A MAD Study of TT301/MW189 in Healthy Volunteers

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Statistical Analysis Plan

TT301/MW189

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A Phase 1b, Double-Blind, Randomized, Placebo-Controlled Multiple Ascending Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetic Profile of TT301/MW189 Administered Intravenously to Healthy Volunteers

Version 2.1

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Sponsor: University of Kentucky

SIGNATURE PAGE AND APPROVALS

Linda J. Van Eldik, PhD Principal Investigator Director, Sanders-Brown Center on Aging University of Kentucky	
Jeffrey Guptill, MD Duke Site Principal Investigator Duke Early Phase Research Unit	
Tracy Spears, MS Biostatistician III Duke Clinical Research Institute	
Christoph Hornik, MD Associate Director Pharmacometrics Duke Clinical Research Institute	

History of Changes to this Document

Date	Version	Changes
1 November 2018	1.0	Original document
11 March 2019	2.0	Addition of Neurological Exam section (10.6) Statistical analyses will be performed with a Linux server-based SAS installation, rather than with PC SAS Removed plan for list of protocol deviations Addition of DCRI names to signature page
22 April 2019	2.1	Study Populations: • Added explanation of population assignment of replaced and replacement subjects • PK analyses and populations not described in this SAP Vital Signs • Temperature added to tables, lists ECG • Determination of baseline value from triplicate results • Figures removed Physical Exam

		• Table added for weight • List will include abnormal findings only Neurological Exam • List will include abnormal findings only

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1.0 LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
DEPRU	Duke Early Phase Research Unit
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
IV	Intravenous
MAD	Multiple Ascending Dose
PK	Pharmacokinetic
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TEAE	Treatment Emergent Adverse Event

2.0 INTRODUCTION

TT301/MW189 (hereafter called MW189) is a small molecule drug candidate developed as a selective suppressor of disease and injury-induced glia proinflammatory cytokine overproduction associated with destructive glia inflammation/neuron dysfunction cycles, and their long-term neurotoxic effects. MW189 treatment attenuates the stressor-induced rise in brain proinflammatory cytokine levels with a downstream improvement in behavior dysfunction and synaptic dysfunction markers in multiple animal models of CNS disorders where up-regulation of proinflammatory molecules is implicated in disease progression. MW189 is being developed for the treatment of acute brain injuries such as traumatic brain injury or intracerebral hemorrhage. The development program is based on nonclinical evidence that MW189 reduces the recruitment of activated microglia, reduces cerebral edema, and improves motor skills and neurocognitive outcomes in animal models of neuroinflammatory disorders.

The present study will provide safety and pharmacokinetic (PK) information on multiple ascending doses (MAD) of MW189 to support decisions for continued clinical development. This study will assess the safety, tolerability, and PK profile of escalating repeated doses of MW189 in healthy adult participants. The trial design will be a double-blind, placebo-controlled, MAD study of MW189 in healthy volunteers. Three dosing cohorts are initially planned. All subjects and the study personnel responsible for assessing adverse events (AEs) will be blinded.
This statistical analysis plan (SAP) provides a more technical and detailed elaboration of the statistical analyses of safety and tolerability data as outlined and/or specified in the most recent amendment of the protocol.

In the study design, 32 healthy male and female volunteers, ages 18-50 years, will be dosed in 1 of 4 dosing cohorts of 8 subjects each (6 MW189, 2 matched placebo). Subjects will be admitted to the Phase 1 unit on the day prior to dosing (Day -1) and will remain in the clinical research unit until discharge on Day 8. Follow-up will be done through a clinic visit at 2 weeks and a phone call 4 weeks from end of dosing.

Subjects will receive 2 doses per day for 5 consecutive days. Doses will be given 12 hours apart.
Cohort 1: 0.075 mg/kg MW189 *iv bid* over 20 minutes (or matched placebo)
Cohort 2: 0.15 mg/kg MW189 *iv bid* over 20 minutes (or matched placebo)
Cohort 3: 0.30 mg/kg MW189 *iv bid* over 20 minutes (or matched placebo)
Cohort 4: 0.25 mg/kg MW189 *iv bid* over 20 minutes (or matched placebo) (added after protocol amendment)

Dose levels selected for the MAD study at 0.075 mg/kg, 0.15 mg/kg, 0.25 mg/kg, and 0.30 mg/kg twice daily for 5 days are based on the safety, PK separation and steady-state PK simulations derived from the phase 1a SAD study of MW189.

