

NCT05605301

**Pharmacokinetics Study of Oral 2-Deoxy-D-Glucose (2DG) in
Subjects with a Confirmed Diagnosis of Epilepsy**

**Version 2.0
February 14, 2023**

FINAL

Study Committee

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DOCUMENT HISTORY (VERSION CONTROL)

Summary of Changes

Version #	Study Protocol (IRB)	Event Date (IRB)	Author	Approved by/Date	Reason
Original	1 May 2021	26 Oct 2021	N.Fountain	IRB 28 Oct 2021	Approval: New Protocol
Amendment 1.0	22 Apr 2022	22 Sep 2022	N.Fountain	IRB 22 Sep 2022	Approval: Expedited Protocol Modifications
Amendment 1.0	22 Apr 2022	11 Oct 2022	N.Fountain	IRB 11 Oct 2022	Approval: Protocol Continuation (1 year); No changes.
Version 2.0 FINAL	22 Apr 2022	14 Feb 2023	N.Fountain	IRB 21 Feb 2023	Approval: Modification: Change: PI to Dr. Mark Quigg; Sub-PI to Dr. N. Fountain

(B.Lewis:28Feb2023)

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1.0 Study Synopsis

NAME OF SPONSOR:	Thomas P. Sutula, MD, PhD Department of Neurology, University of Wisconsin-Madison (Investigator-initiated)
IND No:	114600
Title of Study:	Pharmacokinetics study of oral 2-Deoxy-D-Glucose (2DG) in subjects with a confirmed diagnosis of epilepsy
Investigators and Study Center(s):	Nathan Fountain, MD, University of Virginia
Phase of development:	Phase 2
Study Period (years):	2021 to 2023
Objectives:	The objective of this study is to study the safety, tolerability and pharmacokinetics of 2DG administered orally to adult epilepsy patients.
Methodology:	This is an open-label safety, tolerability, and pharmacokinetics study of 2DG in epilepsy patients. Subjects will be hospitalized during the duration of the study. Subjects are requested to report to the hospital in a fasted state (since midnight) on the morning of the pharmacokinetics study. Subjects will continue to fast until one hour after the initial 2DG dose has been given. Subjects will be given 2DG orally during a single day of dosing at one of three dose levels (a single dose of 40 mg for Dose Level 1, a single dose of 60 mg for Dose Level 2, or 60 mg bid for Dose Level 3). The total dose will not exceed 120 mg/day, or 60 mg maximum as a single dose. Blood samples for pharmacokinetics analysis will be drawn at time 0 (prior to drug administration for baseline), and then at 15, 30, 45, and 60 minutes and at 2, 4, 6, 12, and 24 hours after each 2DG administration. Subjects will be closely monitored for safety during and following dosing with 2DG.
Number of Subjects:	9 (estimated)
Diagnosis and Main Criteria for Inclusion:	Eligible patients will have a confirmed diagnosis of epilepsy.
Drug Product, Dose and Mode of Administration:	2DG will be formulated as an immediate-release, solid dosage form and administered orally. The starting dose will be 40 mg. Three dose levels are planned in sequential cohorts. The maximum dose will not exceed 60 mg bid.
Duration of Treatment:	The subjects will be exposed to either a single dose (40 mg or 60mg of 2DG) or bid dosing (60 mg bid) during one treatment day.
Reference Therapy, Dose and Mode of Administration:	There will be no placebo or reference treatment.
Inclusion Criteria: <ul style="list-style-type: none"> Confirmed diagnosis of epilepsy. For the purpose of inclusion, the seizure types include complex partial, simple partial motor, primary generalized tonic-clonic, secondary generalized tonic-clonic, tonic, clonic and atonic seizures, as well as simple partial and absence seizures. Stable treatment regimen with no change in antiepileptic drugs or antiepileptic drug doses for 28 days prior to enrollment. Age 18 to 60 years old. Women of childbearing potential must be using a standard method of birth control and agree not to become pregnant during the trial. Men must agree to not father a child during the trial. BMI must be between 18 and 35. 	

NAME OF SPONSOR:	Thomas P. Sutula, MD, PhD Department of Neurology, University of Wisconsin-Madison (Investigator-initiated)
Exclusion Criteria: <ul style="list-style-type: none"> • Occurrence of non-epileptic psychogenic spells within 2 years prior to enrollment. • Current or past history of diabetes or any abnormality of glucose metabolism. • Use of glucocorticoids, hypoglycemic agents (e.g., metformin) or any drug that alters glucose levels. • Use of any drug that is expected to alter glucose absorption, metabolism or serum measurements. • Clinically significant psychiatric or medical disease. • Previous therapeutic use of 2DG. • Pregnant or nursing women. • Use of an investigational medication within 2 months prior to enrollment. • Supine systolic blood pressure < 90 or > 160 mm Hg or diastolic > 90 mm Hg, or pulse < 60 or > 110 BPM. • Clinically significant abnormal 12-lead ECG. • Baseline prolongation of the QTc interval > 450 msec. • Clinically significant abnormal result by speckle tracking echocardiography (STE). • Elevated ALT or AST more than 1.5 times upper reference limit. • Baseline fasting glucose < 60 or > 110. • History of status epilepticus within 6 months prior to enrollment. • Progressive structural brain lesion or illness likely to progress during the study. 	
Criteria for Evaluation: Toxicities will be summarized in terms of types and severities by the most recent version of MedRA terminology.	

