

REVISED CLINICAL STUDY PROTOCOL

Document Title: Revised Clinical Protocol #2 for a Phase IIa Open-Label, Multiple Ascending Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Exploratory Efficacy of Vamorolone in Boys with Duchenne Muscular Dystrophy (DMD)

Protocol Number: VBP15-002

Document Number: VBP15-002-R2

FDA IND No.: 118,942

Investigational Product: Vamorolone

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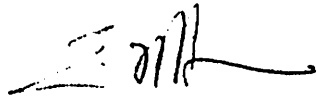
Document Date: 20 January 2017

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SIGNATURES OF AGREEMENT FOR VBP15-002-R2

Revised Clinical Protocol #2 for a Phase IIa Open-Label, Multiple Ascending Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Exploratory Efficacy of Vamorolone in Boys With Duchenne Muscular Dystrophy (DMD)


Reviewed and Approved by:



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20 January 2017


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20 January 2017

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Avital Cnaan, PhD
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20 January 2017

Date

INVESTIGATOR REVISED PROTOCOL AGREEMENT

Revised Clinical Protocol #2 for a Phase IIa Open-Label Multiple Ascending Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Exploratory Efficacy of Vamorolone in Boys with Duchenne Muscular Dystrophy (DMD)

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By my signature, I confirm that my staff and I have carefully read and understand this protocol, protocol amendment, amended protocol, or revised protocol and agree to comply with the conduct and terms of the study specified herein and with any other study conduct procedures provided by ReveraGen BioPharma, Inc.

I agree to conduct the study according to this protocol and the obligations and requirements of clinical Investigators and all other requirements listed in 21 CFR part 312 and all applicable local, state, and federal regulations and ICH guidelines. I will not initiate this study without the approval of an Institutional Review Board (IRB) or Independent Ethics Committee (IEC).

I understand that, should the decision be made by ReveraGen BioPharma, Inc. to terminate prematurely or suspend the study at any time for whatever reason, such decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study, I will communicate immediately such decision in writing to ReveraGen BioPharma, Inc.

For protocol amendments and amended protocols, I agree not to implement the amendment without agreement from ReveraGen BioPharma, Inc. and prior submission to and written approval (where required) from the IRB/IEC, except when necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).

Investigator's Signature

Date

Investigator's Name (Please print)
Address (Please print):

RETURN THE ORIGINAL SIGNED COPY TO REVERAGEN BIOPHARMA, INC. OR DESIGNEE AND KEEP A COPY AT YOUR SITE.

SERIOUS ADVERSE EVENT CONTACT INFORMATION

In the event of a serious adverse event (SAE) (see [Section 7.2.6.1](#)), the Investigator will complete the SAE electronic case report form within 24 hours of first awareness of the event. In the unlikely event that the electronic study database is inaccessible and the Investigator is unable to complete the SAE electronic case report form within 24 hours, the SAE Notification Form (pdf) should be completed and emailed or printed/faxed to the PRA safety management team within 24 hours, using the contact information below:

Email CHOSafety@prahs.com

Drug Safety Fax: 1-888-772-6919 or 1-434-951-3482

SAE Questions: Drug Safety Hotline: 1-800-772-2215 or 1-434-951-3489

PROTOCOL AMENDMENT TRACKING

Document	Document Number	Approval Date
Original Protocol	VBP15-002	16 February 2016
Amended Protocol #1	VBP15-002- A1	31 May 2016
Revised Protocol #1	VBP15-002-R1	01 August 2016
Amended Protocol #2	VBP15-002-A2	19 January 2017
Revised Protocol #2	VBP15-002-R2	20 January 2017

Note: Amended protocols show all changes from last previous document version in bold/strike-through format.

Reason for Revised Protocol #2:

1. to incorporate all changes from Amended Protocol #2 into a new protocol document.

Reasons for Amended Protocol #2:

1. to update the nonclinical toxicology information with results of a 26-week toxicology study in mice;
2. to remove reference to a dose escalation plan based on an mTPI design;
3. to remove the requirement that QT_c must be calculated using Fredericia's formula;
4. to clarify that SAE information is not required to be forwarded to the DSMB within 24 hours of first awareness; and
5. to correct typographical errors.