For this phase 1b MAD study, dosing will start at the lowest dose in the 1st cohort, then escalate in the 2nd cohort, then escalate in the 3rd cohort. Each subject will receive 1 *iv* dose level of MW189 twice daily (*bid*) on Days 1 through 5, inclusive. After all subjects in Cohort 1 have completed the study through the clinic visit at 2 weeks, data will be collected for the safety review. Dosing of Cohort 2 will proceed upon joint recommendation by the Safety and Medical Monitors, after reviewing Cohort 1's safety data including AEs, SAEs, vital signs, safety laboratory tests and ECGs. Dosing of Cohort 3 will proceed upon joint recommendation by the Safety and Medical Monitors, after reviewing Cohort 2's safety data including AEs, SAEs, vital

signs, safety laboratory tests and ECGs. Should the safety data warrant, the 2nd or 3rd dose levels may be reduced or additional cohorts added in order to generate a comprehensive understanding of the clinical dose range, if deemed necessary by the sponsor and recommended by the Data Safety Monitoring Board (DSMB). The maximum dose in this study will not exceed 0.30 mg/kg *bid*. Any changes to dosing will be made during the safety review.

Screening will be conducted within 28 days of first dose. After giving written informed consent, each subject will undergo a medical screen consisting of the following procedures: demographics; medical history; concomitant medication review; body temperature; 12-lead ECG; vital signs; physical exam; alcohol and drug screen; Hepatitis B, Hepatitis C, HIV, and TB screens; and safety laboratory work and pregnancy testing.

One day prior to dosing (Day -1), subjects will be admitted to the Duke Early Phase Research Unit (DEPRU) Phase 1 unit for final qualification assessments. These assessments will include inclusion/exclusion review, medical history, physical exam, infection screen, body temperature, alcohol and drug screen, vital signs, pregnancy test, neurological exam, and safety laboratory tests.

Dosing will be twice daily, starting on Day 1 and ending on Day 5 for a total of 5 days with 10 doses. The second dose of the day will be approximately 12 hours after the morning dose. The morning dose each day will be anchored to the initial dose on Day 1 so that the Day 2 morning dose will be 24 hours after the Day 1 morning dose and the Day 3 morning dose will be 48 hours after the Day 1 morning dose, etc. During dosing, subjects will be continuously monitored with 12-lead ECG, blood pressure, and heart rate. Plasma samples will be drawn at sequential time points for PK analysis.

Subjects will remain in the clinical research unit until discharge on Day 8. Follow-up will be done through a clinic visit at 2 weeks and a phone call 4 weeks from end of dosing. The follow-up visit will consist of safety lab work, body temperature, ECG, vital signs, concomitant medication review, and assessment of adverse events (AEs). The follow-up phone call will include a concomitant medication review, and assessment of any safety issues.

Table 1. Schedule of Activities

PROCEDURES	SCRN(1)	D-1	D1	D2	D3	D4	D5[2]	D6	D7	D8 (ET)(2)	W2	W4
Informed Consent	X											
Inclusion/Exclusion Review	X	X										
Demographics	X											
Medical History	X	X										
Physical Examination(3)	X	X								X		
Infection Screen(4)		X										
Body Temperature(5)	X	X	X	X	X	X	X	X	X	X	X	
Neurological Examination(6)		X			X			X		X		
Safety Laboratory Tests(7)	X	X			X			X		X	X	
Drug Screen (urine)	X	X										
Alcohol Screen (breathalyzer)	X	X										
Hepatitis B & C /HIV Screen	X											
TB Test (blood test)	X											
Pregnancy Test	X	X										
12-lead ECG(8)	X		X	X	X		X	X		X	X	
Vital Signs (BP/HR)(9)	X	X	X	X	X	X	X	X	X	X	X	
PK Blood Sampling(10)			X	X	X	X	X	X	X			
Admit to Unit		X										
Randomization(11)		X										
Dosing(12)			X	X	X	X	X					
Adverse Event Assessment			X	X	X	X	X	X	X	X	X	X
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X
Discharge From Unit										X		

Footnotes to Schedule of Activities

(1) Screening will occur within 28 days of 1st dose

(2) Subjects who discontinue from the study should undergo all Early Termination assessments as per the Day 8 visit.

