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TITLE: A Randomized Clinical Trial of Allopregnanolone for the Treatment of Severe Traumatic Brain Injury

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**14. ABSTRACT**

There is strong experimental support for the concept that allopregnanolone will be safe and have beneficial effects on disability when administered as a treatment following acute traumatic brain injury (TBI). This study will provide initial data on the safety and effectiveness of allopregnanolone in improving neurobehavioral outcome and reducing mortality in adults with moderate and severe TBI. A Phase 2, adaptive, two-stage, placebo controlled, double blind, randomized clinical trial will be undertaken at the UC Davis Medical Center, a Level 1 trauma center, and at additional medical centers with appropriate expertise. Allopregnanolone has been manufactured GMP for this trial. Intravenous product solutions have been developed. Subjects will be allocated to 3 dosing levels (1) placebo, (2) low (50 nM), and (3) high (150 nM) by an innovative adaptive trial design consisting of two stages, in which safety and pharmacokinetics will be assessed in the first stage and adaptive allocation to the low and high doses will occur in the second stage. Key research accomplishments during the reporting period include: (1) finalized allopregnanolone product formulation, (2) Data Safety Monitoring Board constituted, (3) revised protocol to implement adaptive trial design, (4) received approval of IND (111,085) from FDA, and (5) received approval from IRB and HRPO.

**15. SUBJECT TERMS**
traumatic brain injury, clinical trial, allopregnanolone

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## Table of Contents

## Section I – Introduction

This study will provide initial data on the safety and effectiveness of allopregnanolone in improving neurobehavioral outcome and reducing mortality in adults with moderate and severe traumatic brain injury (TBI). There is strong experimental support for the concept that allopregnanolone will have beneficial effects in TBI. Allopregnanolone, a neurosteroid that acts as a powerful modulator of $GABA_A$ receptors, has anticonvulsant and anesthetic activity but is free of the hormonal actions of progesterone (Rogawski and Reddy, 2004), another agent currently being studied in the treatment of TBI. Recent studies have demonstrated that allopregnanolone is efficacious in enhancing neurobehavioral recovery and decreasing TBI-induced neuronal death (Djebaili et al., 2004; 2005; He et al., 2004ab; Ciriza et al., 2006; Sayeed et al., 2006). These studies support the clinical trial to be conducted in this project.

The overall aim of this project is to proved information that will advance the development of allopregnanolone as a treatment for field use to mitigate the effects of TBI in warfighters. In order to demonstrate the safety and efficacy of allopregnanolone for this application, a clinical trial will be conducted in the civilian setting. The main site for the trial is the UC Davis Medical Center, a Level 1 trauma center. Other clinical sites with the capabilities required for conduct of the trial will also be used. It is anticipated that 136 subjects will be enrolled.

The study to be conducted is designated as a phase 2, adaptive, two-stage, placebo controlled, double blind, randomized clinical trial. The primary objective is to determine in adults with moderate or severe TBI (GCS 3–12): (1) the safety of intravenous allopregnanolone compared to placebo during a 5-day continuous infusion starting within 8 hours after the injury; and (2) the efficacy of intravenous allopregnanolone treatment to improve GOS-E at 3 months after injury. Secondary objectives are to determine the clinical benefit of allopregnanolone treatment as assessed through secondary endpoints including mortality, GOS-E at 1 and 6 months, quality of life, global neurobehavioral function, depression, and late post-traumatic epilepsy. Allopregnanolone, the active pharmaceutical ingredient (API), has been manufactured for this trial according to Good Manufacturing Practices (GMP) standards mandated by the U.S. Food and Drug Administration (FDA). Intravenous product solutions have been developed by the UC Davis Good Manufacturing Practice Laboratory. There are 3 dosing levels: (1) placebo, (2) low (50 nM steady-state target level), and (3) high (150 nM steady-state target level). Within 8 hours after the injury, either placebo, low dose allopregnanolone, or high dose allopregnanolone will be administered intravenously as a loading dose over 1 hour followed by a maintenance infusion. After 4 days, the maintenance dose will be tapered by reducing the infusion rate to 75%, 50%, and 25% every 8 hours. Allocation of subjects among the doses will be determined by an innovative adaptive trial design. The study will proceed in two stages. The goal of Stage 1 is to assess product safety and confirm that the dosing regimen achieves the desired steady-state target serum concentrations of 50 nM (low) and 150 nM (high). The sample size of Stage 1 is flexible and enrollment in this stage will be considered complete when there is sufficient evidence that the low and high doses are safe and that the doses achieve the target steady-state concentrations. Data obtained from each Stage 1 study subject will be submitted to the Data Safety Monitoring Board (DSMB). The DSMB will decide when to proceed to Stage 2.