STUDY SYNOPSIS

Protocol Title	A Phase IIa, Open-Label, Multiple Ascending Dose Study to Assess the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Exploratory Efficacy of Vamorolone in Boys with Duchenne Muscular Dystrophy (DMD)
Protocol Number	VBP15-002
Name of Sponsor	ReveraGen BioPharma, Inc.
Drug Substance	delta-1,4,9(11)-pregnatriene-17-alpha,21-dihydroxy-16-alpha-methyl-3,20-dione
Investigational Drug Product	vamorolone, 4% suspension for oral dosing
Phase of Development	Phase IIa
Indication	Treatment of Duchenne muscular dystrophy (DMD)
Objectives	<p>Primary:</p> <ol style="list-style-type: none"> To evaluate the safety and tolerability of multiple ascending oral doses of vamorolone in ambulant boys ages 4-< 7 years with DMD. <p>Secondary:</p> <ol style="list-style-type: none"> To investigate the single-dose and multiple-dose pharmacokinetics (PK) of oral vamorolone at multiple dose levels in ambulant boys ages 4-< 7 years with DMD; To investigate the effects of single and multiple oral doses of vamorolone on serum pharmacodynamic (PD) biomarkers in ambulant boys ages 4-< 7 years with DMD; To evaluate metabolites of vamorolone in Metabolites in Safety Testing (MIST) assessments following administration of multiple ascending oral doses. <p>Exploratory:</p> <ol style="list-style-type: none"> To investigate the effect of multiple oral doses of vamorolone on muscle strength, mobility, and functional exercise capacity, as measured by Quantitative Muscle Testing (QMT), Time to run/walk 10 meters Test (TTRW), Time to Stand Test (TTSTAND), Time to Climb Test (TTCLIMB), North Star Ambulatory Assessment (NSAA), and Six-minute Walk Test (6MWT) in ambulant boys ages 4-< 7 years with DMD.
Study Design	<p>This Phase IIa study is an open-label, multiple ascending dose study to evaluate the safety, tolerability, PK, PD, and exploratory clinical efficacy of oral vamorolone over the course of a 14-day Treatment Period in ambulant boys ages 4-< 7 years with DMD.</p> <p>The study is comprised of a 26-day Pretreatment Screening Period, a 1-day Pretreatment Baseline Period, a 14-day Treatment Period, and a 14-day Post-treatment Follow-up Period.</p> <p>Note: Subjects who complete the 14-day Follow-up Period will be given the option of continuing vamorolone treatment in an extension study under a separate protocol (VBP15-003).</p> <p>Approximately 12 subjects will be enrolled per dose level at up to four ascending dose levels (unless there are dose-limiting toxicities in 2 or more subjects at a given dose level) in order to have minimum data for the dose</p>