2.0 Background

The anticonvulsant and antiepileptic properties of 2-Deoxy-D-Glucose (2DG) were discovered in 2004 at the University of Wisconsin during an investigation of the mechanisms of action that underlie the ketogenic diet, which substitutes fats and proteins for carbohydrates and has been used for decades to treat refractory epilepsy ([Vamecq 2005](#)). A remarkable feature of the ketogenic diet is that in patients who have achieved seizure control on the diet, ingestion of even a small amount of carbohydrate can rapidly reduce the diet's effectiveness and result in seizure recurrence ([Huttenlocher 1976](#)). This clinical observation suggested that glycolysis and carbohydrate metabolism might promote seizure susceptibility, and that inhibition or reduction of glycolysis might have anticonvulsant effects.

2DG, a known inhibitor of glycolysis, differs from glucose by the presence of a hydrogen atom at the 2-position in place of a hydroxyl group. 2DG is taken into cells via glucose transport mechanisms and preferentially accumulates in cells that have high energy demands, including cells in the neural circuitry that are undergoing synchronization during seizures. Although 2DG is phosphorylated by hexokinase, 2DG-6P cannot be metabolized by G6P isomerase ([Wick 1957](#)). 2DG "glycolysis" therefore stops after the first glycolytic step, and 2DG-6P becomes trapped metabolically. The trapped 2DG-6P reversibly inhibits subsequent steps of glycolysis. These properties have enabled the use of radiolabeled ^{18}F -2DG and ^3H -2DG in PET and autoradiographic imaging for measurement of regional glucose utilization ([Newman 1990](#), [Elman 1999](#)).

In early experiments, 2DG in vivo had pronounced anticonvulsant and antiepileptic effects in a common chronic model of limbic epilepsy ([Garriga-Canut 2006](#), [Stafstrom 2009](#)). The antiepileptic effects of 2DG included 2-fold slowing of progression of kindled seizures. The acute anticonvulsant properties of 2DG were subsequently confirmed in acute screening models including 6 Hz seizures and audiogenic seizures in Frings mice, with distinct actions and properties compared to all marketed anticonvulsants ([Stafstrom 2009](#)). Glycolysis inhibition was shown to have anticonvulsant actions in hippocampal slices against epileptic activity induced by elevated extracellular K^+ and the GABA_A antagonist bicuculline ([Stafstrom 2009](#)).

There is evidence that 2DG enhances tonic inhibition mediated by extrasynaptic GABA_A receptors, which may contribute to anticonvulsant actions ([Forte 2016](#)). Shao and Stafstrom ([Shao 2017](#)) investigated the effects of glycolytic inhibition with 2DG on basal membrane properties, spontaneous neuronal firing, and epileptiform network bursts in hippocampal slices. The effect of glycolytic inhibition on basal membrane properties was examined in hippocampal CA1 neurons, which are not ordinarily active spontaneously. Intracellular application of 2DG did not significantly alter the membrane input resistance, action-potential threshold, firing pattern, or input-output relationship of these neurons compared with simultaneously recorded neighboring neurons not treated with intracellular 2DG. The effect of glycolytic inhibition on neuronal firing was tested in spontaneously active hippocampal neurons in CA3 when synaptic transmission was left intact or blocked. Under both intact and blocked conditions, bath application of 2DG (2 mM) blocked spontaneous firing of CA3 pyramidal neurons. In contrast, neuronal firing of CA3 neurons persisted when 2DG was applied intracellularly, suggesting that glycolytic inhibition of

individual neurons is not sufficient to stop neuronal firing. The effects of 2DG on epileptiform network bursts in area CA3 were tested. Bath application of 2DG abolished epileptiform bursts in a dose-dependent and all-or-none manner.

In another recent study ([Pan 2019](#)), bath application of 10 mM 2DG in hippocampal slices reduced epileptic burst currents in CA3 neurons with greater reduction of inward excitatory synaptic currents compared to outward inhibitory currents (71 vs. 41%, respectively), consistent with an anticonvulsant action. The anticonvulsant effects were associated with reduction in frequency but not amplitude of 1) glutamatergic inward currents, 2) pharmacologically isolated outward inhibitory currents, and 3) miniature EPSCs. The findings of reduced frequency but not amplitude of these measures indicates a presynaptic locus of 2DG anticonvulsant action.