Stage 2 utilizes adaptive allocation to the low and high dose to determine the relative efficacy of each dose and if allopregnanolone is superior to a placebo.

The primary outcome measure of the trial is the Glasgow Outcome Scale–Extended (GOS-E). The study is designed to detect a 1 point improvement. A 1 point improvement is clinically significant as such an improvement would place the GOS-E at the level reported for patients with mild TBI (GCS 13–15) (Hudak et al., 2005). Several authors have found that greater disability and handicap as measured by the GOS and GOS-E are associated with subjective self-reports of poorer outcome. Specifically, individuals with poorer outcome as measured by the GOS or GOS-E had a higher frequency of depressive symptomatology (McCleary et al., 1998; Wilson et al., 2000). Poor outcome is also associated with reduced mental well-being and problems in neurobehavioral functioning (Wilson et al., 2000). Overall, the improved outcome contemplated by allopregnanolone treatment is expected not only to be associated with improved neurological function but also with an improved subjective sense of satisfaction with life (Wilson et al., 2000). Since it is difficult to conduct clinical research in a war zone, we have chosen to conduct this research in a civilian setting. Nevertheless, we believe that the results obtained will be applicable to the use of allopregnanolone in a military situation. Such application has the potential to have a dramatically positive impact on the function, wellness, and overall quality of life for military Service members affected by TBI. Caregivers and families will also be positively impacted since affected Service members with less disability will require less demanding care. The Brain Injury Association of America estimates that the long-term cost of care for a person with severe TBI is $4.1 to 9 million. Such an individual may require 5 to 10 years of rehabilitation and follow-up services. Therefore, in addition to providing improved function, well-being and overall quality of life, the improvement contemplated by allopregnanolone treatment should result in substantial societal cost savings.

**Section II – Body**

***Summary of progress.*** Allopregnanolone has not previously been administered to humans for the treatment of any disease and it is not approved by the FDA for human use. FDA requires pharmaceuticals, including pharmaceuticals used in investigational trials, to be manufactured according to GMP. GMP allopregnanolone is not commercially available and a GMP formulation has not been previously manufactured. Therefore, in the initial years of this project we developed a chemical synthetic method to manufacture allopregnanolone according to the strict purity standards required by the FDA. Using this synthetic method, we manufactured sufficient quantities of allopregnanolone for this trial. This valuable material is currently being stored under controlled environmental conditions in the UC Davis GMP Laboratory. The GMP allopregnanolone underwent extensive testing to insure its purity and stability. We next developed intravenous product formulations for use in the clinical trial. This required the development of a method to solubilize allopregnanolone in solution in such a way that it is safe for human intravenous administration. In the course of these studies, we successfully identified excipients and a formulation that met the product requirements. A key requirement of the product is that it is sufficiently stable to be deployed in military field use. We conducted extensive testing to select a container compatible with the product formulations for long-term storage. We next conducted assays of the products by high performance liquid chromatography (HPLC) and we determined the pH, osmolality, and particulates according to USP requirements,