	<p>level. If fewer than two of the approximately 12 subjects at any dose level experience a dose-limiting toxicity, following review of the available safety data for the Treatment and Follow-up Periods for all subjects in the dose cohort by the Study Chair and Medical Monitor, the Study Chair and Medical Monitor will jointly decide whether to recommend dose escalation to the next dose level. If two or more subjects at any dose level experience a dose-limiting toxicity, the Medical Monitor and Study Chair will be notified, and enrollment at that dose level will be halted; the MTD will be defined as the previous dose level, unless the decision is made to study lower intermediate dose level(s). The DSMB will be informed promptly of any dose decisions which are a result of dose-limiting toxicities.</p> <p>If dose escalation is terminated due to dose-limiting toxicities, the remaining dose level groups may be enrolled to evaluate the safety and PK effects of vamorolone at lower intermediate dose level(s). Once safety data from additional dose level group(s) are assessed, the MTD will be defined as the dose level below the dose at which the DSMB determines there to be an unacceptable risk to subjects. This plan allows the maximum number of safe dose level groups to have sufficient numbers for overall estimates of safety, PK, PD and baseline assessments of preliminary efficacy outcomes to be followed via an extension study (VBP15-003).</p>
<p>Planned Sample Size</p>	<p>A total of up to 48 subjects will be enrolled. Approximately 12 subjects in each of 4 dose level groups will be studied, unless 2 or more subjects at a given dose level have dose-limiting toxicities and dose escalation is halted.</p>
<p>Population</p>	<p>Inclusion Criteria:</p> <ol style="list-style-type: none"> 1. Subject's parent or legal guardian has provided written informed consent/Health Insurance Portability and Accountability Act (HIPAA) authorization prior to any study-related procedures; 2. Subject has a confirmed (by Central Genetic Counselor) diagnosis of DMD as defined as: <ol style="list-style-type: none"> a) Dystrophin immunofluorescence and/or immunoblot showing complete dystrophin deficiency, and clinical picture consistent with typical DMD, OR b) Identifiable mutation within the <i>DMD</i> gene (deletion/duplication of one or more exons), where reading frame can be predicted as 'out-of-frame', and clinical picture consistent with typical DMD, OR c) Complete dystrophin gene sequencing showing an alteration (point mutation, duplication, other) that is expected to preclude production of the dystrophin protein (i.e. nonsense mutation, deletion/duplication leading to a downstream stop codon), with a typical clinical picture of DMD; 3. Subject is ≥ 4 years and < 7 years of age at time of enrollment in the study; 4. Subject is able to complete the Time to Stand Test (TTSTAND) without assistance, as assessed at the Screening and Baseline Visits; 5. Clinical laboratory test results are within the normal range at the Screening Visit, or if abnormal, are not clinically significant, in the opinion of the Investigator. (Note: Serum gamma glutamyl transferase [GGT], creatinine, and total bilirubin all must be \leq upper limit of the normal range at the Screening Visit); 6. Subject has evidence of chicken pox immunity as determined by presence of IgG antibodies to varicella, as documented by a positive test result from

	<p>the testing laboratory at the Screening Visit; and</p> <p>7. Subject and parent/guardian are willing and able to comply with scheduled visits, study drug administration plan, and study procedures.</p> <p>Exclusion Criteria:</p> <ol style="list-style-type: none"> 1. Subject has current or history of major renal or hepatic impairment, diabetes mellitus or immunosuppression; 2. Subject has current or history of chronic systemic fungal or viral infections; 3. Subject has had an acute illness within 4 weeks prior to the first dose of study medication; 4. Subject has used mineralocorticoid receptor agents, such as spironolactone, eplerenone, canrenone (canrenoate potassium), prorenone (prorenoate potassium), mexrenone (mexrenoate potassium) within 4 weeks prior to the first dose of study medication; 5. Subject has evidence of symptomatic cardiomyopathy [Note: Asymptomatic cardiac abnormality on investigation would not be exclusionary]; 6. Subject is currently being treated or has received previous treatment with oral glucocorticoids or other immunosuppressive agents [Notes: Past transient use of oral glucocorticoids or other oral immunosuppressive agents for no longer than 3 months cumulative, with last use at least 3 months prior to first dose of study medication, will be considered for eligibility on a case-by-case basis. Inhaled and/or topical corticosteroids prescribed for an indication other than DMD are permitted but must be administered at stable dose for at least 3 months prior to study drug administration]; 7. Subject has used idebenone within 4 weeks prior to the first dose of study medication; 8. Subject has an allergy or hypersensitivity to the study medication or to any of its constituents; 9. Subject has severe behavioral or cognitive problems that preclude participation in the study, in the opinion of the Investigator; 10. Subject has previous or ongoing medical condition, medical history, physical findings or laboratory abnormalities that could affect safety, make it unlikely that treatment and follow-up will be correctly completed or impair the assessment of study results, in the opinion of the Investigator; 11. Subject is taking any other investigational drug currently or has taken any other investigational drug within 3 months prior to the start of study treatment; or 12. Subject has previously been enrolled in the study. <p>Note: Any parameter/test may be repeated at the Investigator's discretion during Screening and/or Day -1 to determine sustainability and reproducibility. In addition, subjects may be rescreened if ineligible due to a transient condition which would prevent the subject from participating, such as an upper respiratory tract infection, or if ineligible due to negative anti-varicella IgG antibody test result.</p>
Number of Centers	The study will be conducted at approximately 10 U.S. and non-U.S. study sites.
Study Period	First subject screened: 1Q 2016 Last subject last visit: 4Q 2016