The data including recent studies demonstrate that altered glucose metabolism profoundly affects cellular and network hyperexcitability and confirm that glycolytic inhibition by 2DG can abrogate epileptiform activity. The anticonvulsant actions of 2DG are distinct from effects of sustained hypoglycemia, including hypoglycemia induced by 2DG, which can induce seizures.

Multiple pre-clinical studies have demonstrated anticonvulsant actions of 2DG against chemoconvulsant-induced status epilepticus in both rats and mice. Administration of 2DG (250 mg/kg intraperitoneal) at 1 hour prior to status epilepticus induced by pilocarpine significantly reduced seizure duration and severity scores, and also significantly delayed the onset of seizures in status epilepticus induced by kainic acid in Sprague Dawley rats ([Lian 2007](#)). In another study, dose-dependent anticonvulsant effects of 2DG against pilocarpine-induced status epilepticus in C57BL/6 mice included significant prolongation of the latency to seizure onset, reduction in seizure severity scores, and reduction in duration of seizures across doses of 100, 250, and 500 mg/kg administered by intraperitoneal injection ([Yang 2013](#)).

2DG has the unique property of activity-dependent delivery to brain regions experiencing high levels of neural activity and increased metabolic demand, as occur during seizures. Activity-dependent delivery is a consequence of the “in vivo” brain mechanism of neurovascular coupling, which regulates local blood flow and delivery of glucose and O₂ with millisecond and micron precision into brain regions with high energy demands ([Zhao 2011](#)). As a consequence of neurovascular coupling, 2DG has novel effects against progressive effects of kindled seizures when administered as long as 10 minutes AFTER a seizure ([Sutula 2008](#)) when local blood flow remains enhanced due to continuing postictal metabolic energy demand and enhanced blood flow to epileptic brain regions. Activity-dependent, seizure-induced enhanced uptake of 2DG will be particularly helpful for anticonvulsant action during status epilepticus because of high metabolic demand in brain regions with a high seizure frequency or continuing seizures.

3.0 Objectives: Specific Aims and Hypotheses

The primary objective is to demonstrate the safety, tolerability and pharmacokinetics of oral 2DG administered to adult epilepsy patients.

Safety Hypothesis: There will be no serious cardiac or metabolic adverse events associated with the administration of a single oral dose of 40 mg or 60 mg, or a 60 mg bid oral dose of 2DG.

4.0 Investigational Plan

4.1 Design Considerations

A 3-level 2DG dose escalation is planned in sequential cohorts of 3 subjects with review of each cohort before proceeding to the next cohort.

On the day of exposure, subjects will receive a single oral dose of 2DG (40 mg or 60 mg) or twice daily (60 mg bid) dosing to assess safety, tolerability, and pharmacokinetics of 2DG.

Eligible patients will have a confirmed diagnosis of epilepsy. For the purpose of inclusion, the seizure types include complex partial, simple partial motor, primary generalized tonic-clonic, secondary generalized tonic-clonic, tonic, clonic and atonic seizures, as well as simple partial and absence seizures.

It is anticipated that 9 subjects will be sufficient to complete the pharmacokinetics study of the 3 dose levels.

4.2 Dosing

In a rat epilepsy model, anticonvulsant activity has been observed at doses of 37.5 mg/kg/day and higher ([Sutula 2008](#)). Based on the body surface area normalization method ([Reagan-Shaw 2007](#)), the rodent efficacy data suggest a potential for antiepileptic 2DG activity to occur in humans at doses above 6 mg/kg/day.

Information about the tolerability of repeated daily and weekly 2DG administration across a wide range of doses has been published for human cancer patients. Threshold Pharmaceuticals sponsored a Phase 1 clinical study of oral 2DG in patients with advanced period cancer ([Stein 2010](#)). Patients were treated with 2DG daily for the first 14 days of the 21-day treatment cycle at doses of 30, 45 or 60 mg/kg/day. 2DG was well tolerated at the lower doses, but grade 3 asymptomatic QTc prolongation was observed in two of four patients treated at the 60 mg/kg/day dose level. Grade 3 QTc prolongation was identified as the dose limiting toxicity (DLT). As none of the patients treated at the 45 mg/kg/day dose developed QTc prolongation and no other adverse events were observed at this dose, 45 mg/kg was identified as the Maximum Tolerated Dose (MTD).

Threshold Pharmaceuticals also evaluated the safety, pharmacokinetics and tolerability of 2DG in combination with docetaxel in patients with advanced solid tumors ([Raez 2013](#)). During dose escalation, 2DG was administered orally once daily for 7 days on an every-other-week schedule (weeks 1 and 3), while docetaxel was administered intravenously (30 mg/m²) for 3 weeks beginning on the first day of week 2 (weeks 2, 3 and 4). When the initial dose escalation was complete, cohorts of patients were treated using two different schedules. In the first, 2DG was administered on the first 21 days of the 28-day cycle, while in the second, 2DG was given on all 28 days. The maximum number of treatment cycles was 12. Of 34 enrolled patients, 21 received

2DG on the every-other-week dose escalation schedule, 6 received 2DG on the 21-day schedule, and 7 received 2DG on the 28-day schedule. 63 mg/kg was identified as the clinically tolerable dose. The most significant adverse effects, noted at 63 and 88 mg/kg, were reversible hyperglycemia (100%), gastrointestinal bleeding (6%) and reversible grade 3 QTc prolongation (22%).