(3) Weight will be measured during all physical examinations and height will be measured at Screening only.

(4) On Day -1, subjects will be screened for the presence of infection as part of physical examination, body temperature, recent/current antibiotic use, recent surgery, evidence of dental abscess, unhealed skin lesions etc. On Day 1 before dosing, subjects will be screened for emergence of acute illness.

(5) Body temperature will be measured at the following time-points:

- Screening
- Day -1
- Day 1 – Day 5 before the morning dose
- Day 6 – Day 7 approximately 24h and 48h, respectively, after the Day 5 morning dose
- Day 8 approximately 72h after the Day 5 morning dose
- Day 14

(6) Neurological Examination will include mental status evaluation, cranial nerves 2-12 testing, motor system and coordination testing, reflex evaluation and sensory system evaluation.

(7) All Safety laboratory samples (Hematology/Coagulation/Biochemistry/Urinalysis) will be taken after the subject has fasted for a minimum of 10 hours and before the morning dose (on dosing days) as follows:

- Screening (all except creatine kinase, amylase, lipase, cholesterol, HDL/LDL/ triglycerides)
- Day -1
- Day 3
- Day 6
- Day 8
- Day 14

(8) A 12-lead ECG will be performed (after lying quietly for 5 minutes). Triplicate tracings (3x) will be taken at Day 1 pre-dose only:

- Screening
- Day 1: Pre-dose (3x) and at approximately 1h, 2h, 4h after the start of the morning dose and pre-dose and 1h, 2h, 4h after the start of the second dose
- Day 2: Pre-dose and at approximately 1h, 2h, 4h after the start of the morning dose and pre-dose and 1h, 2h, 4h after the start of the second dose
- Days 3, 5 and 6 before the morning dose
- Day 8 approximately 72h after the Day 5 morning dose
- Day 14

(9) Vital signs will include BP and HR. Vitals will be performed at the following time-points:

- Screening
- Day -1
- Day 1: Pre-dose and at 1h, 2h, 4h after the start of the morning dose and pre-dose and 1h, 2h, 4h after the start of the second dose

- Day 2: Pre-dose and at 1h, 2h, 4h after the start of the morning dose and pre-dose and 1h, 2h, 4h after the start of the second dose
- Day 3 – Day 5 before the morning dose and before the second dose
- Day 6 – Day 8 approximately 24h, 48h and 72h, respectively, after the Day 5 morning dose
- Day 14

(10) PK samples will be taken at the following time-points, anchored to the start of each injection.

- Day 1 Morning Dose: Pre-dose, 20min (at the end of injection; sample should be collected prior to completion of the injection), 1h, 2h, 4h, 7.5h and 12 hours (immediately before the Day 1 Second Dose) post-dose
- Day 1 Second Dose: 20min (at the end of injection; sample should be collected prior to completion of the injection) 1h, 2h, 4h, 7.5h post-dose
- Day 2 Morning Dose: Pre-dose (approximately 24h after Day 1 morning dose)
- Day 3 Morning Dose: Pre-dose (approximately 48h after Day 1 morning dose)
- Day 4 Morning Dose: Pre-dose (approximately 72h after Day 1 morning dose)
- Day 5 Morning Dose: Pre-dose (approximately 96h after Day 1 morning dose), 20min (at the end of injection; sample should be collected prior to completion of the injection), 1h, 2h, 4h, 7.5h and 12 hours (immediately before the Day 5 Second Dose) post-dose
- Day 5 Second Dose: 20min (at the end of injection; sample should be collected prior to completion of the injection), 1h, 2h, 4h, 7.5h post-dose
- Day 6: approximately 16h after Day 5 second dose, approximately 24h after Day 5 second dose
- Day 7: approximately 48h after Day 5 second dose

(11) Randomization is expected to occur on D-1, but if there are delays in receiving prequalification safety labs randomization may occur on D1 prior to dosing.

(12) Dosing will be twice daily, starting on Day 1 and ending on Day 5 for a total of 5 days with 10 doses. The second dose of the day will be approximately 12 hours after the morning dose. The morning dose each day will be anchored to the initial dose on Day 1 so that the Day 2 morning dose will be 24 hours after the Day 1 morning dose and the Day 3 morning dose will be 48 hours after the Day 1 morning dose, etc.

3.0 OBJECTIVES

Primary Objectives

To assess the safety and tolerability of multiple ascending doses of MW189 when administered *iv* to healthy volunteers.