as mandated by the FDA. Certificates of analysis were generated. Stability testing was also conducted as required by the FDA. At the same time as the product forms for the clinical trial were being developed, we created a protocol for the clinical trial and we constituted a team of clinicians and scientists at UC Davis with the diverse skills required to conduct the clinical trial. We also developed a charter for the Data Safety Monitoring Board (DSMB) and recruited the members of this committee. A site operations manual was also developed. We then prepared a detailed report of the manufacturing process and the product assays. We collected extensive background material to justify the testing of allopregnanolone in a clinical trial for TBI. All of this material was combined with our clinical protocol in a Pre-IND document submitted to the FDA. Following review by the FDA, we participated in a face-to-face Pre-IND meeting with the staff of the FDA Division of Neurology, Center for Drug Research and Evaluation. We received specific guidance from the FDA regarding the requirements for our clinical trial. Among the many requirements imposed by the FDA was the requirement that we carefully monitor blood plasma levels of allopregnanolone during the conduct of the trial to insure that dosing does not exceed limits mandated by the FDA. In addition, the FDA recommended that we examine more than one dose. In order to meet these requirements, we developed a bioanalytical method for the measurement of allopregnanolone in human blood plasma that utilizes an ultrahigh pressure liquid chromatograph (UPLC) system and tandem quadrapole mass spectrometer (MS/MS). To meet the FDA requirement that we examine more than one dose, we developed an innovative adaptive clinical trial design with assistance from Berry Consultants, who will provide ongoing assistance during the course of the trial. We also developed a statistical analysis scheme in consultation with statisticians at UC Davis. A case report system was designed and, in consultation with the UC Davis Clinical and Translational Research Center (CTSC), we designed a database for the secure collection of data from the clinical trial using REDCap (Research Electronic Data Capture). In order to meet the sophisticated requirements of the adaptive trial design, we contracted with Bracket for the development of an interactive web response system to monitor drug supply and carry out randomization. In the course of communication with the FDA through the submission of an IND document and several requests for information, our IND was approved on May 7, 2012 giving us authorization to administer our drug product formulations in the setting of an investigational trial. In addition, we submitted our trial for approval to the UC Davis Institutional Review Board (IRB), ultimately receiving authorization to begin the trial on May 11, 2012. With the approval from the FDA and IRB in hand, we were able to receive approval from the Human Research Protection Office (HRPO) on July 18, 2012. We published the clinical trial on ClinicalTrials.gov. We also enlisted the UC Davis Investigation Drug Services Pharmacy to develop methods for storage and dispensing of the drug product forms. Details of some of these various activities are provided in the sections below.

***Data Safety Monitoring Board (DSMB).*** A chairperson and members of the DSMB were recruited and a DSMB Charter prepared. The DSMB Charter specifies the DSMB objectives and responsibilities; lists the DMSB members; describes the responsibilities of the UC Davis Project Team, the responsibilities of the CTSC, and the responsibilities of the DSMB chairperson. It also describes DSMB meetings and voting including safety data review meetings and unscheduled (ad-hoc) DSMB meetings. Procedures for identification of dose-limiting toxicities and identification of severe adverse events are described. The Charter also provides information on procedures for record retention and audit, the duration and changes to the DSMB membership,

confidentiality, financial disclosure and conflict of interest disclosure, and provides a description of the information that should be stored in the Trial Master File.

***Adaptive Clinical Trial Design.*** The study utilizes a novel adaptive design organized in two stages. The goal of Stage 1 is to assess product safety and confirm that the dosing regimen achieves the desired steady-state target serum concentrations of 50 nM (low) and 150 nM (high). The sample size of Stage 1 is flexible and enrollment in this stage will be considered complete when there is sufficient evidence that the low and high doses are safe and that the doses achieve the target steady-state concentrations. Data obtained from each Stage 1 study subject will be submitted to the DSMB. The DSMB will decide when to proceed to Stage 2. Stage 2 utilizes adaptive allocation to the low and high dose to maximize the likelihood of obtaining a positive treatment effect if there is a beneficial effect of the treatment. The adaptive design was created in consultation with Berry Consultants and UC Davis biomedical statisticians who are members of the study team. During the reporting period, the design was created and simulations were performed, which required defining with the clinical team the assumptions underlying the trial (e.g., the distribution and variability of the patient population and outcomes, the existing pharmacokinetic data and it's uncertainty, patient characteristics to be included in the models, known information regarding the relationship between early and late neurological outcomes, the likely rate of patient recruitment, fraction of patients lost to follow up). In addition, data elements required to inform the adaptive algorithm (e.g., treatment arm, blood drug levels, gender, age, weight, interim and final neurological outcomes) were defined and a strategy for transfer of these data elements was developed and implemented. UC Davis statisticians will primarily be involved in managing Stage 1 whereas Berry Associates will periodically revise randomization probabilities in Stage 2.

***Publication on ClinicalTrials.gov.*** On August 20, 2012, an entry on ClinicalTrials.gov, a registry of clinical trials operated by the National Library of Medicine was created for the trial. The ClinicalTrials.gov identifier is NCT01673828.Extensive information on the purpose, outcome measures, and eligibility criteria are included as well as key contacts. The University of California, Davis is listed as a sponsor and the Department of Defense is acknowledged.

***Interactive Web Response System (IWRS).*** During the reporting period work was begun on the development of a web-based system for patient randomization, patient deactivation, GOS-E score recording, drug dispensation, drug shipment receipt, tracking of lost or damaged IV bags, and unblinding. A key aspect of the system is to provide web-based reports for study management and control of drug inventory. The system interfaces with the UC Davis Good Manufacturing Practices Laboratory and the Investigational Drug Services Pharmacy. A vendor built the system to our specifications and was tasked to conduct validation and investigator training in the use of the system.