Study Duration	Up to approximately 12 months total duration, or longer in the event dosing or follow-up of a dose level group is extended, dose level group(s) repeated, or additional subjects added.																				
Individual Subject Study Duration	<p>Up to 8 weeks:</p> <ul style="list-style-type: none"> • Screening Period: up to 26 days • Baseline Period: 1 day • Treatment Period: 2 weeks • Post-treatment Follow-up Period: 2 weeks <p>Subjects who complete the 2-week Follow-up Period will be given the option of continuing vamorolone treatment in an extension study under separate protocol.</p>																				
Study Drug Formulation, Dosage & Administration	<p>Vamorolone 4% oral suspension administered once daily over a 14-day Treatment Period (total of 14 doses) at the following planned dose levels: 0.25 mg/kg/day, 0.75 mg/kg/day, 2.0 mg/kg/day, or 6.0 mg/kg/day.</p> <p>Four dosing groups of approximately 12 subjects each will receive vamorolone once daily for 14 days. The planned dose levels are 0.25 mg/kg (Dose Level Group 1), 0.75 mg/kg (Dose Level Group 2), 2.0 mg/kg (Dose Level Group 3), and 6.0 mg/kg (Dose Level Group 4):</p> <table border="1" data-bbox="557 903 1396 1161"> <thead> <tr> <th>Dose Level Group</th> <th>Number of Subjects in Dose Level Group</th> <th>Vamorolone Dose Level</th> <th>Treatment Duration</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>12</td> <td>0.25 mg/kg</td> <td>14 days</td> </tr> <tr> <td>2</td> <td>12</td> <td>0.75 mg/kg</td> <td>14 days</td> </tr> <tr> <td>3</td> <td>12</td> <td>2.0 mg/kg</td> <td>14 days</td> </tr> <tr> <td>4</td> <td>12</td> <td>6.0 mg/kg</td> <td>14 days</td> </tr> </tbody> </table> <p>Study drug will be administered at a participating study site on Days 1 and 14; all other doses will be administered at home. Study drug will be administered by mouth using a volumetric syringe. Following administration of the dose of study drug, the syringe will be filled once with water and the water will be administered by mouth using the volumetric syringe. The subject will then drink approximately 50 mL (approximately 2 ounces) of water to ensure the full dose has been ingested.</p> <p>Subjects will be asked to take the dose following ingestion of 8 ounces of whole milk. If whole milk is not tolerable to the subject, a serving of another high fat food can be substituted. There are no other food or drink restrictions during the study.</p>	Dose Level Group	Number of Subjects in Dose Level Group	Vamorolone Dose Level	Treatment Duration	1	12	0.25 mg/kg	14 days	2	12	0.75 mg/kg	14 days	3	12	2.0 mg/kg	14 days	4	12	6.0 mg/kg	14 days
Dose Level Group	Number of Subjects in Dose Level Group	Vamorolone Dose Level	Treatment Duration																		
1	12	0.25 mg/kg	14 days																		
2	12	0.75 mg/kg	14 days																		
3	12	2.0 mg/kg	14 days																		
4	12	6.0 mg/kg	14 days																		
Study Evaluation and Sample Collection	<p>This is a Phase IIa, multiple center, open-label, multiple ascending dose study of vamorolone administered orally once daily for 14 days to ambulant boys ages 4-< 7 years with DMD.</p> <p>Each dose level group of approximately 12 subjects will be assessed for safety and tolerability, PK, clinical efficacy, and PD biomarker response during the 14-day Treatment Period and a 14-day Follow-up Period. Treatment Period study visits will occur on Day 1, Week 1 (Day 7) and Week 2 (Days 13 and 14). Once-daily study drug dosing on Days 2-13 will occur at home. During the Follow-up Period, a scheduled telephone contact will be made by site study staff at Week 3 (Day 21), and a final study visit will occur at Week 4 (Day 28). Subjects will receive their first and final doses of study medication on Day 1</p>																				