Taken together, the Threshold studies suggest that 2DG can be administered to humans at dose levels lower than 45 mg/kg/day for a duration of two weeks without inducing QTc prolongation.

The Maximum Recommended Starting Dose (MRSD) has been calculated using data from toxicity studies conducted by Battelle Laboratories in dogs and rats. In these studies, 2DG was administered daily for 28 consecutive days.

In a 28-day good laboratory practice (GLP) study in beagle dogs, 2DG was well tolerated up to the maximum tested dose of 180 mg/kg/day which was designated as the NOAEL. No cardiac myocyte vacuolation was detected. Based on body surface area considerations ([Reagan-Shaw 2007](#)), the 180 mg/kg/day dose in dogs was equivalent to 600 mg/kg/day in rats.

In a 28-day GLP study in Sprague-Dawley rats, 2DG was administered at dose levels of 60, 120 and 250 mg/kg/day. At the low dose level of 60 mg/kg/day, 1 of 10 animals scored positive for cardiac myocyte vacuolation, an anticipated toxicity. At the mid dose level of 120 mg/kg/day, 6 of 10 animals scored positive, while at the high dose of 250 mg/kg/day, 9 of 10 animals scored positive. Based on these data, the NOEL could not be determined, and the MRSD could not be calculated.

The study in Sprague-Dawley rats was redesigned and repeated using dose levels of 20, 50, 125 and 375 mg/kg/day. The doses were chosen to provide two dose levels (20 and 50 mg/kg/day) expected to be near or below the NOEL, one dose level previously associated with a moderate level of cardiac histopathology (125 mg/kg/day), and one dose level expected to produce a significant level of cardiac histopathology (375 mg/kg/day). At 20 and 50 mg/kg/day, no animals scored positive for cardiac myocyte vacuolation. At 125 mg/kg/day, cardiac myocyte vacuolation was observed in male but not female animals, while at 375 mg/kg/day, animals of both sexes scored positive. Thus, 50 mg/kg/day was identified as the NOEL. Myocyte vacuolation was demonstrated to be reversible including partial resolution in all rats and complete resolution in a subset of rats by 2 weeks after cessation of dosing.

The MRSD calculation was based on the observed NOEL of 50 mg/kg/day in Sprague-Dawley rats, the more sensitive species, and includes a body surface area correction and a safety margin of ten-fold according to the FDA guidance:

- $\text{NOEL (mg/kg/day)} \div [k_m \text{ human}/k_m \text{ animal}] = \text{Human Equivalent Dose (HED)}$

where the K_m factor, body weight (kg) divided by body surface area (BSA, m^2), is used to convert the mg/kg dose to a mg/m^2 dose. The K_m factor for rats is 6; the K_m factor for humans is 37 ([Reagan-Shaw 2007](#))

- $\text{HED} \div \text{safety factor (10)} = \text{MRSD in humans}$

where the safety factor is from the FDA guidance.

- $\text{NOEL (50 mg/kg/day in rats)} \div (37 \div 6) = 8.1 \text{ mg/kg/day (HED)}$
 $8.1 \div 10 \text{ (safety factor)} = \sim 0.8 \text{ mg/kg/day (MRSD)}$

With the application of a 10-fold safety factor, the safe starting dose is calculated to be 0.8 mg/kg/day (56 mg for a 70 kg human).

The starting dose will be rounded down from 56 mg to 40 mg to provide an additional margin of safety and to be consistent with the solid dosage forms (20, 100 and 500 mg capsules).

The following dose escalation scheme will be used in sequential 3-subject cohorts to achieve the three dose levels planned for this study:

Dose Level 1: 40 mg	(0.57 mg/kg for a 70 kg human)
Dose Level 2: 60 mg	(0.86 mg/kg for a 70 kg human)
Dose Level 3: 60 mg bid	(0.86 mg/kg bid or 1.71 mg/kg/day for a 70 kg human)

Dose Level 3 is the maximum planned dose. In multiple dose studies conducted in cancer patients by Threshold Pharmaceuticals, it was shown that 2DG can be administered to humans at dose levels of 45 mg/kg/day for a duration of at least one week without inducing QTc prolongation.

After 3 subjects have completed enrollment at Dose Level 1, the results will be reviewed. The Study Committee will determine if the next cohort should be enrolled at Dose Level 2. The same procedure will be repeated after each cohort to review data to determine if the subsequently higher Dose Level should be enrolled. If the Study Committee determines that the most recent dose is not tolerated or that there are significant adverse events, the subsequent Dose Level will not be enrolled.