***Investigational New Drug Application (IND) Approval.*** The initial IND document was prepared by Dr. Rogawski and submitted for review on September 6, 2011. The FDA acknowledged the date of receipt as September 13, 2011. The IND number assigned was 111,085. The sponsor is designated as Dr. Rogawski. The FDA required additional detailed information on the procedure for drug product manufacturing; certificate of analyses for a representative batches of drug product, including assay, impurity/degradant levels, sterility results, pyrogenicity results, pH,

osmolality and particulates; stability data and plans for continuing stability testing; storage details for the drug products; and a description of the infusion bags. This information was provided to the FDA on March 22, 2011. On May 7, 2012 the FDA approved the IND.

***Change in Manufacturing.*** We instituted a revised manufacturing process utilizing a new excipient to increase the efficiency and reduce the cost of manufacturing. The new procedures for manufacturing of the placebo and allopregnanolone product formulations were finalized by the UC Davis Good Manufacturing Products Laboratory during the reporting period. Stability testing was accomplished on the frozen concentrate used as an intermediate in manufacturing and the final product forms. On November 15, 2012, we submitted an IND amendment notifying the FDA of a change in product excipient and we also provided updated information on the manufacturing procedure. Specifically, we changed the solubilizing agent to Captisol® [β-cyclodextrin sulfobutyl ethers, sodium salts] (CyDex Pharmaceuticals Inc., Lenexa, KS), manufactured and tested in conformance with USP<1078> (GMP for Bulk Pharmaceutical Excipients). In addition, we notified the FDA of a new manufacturing procedure in which we stored 4-fold stock solutions of the product forms frozen at –20 º for extended periods (up to 56 days). Stability data for the final product formulation was provided at 2–8 ºC (for up to 31 days). The certificates of analyses for the final product forms are given in Appendix I. The certificates of analyses for the 4-fold concentrates are given in Appendix II. The certificate of analysis of the Captisol® excipient is given in Appendix III. The new excipient improves manufacturing efficiency. The new excipient (Captisol®) is a component of several marketed products and replaces an excipient that we believe is no longer used in any marketed products. The revised product label is shown in Appendix IV.

***Institutional Review Board (IRB) Approval.*** The protocol was initially approved by the University of California, Davis IRB on May 10, 2012 and modifications were approved on June 13, 2012.

***Human Research Protection Office (HRPO) Approval.*** The protocol was assigned HRPO Log Number A-15737 and reviewed by HRPO, a unit of the U.S. Army Medical Research and Materiel Command (USAMRMC). On July 18, 2012, notification was received that HRPO had determined that the protocol complies with applicable DoD, US Army and USAMRMC human subject protections requirements. HRPO initial approval was granted.

***Investigator's Brochure.*** An Investigator's Brochure was produced with information on the drug substance and drug product, including chemical properties, manufacturing and formulation. Drug related risks were summarized. Extensive pharmacology information was provided. A complete table of dosing based on body weight is also provided in this document.

***Study Team, Study Monitoring and Quality Assurance.*** During the reporting period, the study team was re-constituted in anticipation of the transition from product development and study design to subject enrollment and study conduct. The study team is led by the principal and co-principal investigators. Two medical monitors were recruited to share monitoring responsibilities, each providing coverage half-time. Since the study requires active participation of the Emergency Department (ED), two ED physicians were recruited to supervise the ED staff and insure adequate recruiting and compliance with all study provisions at the point of entry into

the study. Similarly, a neurosurgeon was recruited to the study team to coordinate the neurosurgical medical staff to insure active participation and compliance with all study requirements when study subjects are cared for by the neurosurgical service. Full time clinical research coordinator coverage is provided by two research coordinators who share responsibilities of around the clock coverage. The clinical coordinators manage the implementation, quality control and completion of the trial, including patient recruitment. They provide a point-of-contact familiar with all aspects of the trial and provide oversight and direction to ensure that all protocol and regulatory requirements are fully met in the conduct of the trial and that the data for each study subject is managed strictly according to the study protocol. The clinical research coordinators are assisted by a study assistant (junior specialist) who is available in the ED to ensure enrollment of all eligible and interested patients. The study assistant also assists with informed consent and with blood collection and processing. The research coordinators and study assistant are supervised by a nurse supervisor with extensive experience in the conduct of TBI trials. This individual provides critical knowledge and judgment in the day-to-day operation of the trial and in patient recruitment.