The hypothesis is that MW189 will be safe in humans when administered in a MAD paradigm, similar to its already documented safety in a SAD study, with no severe or serious adverse events.

Secondary Objectives

To assess the PK profile of multiple ascending doses of MW189 when administered *iv* to healthy volunteers.

4.0 SUBJECT POPULATIONS

Any subject who discontinues the study prior to dose administration or prior to receiving all doses for reasons other than AEs may be replaced by a different subject. The replacement subject will receive the same treatment assignment as the original subject.

4.1 RANDOMIZED POPULATION

The Randomized Population will consist of all subjects who sign informed consent and who are randomized to a treatment group in the study. Both the original and replacement subject will be included.

4.2 SAFETY POPULATION

The Safety Population will consist of all subjects in the Randomized Population who receive any double-blind study medication (MW189 or placebo). Both the original and replacement subject will be included.

4.3 PK POPULATION

PK Population and analyses will be defined in the PK Analysis Plan.

The Randomized and Safety Populations will be summarized by study dose cohort.

5.0 STUDY DISPOSITION

The number of subjects included in each study population (i.e., Randomized, Safety) will be summarized by cohort, and active drug vs. placebo.

The number and percentage of subjects who completed and prematurely discontinued the study during the double-blind periods will be presented for each treatment group and overall for the Randomized Population. Reasons for premature discontinuation from the double-blind periods as recorded on the completion page of the CRF will be summarized, as applicable.

6.0 DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

Demographic parameters (e.g. age, race, sex, weight, height, BMI) and other baseline characteristics, such as medical history, will be summarized by treatment group for the Safety Population. In addition, data listings will be generated.

7.0 STATISTICAL ISSUES

7.1 GENERAL ANALYSIS CONVENTIONS

Detailed statistical analysis plans will be created for the study and signed off in advance of database lock. One plan will be created for the analysis of PK data and a separate plan will be created for the analysis and presentation of all other study data. General statistical approaches are presented below.

All subjects who receive any study treatment will be included in the safety analysis grouped by treatment received. Statistical analysis of safety data will be descriptive.

Baseline is defined as the last assessment prior to first dose of study medication.

For continuous variables, summaries will include sample size, mean, standard deviation, minimum, and maximum. For categorical variables, the summaries will include frequencies and corresponding percentages. No inferential hypothesis testing will be performed on the safety variables. Repeat or unscheduled results will not be included in the summaries, but will be listed. Data from placebo subjects will be pooled for final presentations.

Statistical analyses will be performed on Linux sever installation of-SAS, Version 9.4.

8.0 EXTENT OF EXPOSURE

8.1 STUDY MEDICATION

Exposure to study medication for the Safety Population will be summarized in terms of the amount of dose administered (in mg/kg) for each dose cohort and treatment group. Concomitant medications will be summarized. Any instance of a subject receiving a dose other than the assigned dose will be listed. Descriptive statistics will be presented by treatment group.

9.0 PHARMACOKINETIC ANALYSIS

The plasma concentrations of MW189 will be determined, and used to estimate appropriate pharmacokinetic (PK) parameters. The PK analysis is outside the scope of this SAP and is described in a separate PK statistical analysis plan.

10.0 SAFETY ANALYSES

The safety analysis will be performed using the Safety Population. Safety and tolerability will be assessed on the basis of physical examination, neurological examination, vital signs, clinical laboratory values, ECG readings, and adverse event (AE) reports. The frequencies of AEs and SAE (serious adverse events) will be tabulated by dose as will all other safety measurements. For each safety parameter, baseline is defined as the last evaluation before dosing.

All subjects who receive any study treatment will be included in the safety analysis grouped by treatment received. Statistical analysis of safety data will be descriptive.

10.1 ADVERSE EVENT ASSESSMENTS

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) v21.0 – 5 Level. AEs will be listed and summarized by treatment group and dose level. Where applicable, AEs may also be summarized by severity, seriousness, relationship to study treatment and by system organ class and preferred term. The number and percentage of subjects who experience events will be shown in the summaries. Listings for all AEs will be presented.

A treatment-emergent AE (TEAE) is an AE, regardless of relationship to study drug, which occurs during or after the first dose of study treatment. TEAEs will be summarized by MedDRA System Organ Class and Preferred Term.

All AEs will be listed.