4.3 Study Design

The study will be conducted as a 1-day Monitored Dosing and Pharmacokinetics Period in an inpatient setting. Subjects will report to the hospital in a fasted state (since midnight) on the morning of the pharmacokinetics study. Subjects will continue to fast until one hour after the 2DG dose has been given. 2DG will be given as either a single oral dose (40 mg for Dose Level 1; 60 mg for dose level 2) or 60 mg bid (Dose Level 3). Blood for pharmacokinetic analysis will be drawn at time 0 (prior to drug administration), and then at 15, 30, 45, and 60 minutes and at 2, 4, 6, 12, and 24 hours after single dose 2DG administration. Blood for pharmacokinetic analysis will be drawn at time 0 (prior to drug administration) and then at 15, 30, 45, and 60

minutes and at 2, 4, 6, 12, and 24 hours after the last dose for Dose Level 3. Patients will be closely monitored for safety during and following dosing with 2DG.

4.4 Inclusion Criteria:

- Confirmed diagnosis of epilepsy. For the purpose of inclusion, the seizure types include complex partial, simple partial motor, primary generalized tonic-clonic, secondary generalized tonic-clonic, tonic, clonic and atonic seizures, as well as simple partial and absence seizures.
- Stable treatment regimen with no change in antiepileptic drugs or antiepileptic drug doses for 28 days prior to enrollment.
- Age 18 to 65 years old.
- Women of childbearing potential must be using a standard method of birth control and agree not to become pregnant during the trial. Men must agree to not father a child during the trial.
- BMI must be between 18 and 35.

4.5 Exclusion Criteria:

- Occurrence of non-epileptic psychogenic spells within 2 years prior to enrollment.
- Current or past history of diabetes or any abnormality of glucose metabolism.
- Use of glucocorticoids, hypoglycemic agents (e.g. metformin) or any drug that alters glucose levels.
- Any drug that is expected to alter glucose absorption, metabolism or serum measurements.
- Clinically significant psychiatric or medical disease.
- Previous therapeutic use of 2DG.
- Pregnant or nursing women.
- Use of an investigational medication within 2 months prior to enrollment.
- Supine systolic blood pressure < 90 or > 160 mm Hg or diastolic > 90 mm Hg, or pulse < 60 or > 110 BPM.
- Clinically significant abnormal 12-lead ECG.
- Baseline prolongation of the QTc interval >450 msec.
- Clinically significant abnormal result by speckle tracking echocardiography (STE).
- Elevated ALT or AST greater than 1.5 times the upper reference limit.
- Baseline fasting glucose < 60 or > 110.
- History of status epilepticus within 6 months prior to enrollment.
- Progressive structural brain lesion or illness likely to progress within the duration of the trial.

4.6 Informed Consent

In accordance with institutional policies approved by the U.S. Department of Health and Human Services, the subject must acknowledge consent for treatment as a human subject in this study. The study will be approved by the University of Virginia Institutional Review Board for Biomedical Sciences. Informed consent will be obtained from each subject prior to enrollment in the study.

5.0 Protocol

5.1 Patient Registration and Screening Visit

The Principal Investigator or sub-investigators will identify potential study subjects and review the study with them. Subjects who meet all eligibility requirements will be eligible to enroll in the study. After each potential subject has been informed of the study, reviewed the study information, reviewed the consent form, and had all questions answered, the subject will be asked to sign the IRB-approved informed consent.

A complete medical and neurological history will be obtained with specific reference to epilepsy and to the presence of diabetes or any abnormality of glucose or acid-base metabolism.

The ILAE seizure type and epilepsy syndrome will be determined.

The safety assessments outlined in Section 5.2 will be performed.

5.2 Safety Assessments

Safety Assessments, including Cardiac Safety Assessments, will be performed at the Patient Registration and Screening Visit, and at specified times during the Pharmacokinetics Study.

- Assessment of suicidality (Columbia-Suicide Severity Rating Scale (C-SSRS)).
- Complete neurological examination (with full sensory and gait testing).
- Blood work. CBC and CMP. Testing will be performed to check for evidence of systemic disease and for abnormalities in glucose metabolism, electrolyte balance, and hepatic enzymes.
- Urinalysis. Tests will be performed to check for glucose and ketones. A urine pregnancy test will be given to women of child bearing potential.
- Cardiac Safety Assessments. 2DG-related, dose-dependent, reversible cardiac myocyte vacuolation has been observed in toxicity studies of repeated dosing in rats at systemic drug exposures higher than expected to occur in this clinical study. At much higher 2DG exposures and with repeated dosing, QTc interval prolongation was the dose limiting toxicity (DLT) in clinical trials with human cancer patients. Because reversible cardiac myocyte damage has been associated with exposure to 2DG in animal and human studies with repeated dosing, the following cardiac safety assessments will be performed.
 - STE (speckle tracking echocardiography). Among imaging modalities, STE has emerged as a preferred method for measuring myocardial deformation (strain),