A neuropsychologist was recruited to supervise the conduct of the neuropsychological testing of study subjects. This individual was tasked with recruiting a psychometrician to conduct the neuropsychological testing. Additional personnel recruited to the study team include a senior supervising biostatistician responsible for the overall statistical conduct of the trial in conjunction with the adaptive trial consultants. A principal statistician was recruited with the responsibility for the conduct of the ongoing statistical analysis as the trial proceeds and for interaction with the DSMB.

An analytical chemist was recruited for chemical analytical studies in support of drug product manufacturing and bioanalytical support in the conduct of the clinical study. He is responsible for analytical methods development and quality control of the drug product, and for ongoing analysis of patient samples. The FDA had mandated ongoing plasma measurements to insure compliance with designated limits on exposure to the study drug. The analytical chemist will allow this mandate to be fulfilled.

***Role of UC Davis Clinical Translational Science Center (CTSC).*** Substantial cost savings are being achieved by the use of the resources of the UC Davis CTSC for quality assurance monitoring and FDA reporting instead of contracting with a commercial vendor for these services as originally anticipated. The Clinical Trial Resource Group within the CTSC will provide quality assurance monitoring and serious adverse event reporting (SAE) to regulatory authorities. Monitoring insures compliance with protocol, SOPs, Good Clinical Practice requirements and applicable regulatory requirements. In addition, accuracy and completeness of case report form data entries are verified against source documents. A MedDRA subscription provides access to official terminology for regulatory reporting. The CTSC has also been tasked to provide database services. Study data will be inputed and stored in an open source REDCap database. Programming of the database was completed during the reporting period. The CTSC provides ongoing maintenance.

***Provisions to Recruit Additional Study Sites.*** Due to the demands of the study, it is unlikely that we will be able to enroll all of the required subjects from the head injury patients admitted to the

UC Davis Medical Center. Therefore, during the reporting period we began to explore mechanisms to utilize additional study sites. We have had discussions with several potential sites and we have identified individuals on our team who can assist with the evaluation, recruitment, liaison and monitoring of the additional sites.

***Revision of Statement of Work (SOW) and Budget.*** Prior to obtaining HRPO approval, funding as mandated by the terms of the award was interrupted. Upon obtaining HRPO approval it was possible to resume funding. Changes to the study design in response to FDA mandates were required. In particular, the FDA required frequent plasma measurements to insure compliance with limits on exposure. This necessitated the creation of a bioanalytical facility with the capability to perform the required plasma sample analyses. At the same time, we were able to achieve substantial cost savings by utilizing the UC Davis CTSC for databasing, quality assurance monitoring and FDA reporting instead of an outside contract research organization as originally proposed and budgeted. In addition, we obtained cost savings through an optimized manufacturing process. It was also necessary to realign our study team to meet the requirements of the study. Certain members of the original study team were no longer available to participate; qualified individuals from Emergency Medicine and Neurological Surgery were substituted. It was also necessary to include medical monitors in the study team to meet FDA requirements for safety monitoring and reporting. The study team was also enhanced by addition of clinical research coordinators and a study assistant to provide around-the-clock coverage. These changed required a new SOW and budget. We began the preparation of these documents in August 2012 and submitted a revised budget and SOW in early September 2012. Program review and revision occurred in the subsequent months and the revised grant award was received on March 15, 2013.

**Section III – Key Research Accomplishments**

- Finalized allopregnanolone product formulation development and verification of stability.
- Data Safety Monitoring Board constituted.
- Implemented novel adaptive trial design to meet FDA requirements and to optimize study.
- Received approval of IND from FDA providing authorization to begin enrollment.
- Received approval from IRB and HRPO.

**Section IV – Reportable Outcome**

None.