	<p>and Day 14, respectively, at participating study sites.</p> <p>Blood will be drawn for PK analysis pre-dose and 1, 2, 4, 6, and 8 hours post-dose on Study Days 1 and 14. Blood will be collected for Metabolites in Safety Testing (MIST) assessment pre-dose and 1, 2, 4, 6, and 8 hours post-dose on Study Day 14. Once-daily study drug dosing on Days 2-13 will occur at home. Additional blood and urine samples for clinical laboratory tests will be collected and results will be used for dose escalation decisions. In addition, samples will be collected at scheduled visits throughout the study to assess safety and PD biomarker levels (see Section 6.3 for detailed assessment schedule).</p> <p>A physical examination and 12-lead electrocardiogram (ECG) will be recorded at Screening, Week 2 (Day 14), and the final Week 4 (Day 28) Follow-up Visit. Exploratory clinical efficacy will be assessed by QMT, TTSTAND, TTCLIMB, TTRW, NSAA, and 6MWT conducted at Screening and Baseline, Week 2 (Day 13), and the final Week 4 (Day 28) follow-up Visit. Vital signs will be recorded at each study visit. Adverse events (AEs), including serious AEs (SAEs), and concomitant medications will be recorded throughout the study.</p> <p>Subjects will be discharged from the study following completion of all Follow-up Week 4 (Day 28) assessments. In the event that any clinical or laboratory parameters remain abnormal at the time of discharge from the study, the subject will be followed medically as clinically indicated.</p>
Dose Escalation Plan, Dose-Limiting Toxicities, and Stopping Criteria	<p>In this multiple ascending dose study, subjects will be enrolled by dose level beginning with Dose Level Group 1, to receive vamorolone at the lowest dose level, after completion of all Pretreatment Screening and Baseline assessments. Dosing will continue through completion of Dose Level Group 4, or until a lower MTD is identified. If dose-limiting toxicities occur at any time during the Treatment or Follow-up Periods, the study design may be modified, in consultation with the Study Chair, Medical Monitor, Data and Safety Monitoring Board (DSMB), and the Sponsor, depending on the timing and nature of the toxicities.</p> <p>The Study Chair and the Sponsor's Medical Monitor will jointly review safety and available PK data for all subjects who have completed the 2-week Treatment Period at a given dose level, to make dose escalation decisions.</p> <p>The DSMB will review at a minimum the PK and safety data from the Screening and Treatment Periods for each subject in each dose level group. DSMB data review will be cumulative to include all data from previous dose level groups and any data from the 14-day Follow-up Periods available at the time of review. The DSMB may make recommendations to the Study Chair, Medical Monitor, and Sponsor regarding conduct.</p> <p>Subjects may be screened for eligibility for each dose level group up to 26 days in advance of the anticipated start of dosing in that dose level group. Screening for the next dose level group may occur when all subjects in the current dose level group have completed the 14-day Treatment Period and are being monitored in the 14-day Follow-up Period.</p> <p>Clinical AEs and clinical laboratory AEs will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, dated June 14, 2010.</p> <p>Dose-limiting toxicities will be defined as follows:</p>