10.2 CLINICAL LABORATORY TESTING

Summary statistics of observed and change from baseline data will be presented for each treatment group and dose level where applicable. Summary statistics will include number of subjects, mean, median, standard deviation, minimum, and maximum. Shift tables summarizing the numbers of subjects shifting from normal pre-dose values to abnormal post-dose values will also be presented where applicable. Listings for all laboratory assessments will be presented.

10.3 VITAL SIGNS

Vital signs (systolic and diastolic blood pressure, heart rate/pulse, body temperature) and changes from baseline at each time point will be presented by cohort and treatment group. Summary statistics for systolic blood pressure, diastolic blood pressure, temperature, and change from baseline (pre-dose) summaries will be presented for each treatment group and dose level where applicable. Summary statistics will include number of subjects, mean, median, standard deviation, minimum, and maximum. Listings for all vital signs will be presented.

Post-dose clinically notable vital signs will be listed and summarized by treatment and dose. Criteria for Clinically Notable Vital Sign abnormalities are indicated in Table 2 below.

Table 2: Criteria for Clinically Notable Vital Sign Abnormalities

Parameter	High Threshold	Low Threshold
Systolic BP (supine)	> 30 mmHg increase from baseline	> 30 mmHg decrease from baseline
Diastolic BP (supine)	> 20 mmHg increase from baseline	> 30 mmHg decrease from baseline
Heart Rate	> 120 bpm and an increase in HR of ≥ 15 bpm from baseline	< 40 bpm and a decrease in HR of ≥ 15 bpm from baseline
Body Temperature	> 39.0°C (> 102.2°F)	< 35.0°C (< 95.0°F)

10.4 ELECTROCARDIOGRAM MONITORING

Descriptive statistics for ECG parameters (e.g. Ventricular Heart Rate, PR interval, QRS interval, QTc interval, QTcB, and QTcF interval, RR interval) at baseline and changes from baseline at each assessment time point will be presented by cohort and treatment group. Baseline will be the median of each subject's available Day 1 pre-dose results for each parameter.

Shift tables summarizing the numbers of subjects shifting from normal pre-dose values to abnormal post-dose values will also be presented. Numeric ECG data will be summarized by treatment group and dose level and mean summary tables, including change from baseline will be presented. The ECG results will be listed by treatment group and dose level. Post-dose clinically notable ECGs will be listed and summarized by treatment and dose. Notable abnormalities are indicated in Table 3 below:

Table 3: Criteria for Clinically Notable ECG Abnormalities

Parameter	High Threshold	Low Threshold
PR interval	PR ≥ 220 ms	PR ≤ 120 ms
QRS duration	QRS duration ≥ 120 ms and an increase of ≥ 20 ms from baseline	-
QT interval	QT interval > 500 ms	-
QTcF interval	Men: QTcF interval ≥ 450 ms and an increase of ≥60 ms from baseline OR QTcF interval >500 ms Women: QTcF interval ≥ 470 ms and an increase of ≥60 ms from baseline OR QTcF interval >500 ms	-

10.5 PHYSICAL EXAMINATIONS

Weight will be measured during all physical examinations (Screening, Day -1, Day 8) and height will be measured at Screening only. Descriptive statistics for weight at baseline and changes from baseline at each assessment time point will be presented by cohort and treatment group. A listing of abnormal physical examination data for all subjects will be provided.

10.6 NEUROLOGICAL EXAMINATIONS

Neurological examinations include mental status, cranial nerves 2-12, motor system and coordination, reflexes, and sensory system. Shift tables summarizing the numbers of subjects shifting from normal pre-dose values to abnormal post-dose values will be created. A listing of abnormal neurological examination data for all subjects will be provided.

11.0 DETERMINATION OF SAMPLE SIZE

This trial is designed to investigate the safety, tolerability, and PK properties of the drug MW189 in a dose escalation Phase Ib study. The number of subjects is based upon the need to gain this knowledge in healthy individuals using as few subjects as possible. Since this is the first MAD study of MW189 in humans, there is no prior experience upon which to base estimates of variability for the PK responses to MAD treatment with MW189. No power analysis is conducted but the cohort size is based on accepted standards in similar Phase I studies: 8 subjects per dose with 6 randomly assigned to active drug while the remaining 2 subjects receive placebo. Eight subjects per cohort was deemed sufficient for obtaining an adequate preliminary description of the responses of interest.