**Section V – Conclusion**

This project seeks to provide initial data on the safety and effectiveness of allopregnanolone in improving neurobehavioral outcome and reducing mortality in adults with moderate to severe TBI through a two-stage, adaptive, placebo-controlled, double blind,

randomized clinical trial. During year 1, we located a manufacturer for the API (allopregnanolone) and began manufacturing. We also undertook extensive development work on the clinical trial protocol. During year 2, we finalized API manufacturing, primarily focusing on assessment of chemical purity and stability. We also created product formulations and assembled the large base of information required for IND filing. We met with the FDA in a Pre-IND meeting and in response to the agency's comments we modified our protocol to meet the requirements defined by the agency. We developed a novel adaptive trial design that provides a means to meet the FDA requirements regarding pharmacokinetics and allows us to address FDA guidance to assess more than one dose. Using the protocol and an extensive base of information on allopregnanolone, an IND package was developed and submitted to the FDA. We also submitted our protocol for review by our local IRB and to HRPO. During year 3, our IND was approved. We also received IRB and HRPO approval. Under the terms of our clinical study award, HRPO approval was required for payments to be made after the first year of the award. Therefore, no funding beyond the first year allocation was received. The unavailability of funding substantially slowed our progress during year 3. Because of the altered timeline and also key changes in the study because of requirements mandated by the FDA, at the time we obtained HRPO approval, a major budget revision was required. This occupied our attention beginning in August 2012 and continued until the revised grant award was received on March 15, 2013. Resumption of funding allowed us to rapidly finalize plans for study initiation. However, the interruption in funding and the focus on budget revision substantially delayed the submission of this annual report.

In sum, the research conducted to date under this award has advanced the development of a potential treatment approach for adults with moderate and severe TBI. There has been substantial progress toward meeting the primary objective of the award, which is to conduct a UC Davis-sponsored clinical study of a promising therapeutic agent. While there were many challenges and uncertainties in the program to date, all barriers to progress have now been successfully overcome. Our research and development activities provide many beneficial spin-offs apart from the conduct of the clinical study itself. The novel methods we have developed for the GMP manufacturing of pharmaceutical grade allopregnanolone and the production of intravenous product formulations can be applied by others who seek to investigate allopregnanolone in clinical trials for the treatment of TBI or other conditions. The methods are also applicable to the eventual production of allopregnanolone for deployment as a treatment agent if approved for use by regulatory authorities. Our approved IND defines the regulatory requirements for allopregnanolone product forms and for the clinical study of allopregnanolone. Our activities under this award have led to the creation of allopregnanolone product forms that are FDA approved for investigational use. In addition, the novel clinical trial design we have developed could also be adapted by other researchers seeking to study allopregnanolone or other agents in the treatment of TBI.

**Section VI – References**

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## Appendix I. Certificates of Analysis: 31-Day Stability of Product Solution

**University of California, Davis**
**University of California Davis Medical Center**
**4635 2nd Avenue, Research I, Suite 1204**
**Sacramento, California 95817, USA**

**CERTIFICATE OF ANALYSIS**

| | |
|---|---|
| MATERIAL: | Product L (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 6% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 0.500 mg/mL) |
| LOT NO.: | 1 |
| MANUFACTURE DATE: | July 20, 2012 |
| APPEARANCE: | Clear solution |
| ASSAY: | 0.485 ± 0.003 mg/mL |
| IMPURITY LEVELS: | No peaks were seen that were not present in 0.9% sodium chloride, USP |
| pH: | 5.447 ± 0.025 |
| OSMOLALITY: | 412.3 ± 3.51 mmol/kg |
| PARTICULATES: | No visible particles at 400x magnification |
| 31-DAY STABILITY | Pass (+3.12% pH, +2.59% osmolality, and +4.92% concentration after 31 day storage at 4 °C) |

Values are reported as mean ± standard deviation of triplicate measurements.

This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817

Gerhard Bauer
Laboratory Director GMP Facility
Adjunct Assistant Professor
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Analytical Chemist
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August 22, 2012

**University of California, Davis**
**University of California Davis Medical Center**
**4635 2nd Avenue, Research I, Suite 1204**
**Sacramento, California 95817, USA**

## CERTIFICATE OF ANALYSIS

| | |
|---|---|
| MATERIAL: | Product H (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 6% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 1.500 mg/mL) |
| LOT NO.: | 1 |
| MANUFACTURE DATE: | July 20, 2012 |
| APPEARANCE: | Clear solution |
| ASSAY: | 1.664 ± 0.003 mg/mL |
| IMPURITY LEVELS: | No peaks were seen that were not present in 0.9% sodium chloride, USP |
| pH: | 5.437 ± 0.042 |
| OSMOLALITY: | 412.3 ± 1.53 mmol/kg |
| PARTICULATES: | No visible particles at 400x magnification |
| 31-DAY STABILITY | Pass (-2.51% pH, +1.70% osmolality, and +0.88% concentration after 31 day storage at 4 °C) |

Values are reported as mean ± standard deviation of triplicate measurements.