- and for detection of cardiac toxicity during chemotherapy for cancer ([Plana, 2014](#)). A clinically significant abnormal result will be an exclusion criterion.
- QTc interval (supine 12-lead ECG). QTc interval prolongations were the dose-limiting toxicity in cancer patients exposed to very high 2DG doses in clinical trials ([Stein 2010](#), [Raez 2013](#)). A baseline prolongation of the QTc interval >450 msec will be an exclusion criterion.
 - hs-cTn (high sensitivity troponins). High sensitivity troponins are the analytes of choice for detection of cardiac injury in clinical settings ([Jaffe 2011](#), [Korley 2013](#)) and during drug development ([Newby 2008](#)). Troponins measured with earlier, low sensitivity methods have usually been interpreted as biomarkers for cardiac necrosis rather than increased wall stress and ventricular distention detected by NT-proBNP and other cardiac natriuretic peptides, but there is documented evidence that hs-cTns can be detected in association with CHF and a variety of other conditions where subtle cardiac injury is present ([Conrad 2014](#)).
 - NT-ProBNP (N-terminal pro brain natriuretic peptide). Elevations in NT-proBNP occur in response to increases in end diastolic volume, ventricular distension caused by volume expansion and pressure overload, acute myocardial infarction, and congestive heart failure ([Clerico 2006](#), [Hickman 2009](#), [Hama 1995](#), [Omland 1996](#)). NT-proBNP is being evaluated as a biomarker for 2DG-related cardiac damage associated with myocyte vacuolation. In a toxicity study in rats, statistically significant NT-proBNP elevations on treatment day 15 corresponded to the initial emergence of cytoplasmic vacuoles in myocytes in the high (375 mg/kg/day) dose group in males and in association with development of vacuolation on treatment day 29 in the mid (125 mg/kg/day) dose group in males.

5.3 Pharmacokinetics Study: Inpatient

Subjects will report to the hospital in a fasted state (since midnight) on the morning of the pharmacokinetics study. Subjects will continue to fast until one hour after the single or initial oral 2DG dose has been given. Subjects receiving 2DG at Dose Level 3 (60 mg bid) will be allowed access to food beginning at 1 hour after the initial dose at 8AM and will be required to fast from noon until 1 hour after administration of the second dose at 8PM.

Each subject must have signed the IRB-approved informed consent form before any study procedures are performed.

An IV catheter will be placed for blood draws. 2DG will be given as a single oral dose at 08:00 hours (40 mg for Dose Level 1; 60 mg for Dose Level 2). 2DG will be given as 60 mg bid at 08:00 and 20:00 hours for Dose Level 3. For Dose Levels 1 and 2, blood for pharmacokinetic analysis will be drawn at time 0 (prior to drug administration), and then at 15, 30, 45, and 60 minutes and at 2, 4, 6, 12 and 24 hours after single doses. For Dose Level 3 (60 mg bid administered at 8AM and 8PM), blood for pharmacokinetic analysis will be drawn at time 0

(prior to drug administration) and at 15, 30, 45, 60 minutes and 2, 4, 6, 12 hours after the initial dose at 8AM (prior to the 8PM dose), and then at 15, 30, 45, and 60 minutes and 2, 4, 6, 12 and 24 hours after the second 2DG dose given at 8PM.

Subjects will be under close observation throughout the duration of the pharmacokinetics study. Blood glucose will be monitored at each pharmacokinetics time point.

Cardiac Safety Assessments (Section 5.2) will be performed at the 24-hour time point. Liver enzymes will be monitored at the 24-hour time point (single doses) and at 36 hours (bid doses).

Subjects will be eligible for discharge from the Clinic after the last 24-hour pharmacokinetics sample has been collected and end-of-study safety assessments have been performed.

5.4 Dose Escalation and Modification

Dose Level 1	40 mg
Dose Level 2	60 mg
Dose Level 3	60 mg bid

Dosing will start with Dose Level 1 and will escalate according to the dose escalation rules described below. Cohorts of three patients will be entered at each dose level.

For the purposes of dose escalation, the significant treatment-related adverse events described in Section 5.5 will be considered dose-limiting toxicities (DLT).

Dose escalation decisions will be made by the Study Committee (see Section 11). The decision to escalate will require a unanimous decision by the Study Committee members.

- If none (0/3) of the subjects in the cohort experience a DLT, then the dose may escalate to the next higher level.
- If one (1/3) of the subjects in the cohort experiences a DLT, then three additional subjects must be treated at the same dose level. The dose may escalate to the next higher level only if 0/3 of the additional subjects experience a DLT. If two of the six subjects experience a DLT, then the maximum tolerated dose (MTD) has been exceeded and further dose escalation will not be permitted. At the discretion of the Study Committee, an additional cohort of three subjects may be enrolled at the next lower dose level to determine the MTD
- If two (2/3) of the three subjects in the cohort experience a DLT, then the MTD has been exceeded and dose escalation will not be permitted. At the discretion of the Study Committee, an additional cohort of three subjects may be enrolled at the next lower dose level to determine the MTD.