	<ol style="list-style-type: none"> 1. The presence of a CTCAE Grade \geq 3 AE, considered to be probably or definitely related to study drug, in the opinion of the Investigator; 2. The presence of a CTCAE Grade \geq 3 clinical laboratory AE considered to be probably or definitely related to study drug, in the opinion of the Investigator; or 3. Deterioration of the muscle condition, unexpected for the natural course of DMD and without other clear cause, in the opinion of the Investigator. The possibility to consider the event as a dose-limiting toxicity will be discussed by the Study Chair, the Medical Monitor and the DSMB. The SAE data collection tool must be completed if the event also meets the criteria for an SAE. <p>If a dose-limiting toxicity occurs in two or more subjects in any dose group during the Treatment or Follow-up Periods, the Study Chair and Medical Monitor will be notified, and enrollment and dosing at that dose level will be halted; the MTD will be defined as the previous dose level, unless the decision is made to study lower intermediate dose level(s). The DSMB will be informed promptly of any dose decisions which are a result of dose-limiting toxicities.</p> <p>If dose escalation is terminated due to dose-limiting toxicities, the remaining dose level groups may be enrolled to evaluate the safety and PK effects of vamorolone at lower intermediate dose levels. Once safety data from additional dose level group(s) are assessed, the MTD will be defined as the dose level below the dose at which the DSMB determines there to be an unacceptable risk to subjects.</p>
<p>Safety Measures</p>	<ul style="list-style-type: none"> • Physical examination • Weight • Vital signs (supine blood pressure, heart rate, respiratory rate, oral temperature) • Clinical laboratory tests: <ul style="list-style-type: none"> ○ Hematology and biochemistry ○ Urinalysis (urine protein and glucose) ○ Lipid profile (triglycerides, total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL]) • 12-lead ECG • Clinical signs and symptoms (AEs and SAEs) • Grading of clinical and clinical laboratory AEs will be according to the CTCAE, v.4.03 • MIST assessment
<p>Pharmacokinetics</p>	<p>Blood will be collected from subjects for vamorolone PK analysis prior to and following dosing on Day 1 and Day 14. On Day 1, sampling times will be 0.5 hours pre-dose (prior to administration of the first dose of study medication), and at 1, 2, 4, 6, and 8 hours post-dose. On Day 14, sampling times will be 0.5 hours pre-dose (prior to administration of the final dose of study medication), and at 1, 2, 4, 6, and 8 hours post-dose.</p> <p>Subjects will have fasted \geq 6 hours prior to the pre-dose blood draws on Day 1 and Day 14.</p>

<p>Pharmacodynamic Measures</p>	<p>Blood will be collected for serum PD biomarker testing to explore the effect of vamorolone on biomarkers of muscle cellular pathology, and biomarkers associated with acute and chronic glucocorticoid treatment. Samples for analysis of acute and chronic PD biomarker response will be collected at Screening, Day 1 (pre-dose and 6 hours post-dose), Week 2 (Day 14) (pre-dose and 6 hours post-dose), and Week 4 (Day 28). Blood will also be collected and stored for future proteomics profiling and SomaScan studies.</p> <p>Subjects will have fasted ≥ 6 hours prior to the pre-dose blood draws on Day 1 and Day 14 for determination of insulin and glucose levels.</p>
<p>Exploratory Clinical Efficacy Measures</p>	<ul style="list-style-type: none"> • Quantitative Muscle Testing (QMT) • Time to Stand Test (TTSTAND) • Time to Climb 4 steps Test (TTCLIMB) • Time to Run/Walk 10 meters Test (TTRW) • North Star Ambulatory Assessment (NSAA) • Six-minute Walk Test (6MWT)
<p>Statistical Methods</p>	<p>Sample Size: A sample of 12 in each cohort is large enough to have a confidence interval of no wider than 50% for the proportion of adverse events assessed or any other event outcome. For the continuous outcomes such as safety laboratory markers or pharmacodynamics markers, a two-sided 95.0% confidence interval for the mean will be no wider than 0.8 standard deviation (SD) from the observed mean, with 90.0% coverage probability, based on the t statistic; thus, the total confidence interval will be approximately 1.6 SDs wide for any continuous parameter if its underlying distribution is approximately normal.</p> <p>Analysis Sets: <u>Safety Population</u> All subjects who receive at least one dose of vamorolone study medication will be included in the Safety Population.</p> <p><u>PK Population</u> All subjects who receive at least one dose of vamorolone study medication and have sufficient data for PK analysis will be included in the PK Population.</p> <p>Statistical Analysis: Owing to the small sample size, no inferential statistical analyses are planned. Individual subject listings of endpoints, sorted by escalating dose level group, will be reviewed for any evidence of dose-related differences or trends.</p> <p>Safety and Tolerability Evaluation: Safety analyses will be performed using the Safety Population. Vital signs, 12-lead ECG, and clinical laboratory test results will be summarized by dose level using descriptive statistics. Treatment-emergent AEs (TEAEs) will be summarized by dose level, system organ class (SOC) and preferred term (using the Medical Dictionary for Regulatory Activities [MedDRA] version 18.1), by dose level and relationship to study medication, and by dose level and intensity (CTCAE grade) as well as AE outcome. All safety data will be listed by dose level.</p> <p>Pharmacokinetic Evaluation: All PK analyses will be performed using the PK Population. Plasma concentrations and the derived plasma PK parameters will be summarized and</p>