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This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817

Gerhard Bauer
Laboratory Director GMP Facility
Adjunct Assistant Professor
Stem Cell Program
School of Medicine
University of California, Davis

Chun-Yi Wu, Ph.D.
Analytical Chemist
Department of Neurology
University of California, Davis

August 22, 2012

## Appendix II. Certificates of Analysis: 56-Day Stability of Concentrate

**University of California, Davis**
**University of California Davis Medical Center**
**4635 2nd Avenue, Research I, Suite 1204**
**Sacramento, California 95817, USA**

**CERTIFICATE OF ANALYSIS**

| | |
|---|---|
| MATERIAL: | Product L, 4X concentrate (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 24% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 2.000 mg/mL) |
| LOT NO.: | 2 |
| MANUFACTURE DATE: | August 24, 2012 |
| APPEARANCE: | Clear solution |
| ASSAY: | 0.551 ± 0.001 mg/mL, 1X final product, diluted from the 4X concentrate with 0.9% sodium chloride, USP, on October 19, 2012 |
| IMPURITY LEVELS: | No peaks were seen that were not present in 0.9% sodium chloride, USP |
| pH: | 5.007 ± 0.058 |
| OSMOLALITY: | 408.0 ± 3.00 mmol/kg |
| PARTICULATES: | Pass [USP<788>, Light Obscuration Method (Method 1, test 1.A.), performed at Nelson Laboratories, Salt Lake City, UT], >10 μm particles: 1 particle/ml; >25 μm particles: 0 particles/ml. |

The 4X concentrate was stored at –20 °C for 56 days.
Values are reported as mean ± standard deviation of triplicate measurements.

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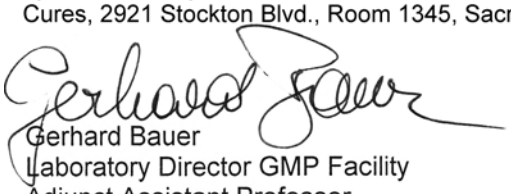
This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817



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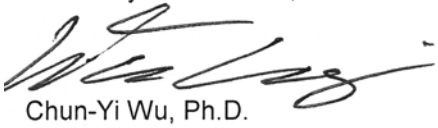

Chun-Yi Wu, Ph.D.
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October 19, 2012

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## CERTIFICATE OF ANALYSIS

| | |
|---|---|
| MATERIAL: | Product H, 4X concentrate (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 24% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 6.000 mg/mL) |
| LOT NO.: | 2 |
| MANUFACTURE DATE: | August 24, 2012 |
| APPEARANCE: | Clear solution |
| ASSAY: | 1.530 ± 0.008 mg/mL, 1X final product, diluted from the 4X concentrate with 0.9% sodium chloride, USP, on October 19, 2012 |
| IMPURITY LEVELS: | No peaks were seen that were not present in 0.9% sodium chloride, USP |
| pH: | 5.047 ± 0.015 |
| OSMOLALITY: | 409.3 ± 1.53 mmol/kg |
| PARTICULATES: | Pass [USP<788>, Light Obscuration Method (Method 1, test 1.A.), performed at Nelson Laboratories, Salt Lake City, UT], >10 μm particles: 2 particles/ml; >25 μm particles: 1 particle/ml. |

The 4X concentrate was stored at –20 °C for 56 days.
Values are reported as mean ± standard deviation of triplicate measurements.

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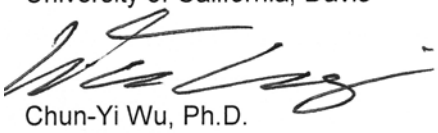

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## CERTIFICATE OF ANALYSIS

| | |
|---|---|
| MATERIAL: | Product Placebo, 4X concentrate (0.9% sodium chloride, USP with 24% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts) |
| LOT NO.: | 2 |
| MANUFACTURE DATE: | August 24, 2012 |
| APPEARANCE: | Clear solution |
| ASSAY: | ND mg/mL, 1X final product, diluted from the 4X concentrate with 0.9% sodium chloride, USP, on October 19, 2012 |
| IMPURITY LEVELS: | No peaks were seen that were not present in 0.9% sodium chloride, USP |
| pH: | 4.913 ± 0.058 |
| OSMOLALITY: | 420.0 ± 1.00 mmol/kg |
| PARTICULATES: | Pass [USP<788>, Light Obscuration Method (Method 1, test 1.A.), performed at Nelson Laboratories, Salt Lake City, UT], >10 μm particles: 0 particles/ml; >25 μm particles: 0 particles/ml. |

The 4X concentrate was stored at –20 °C for 56 days.
Values are reported as mean ± standard deviation of triplicate measurements.

