic findings in kidney and thyroid tissue. Repeat dosing for 28 days in dogs showed microscopic findings in mesenteric lymph nodes and thyroid.

Uracil, the free base of uridine, is demonstrated to be a urinary bladder cancer promoting agent in rats, with a TD50 of 671 mg/kg/day (TD50 is the daily dose required to induce tumors in half of the animals that would have remained tumor-free at zero dose). Bladder carcinoma in rats is associated with the formation of calculi [52].

Previous experience in man with oral dosing of uridine and uridine based prodrugs have shown mild-to moderate side effects at high doses including diarrhea and nausea.

Uridine/RG2417 in healthy human volunteers at repeated single dosing of 1 g, 2 g and 4 g was well tolerated with minimal side effects.

Uridine/RG2417 given as a daily dose of 2 g or 4g day was safe and well tolerated over a 6 week period in bipolar depression.

5.3 Adverse Reactions

Transient gastrointestinal discomfort and diarrhea are the most common adverse events noted in conjunction with treatment with uridine and its prodrugs.

In subjects dosed with RG2417, the most common adverse reactions included dizziness, dry mouth, nausea, fatigue and headache. (Table 11 and Table 14). Less common adverse reactions included dyspepsia, haematochezia, weight decrease, myalgia, clumsiness, dysphasia, bruxism, nightmare, pollakiuria, rhinorrhea, increased bruising, rash, and hypertension (Table 15).

There was only one serious adverse event reported that was possibly related to uridine/RG2417. This subject, with a co-morbid hypertension experienced a worsening of this pre-existing condition and required inpatient monitoring. There were no other ischemic or cardiopulmonary sequelae of this event.

5.4 Method of Administration

Uridine is intended for oral dosing.

5.5 Contraindications

None known.

5.6 Special Warning and Special Precautions for Use

There is an increased lactose content in the placebo formulation which may cause clinical symptoms in patients that are lactose intolerant.

5.7 Interactions with Other Medications and Other Forms of Interaction

None known.

5.8 Undesirable Effects

None known.

5.9 Overdose

None known.

5.10 Shelf life

Uridine/RG2417 drug product is expected to remain stable for the duration of clinical studies. All lots used in clinical studies will remain on stability testing for the duration of the studies. RG2417

drug product lot RCM-05-0001 has remained stable for 2 years when stored at 25°C. The stability testing is ongoing.

5.11 Special Precautions for Storage

Avoid exposure to temperatures < 20°C and > 30°C.

5.12 Nature and Contents of Container

Tablets are packaged in white HDPE bottles (400cc) with cotton fill. Bottles are induction sealed and closed with a childproof cap.

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