The MTD is defined as the dose level at which 0/6 or 1/6 subjects experience a DLT, with the next higher dose having at least 2/3 or 2/6 patients who experience a DLT.

There are no plans to escalate the dose beyond 60 mg bid (total dose of 120 mg /day). If the study escalates to Dose Level 3, and if none of the first three subjects at this dose level experience a DLT, the study will stop and no MTD will be declared.

5.5 Dose-Limiting Toxicities (DLT)

Seizure Exacerbation:

Development of new seizure clusters or acute repetitive seizures (ARS) of >5 minutes duration without return to normal consciousness, or ARS clusters during which seizures are too numerous to count, will be a DLT.

Liver Enzymes:

A confirmed elevated ALT or AST greater than two times the upper reference limit will be a DLT.

Cardiac Assessments:

A clinically significant abnormal 12-lead ECG or a QTc interval prolongation > 500 msec will be considered a DLT. QTc interval prolongation associated with 2DG treatment will be evaluated according to FDA Guidance ([E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs, 2005](#)).

A reduction in ejection fraction greater than 50% from baseline or increase in global longitudinal strain (GLS) greater than 15% from baseline or any other clinically significant abnormality detected by echocardiography will be considered a DLT.

An elevated NT-ProBNP plasma level greater than 2 times the upper reference limit or a 2-fold increase from the subject's baseline level will be considered a DLT.

An elevated hs-cTn plasma level greater than the upper reference limit or a 2-fold increase from the subject's baseline level, will be considered a DLT.

As elevations of cTns after seizures are common and well-documented ([Hocker 2013](#), [Fawaz 2014](#), [Chatzikonstantinou 2015](#)), increases in NT-proBNP or hs-cTns during the 24 hours following a sporadic seizure during the outpatient screening or inpatient phases of the study will not be regarded as signals of potential cardiac toxicity, but will be followed with repeat measurements. Return of NT-proBNP or hs-cTns levels to baseline within 3 days after a seizure will be regarded as seizure-related rather than 2DG-related. In all cases with reduced ejection fraction or sustained elevations in NT-proBNP or hs-cTns indicating a DLT, repeat echocardiography or cardiac magnetic resonance imaging (cMRI) at 1-2 weeks will be obtained.

Additional clinical follow-up will be undertaken if considered appropriate by the Study Committee and cardiology consultants.

Subjects will have a safety visit 2 weeks after dosing (Day 14) in the hospital. They will be queried about any adverse events that occur in the interval.

6.0 Required Observations

6.1 Pre-Treatment Observations at Screening Visit

See Sections 5.1 and 5.2.

6.2 Observations during Treatment

See Sections 5.3 and 5.4

6.3 Post-Study Observations

See Section 5.5.

6.4 Table of Time and Events:

	Outpatient	Inpatient	Inpatient	Inpatient	Outpatient
Study Day	-14 to -1	1	2	2-3	14
			Dose levels 1,2 (single dose)	Dose level 3 (bid dose)	
Informed Consent	x				
Screening Procedures	x				
Neurologic Exam	x				
Vital Signs	x	x	x	x	
Suicidality (C-SSRS)	x				
Clinic Inpatient Check-in	-1	x			
2DG administration		8AM (dose levels 1,2) 8AM, 8PM (dose level 3)			
ECG	x		At each PK time point	At each PK time point	
STE	x		24-hr time point	24-hr time point	
NT-ProBNP	-1		24-hr time point	24 & 36-hr time points	
hs-cTn	-1		24-hr time point	24 & 36-hr time points	
Blood Glucose	-1	each PK time point	each PK time point	each PK time point	
Blood Work*	x		24 hr time point	36 hr time point	
Urinalysis	x		24 hr time point	36 hr time point	
Urine Pregnancy Test	x				
Pharmacokinetics Study (duration)		baseline begins just prior to 8AM dose	To 24 hr after 1 st dose and from baseline	To 24 hr after 2 nd dose (36 hr from baseline)	
Follow up phone call					x

* CBC & CMP to check for evidence of systemic disease and changes in glucose metabolism, electrolyte balance, and hepatic enzymes.