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October 19, 2012

## Appendix III. Certificates of Analysis for Captisol® Excipient

CYDEX PHARMACEUTICALS
A Ligand Company

**CAPTISOL®** [β-Cyclodextrin Sulfobutyl Ethers, Sodium Salts]
Certificate of Analysis Rev 0
**Batch Number: 17CX01.HQ00078**

| Test | Specification | Result |
|---|---|---|
| Appearance | White to off-white solid essentially free from foreign matter | Pass |
| Solution Clarity | A 30% w/v solution in water is clear and essentially free from particles of foreign matter | Pass |
| Identification (IR) | Spectrum is consistent with the SBECD standard | Pass |
| Sodium Identity | Identity tests are positive for Sodium | Pass |
| Solution pH | The pH of a 30% w/v solution in water is within the range of 4.0 - 6.8 | 6.3* |
| Heavy Metals | Maximum 5 ppm | < 5 ppm |
| Water (by KF) | Maximum 10.0% | 3.6%* |
| Average Degree of Substitution (CE)<br>SBE I Content<br>SBE II Content<br>5 Largest Fractions | 6.2-6.9<br>Maximum 0.2%<br>Maximum 0.8%<br>Not Less Than 85% | 6.7<br>0.0%<br>0.3%<br>91% |
| β-cyclodextrin Content | Maximum 0.1% | < 0.05% |
| Sodium Chloride | Maximum 0.2% | < 0.05% |
| 1,4-Butane Sultone | Maximum 0.5 ppm | < 0.5 ppm |
| 4-Hydroxybutane-1-sulfonic acid | Maximum 0.09% | < 0.03% |
| Disodium bis-(4-sulfobutyl) ether | Maximum 0.05% | < 0.02% |
| Assay (anhydrous basis) | Minimum 95.0% to Maximum 105.0% | 101.1.% |
| Bacterial Endotoxins | Maximum 25 EU/g | < 2.4 EU/g |
| Microbiology<br>Aerobic microorganisms<br>*Escherichia coli*<br>*Salmonella* species<br>Molds & Yeasts | <br>Maximum 50 CFU/g<br>Meets test requirements for absence<br>Meets test requirements for absence<br>Maximum 25 CFU/g | <br>0 CFU/g<br>Absent<br>Absent<br>0 CFU/g |

***Results obtained at time of testing, values may change depending upon exposure to atmospheric conditions.**
**STORAGE: Store at ambient temperature in sealed containers. Protect from moisture.**

References: 17CX01.HQ00078 Re-evaluation Date: February 2016 Date of Manufacture: February 2011
Manufactured by: Hovione SA,
Sete Casas Loures 2674-506 Portugal

Vincent Antle

Released By:_____ Date: 04 April 2011
Vincent Antle, PhD
Sr. Director of Technical Operations &
Quality Assurance

QA APPROVED

*"Captisol is manufactured and tested in conformance with the principles of Chapter <1078> of the United States Pharmacopeia (Good Manufacturing Practices for Bulk Pharmaceutical Excipients)."*

CyDex Pharmaceuticals Inc. 10513 W. 84th Terr., Lenexa, KS 66214-1643 ● (913) 685-8850 ● FAX (913) 685-8856 ● www.cydexpharma.com

## Appendix IV. Product Label

**I.V. Bag #:_____ Subject #: _____**

**Allopregnanolone or Placebo I.V. Solution in 0.9% sodium chloride injection, USP with 6% sulfobutyl ethers β-cyclodextrin sodium salts**

| BAG SEQUENCE # ___ of ___ | | |
|---|---|---|
| ☐ Load | ☐ Maintenance | ☐ Taper |

This container contains _____ mL

To Be Completed by Pharmacy:

Administer at _____ mL/min

Expiration Date: _____/____/_____

Protocol Name: IV Allopregnanolone Treatment of Patients with Acute Traumatic Brain Injury.
UC Davis IRB Protocol # 27314; IND 111,085.
Store at 2–8 ºC; dispense at room temperature.
For intravenous use only.
Caution: New Drug – Limited by Federal Law to Investigational Use
*Manufactured by University of California, Davis*