7.0 Drug Information

7.1 Active Pharmaceutical Ingredient (API)

The 2-Deoxy-D-Glucose conforms to specifications that include the following criteria:

<u>Tests</u>	<u>Specifications</u>
Appearance	white to off white powder
Assay	$\geq 98.0\%$ (w/w, anhydrous & solvent free basis)
Total Impurities	$\leq 2\%$
1-(2-furanyl)-(1R)-1,2- ethanediol	$\leq 0.5\%$
D-Glucal	$\leq 0.5\%$
Tri-O-Acetyl-D-Glucal	$\leq 0.5\%$
Specified Impurity (RRT ~0.79)	$\leq 1.0\%$ AUC
Individual Unspecified Impurities	$\leq 0.5\%$ AUC

7.2 Finished Drug Product

The 2-Deoxy-D-glucose finished dosage form conforms to the following specifications:

Test	Specification
Appearance of Dosage Form	20 mg Capsule: White opaque HPMC size 5 capsules with desiccant; packaged in a child-resistant sealed 60 cc HDPE white bottle. 100 mg Capsule: White opaque HPMC size 4 capsules with desiccant; packaged in a child-resistant sealed 60 cc HDPE white bottle. 400 mg Capsule: White opaque HPMC size 0 capsules with desiccant; packaged in a child-resistant sealed 100 cc HDPE white bottle.
Appearance of Solid Content	White to off-white powder
Identification	The retention time of 2DG in the RID chromatogram of the sample is within 3% if the 2DG reference standard.
Assay	100 +/- 5%
Related Substances / Impurities	Individual(s) - Report value and RRT of each identified impurity $\geq 0.1\%$. Report unidentified impurities $\geq 0.1\%$ Total - Report value impurity $\geq 0.1\%$
Uniformity of Dosage Units	Meets USP Requirements. Report Acceptance Value and Relative Standard Deviation.
Moisture Analysis	Report Value
Dissolution in simulated gastric fluid, pH 1.2 without enzyme (15, 30, 45 & 60 min).	Average not less than 75% at 45 min. Report dissolution values for 15, 30 and 60 min.
Overall Closed Capsule Length	20 mg Capsule: 10.8 - 11.4 mm 100 mg Capsule: 14.0 - 14.6 mm 400 mg Capsule: 21.3 - 22.1 mm

8.0 Removal from Protocol Therapy and Off Study Criteria

8.1 Criteria for removal from protocol therapy

- a) Refusal of further protocol therapy by the subject.
- b) Physician determines it is in the best interest of the patient.
- c) Completion of protocol therapy, including follow up.
- d) Occurrence of a protocol-specified, significant treatment-related adverse event or dose-limiting toxicity (DLT). See Section 5.5.

8.2 Off study criteria

- a) Death
- b) Lost to follow-up (subjects who do not complete the study assessments).
- c) Withdrawal of consent for any further data submission.

9.0 Safety Monitoring and Reporting

Adverse event reporting will follow standard procedures as specified by the University of Virginia Institutional Review Board for Health Sciences Research (IRB-HSR). All adverse events will be recorded. Adverse events will be classified as serious or non-serious. They will be further classified as either drug related, possibly related, or unrelated. All serious adverse events and all unexpected severe adverse events will be reported to the IRB-HSR.

Adverse events will be continuously monitored by the principle investigator.

The tables below describe the reporting procedures and requirements for adverse events.

Internal IRB Reporting:			
Type of Event	To whom will it be reported:	<u>Time Frame for Reporting</u>	<u>How reported?</u>
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation (Note: An internal event is one that occurs in a subject enrolled in a UVA protocol.)	IRB-HSR	Within 24 hours	IRB Online and phone call www.irb.virginia.edu/
Internal, Serious, Unexpected adverse event	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event. Timeline includes submission of signed hardcopy of AE form.	IRB Online www.irb.virginia.edu/
Unanticipated Problems that are not adverse events or protocol violations	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. http://www.virginia.edu/vprgs/irb/HSRdocs/Forms/ReportingRequirements-UnanticipatedProblems.doc
Protocol Violations (Note the IRB-HSR only requires that MAJOR violation be reported) Or Enrollment Exceptions	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form http://www.virginia.edu/vprgs/irb/hsr_forms.html

FDA Reporting:			
Life-threatening and/or fatal unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IND annual report

10.0 Statistical Considerations

10.1 Statistical analysis plan

The primary objective of this study is safety. Toxicities will be summarized in terms of types and severities by the most recent version of MedRA terminology. Changes in glucose, vital signs and other measured parameters will be closely monitored. Adverse events will be graded as mild, moderate, or severe and by whether the relationship to study drug is related, possibly related, or unrelated. Toxicities will be graded as serious following standard guidelines. Adverse events will be tabulated by their original description, organ system, and by standardized MedRA terminology, as well as by severity and whether they were likely to be drug-related.

As the study involves a small number of subjects and is not powered to enable statistical analysis, it is anticipated that the reporting of results will be anecdotal and descriptive.

10.2 Sample size

It is anticipated that approximately 9 subjects (3 per dose level) will be sufficient to demonstrate that a single oral administration of the 40 mg or 60 mg doses, or 60 mg bid doses, are safe and well tolerated, and to complete the pharmacokinetics study.

11.0 Study Committee

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12.0 References

(hyperlinks to reference pdfs are available from the text)

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