	<p>listed for vamorolone, as appropriate. Individual and mean vamorolone concentrations versus time will be plotted on linear and semi-logarithmic scales by dose for vamorolone, as appropriate.</p> <p>Summary statistics (number of subjects, mean, standard deviation [SD], minimum, median, maximum, geometric mean, and percentage coefficient of variation [%CV]) for the derived PK parameters of vamorolone will be presented by dose level for the PK Population. Plots of plasma concentration-time profiles will be provided by dose level. All available concentration-time data will be listed. Derived PK parameters will be listed by treatment.</p> <p>A (compartmental) population PK model will be developed based on data obtained from VBP15-001 (a study in healthy adults). As part of this procedure, the number of systemic compartments will be determined. In addition, linearity with respect to dose and time and appropriateness of a first-order absorption model will be evaluated. The parameters of this model, with systemic parameters scaled either by weight or allometrically (clearances scaled by weight raised to the 0.75 power), will be applied to the dosing history in VBP15-001 to predict the plasma concentration profile for pediatric subjects. The observed values will then be compared to the predicted concentration and the model adjusted as necessary.</p> <p><i>Pharmacodynamic and Exploratory Efficacy Evaluation:</i></p> <p>The Safety Population will be used for all PD and exploratory efficacy evaluations. Standard descriptive statistics will be used to summarize the PD and clinical efficacy endpoints by dose level and overall.</p>
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LIST OF ABBREVIATIONS

Abbreviation	Definition/Term
ACTH	adrenocorticotrophic hormone
ADL	activities of daily living
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
AUC _{0-24hr}	area under the concentration-time curve from time 0 to 24 hours
AUC _{0-t}	area under the concentration-time curve from time 0 to time t
AUC _{inf}	area under the concentration-time curve from time 0 to infinity
AUC _{last}	area under the concentration-time curve from time 0 to the last observed measurable concentration
BL	baseline
BMI	body mass index
BP	blood pressure
BUN	blood urea nitrogen
C	Celsius
CD23	cluster designation 23
CFR	Code of Federal Regulations
CINRG	Cooperative International Neuromuscular Research Group
CK	creatine kinase
CL	clearance
ConA	concanavalin A
cm	centimeter
C _{max}	maximum observed plasma concentration
CQMS	CINRG Quantitative Measurement System
CTCAE	Common Terminology Criteria for Adverse Events
CTM	Clinical Trial Material
CTX	carboxy-terminal telopeptide
CYP	Cytochrome P450
dL	deciliter
DMD	Duchenne muscular dystrophy
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram
eCRF	electronic case report form

Abbreviation	Definition/Term
EDC	electronic data capture
F	Fahrenheit
F, F%	bioavailability; percent bioavailability
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
HbA1c	hemoglobin A1c
HDL	high density lipoprotein
HEENT	head, eyes, ears, nose and throat
HIPAA	Health Insurance Portability and Accountability Act
hr	hour
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IGFBP-2	insulin-like growth factor-binding protein 2
IGFBP-5	insulin-like growth factor-binding protein 5
IL-22BP	interleukin-22 binding protein
IND	Investigational New Drug
IRB	Institutional Review Board
K ₂ -EDTA	dipotassium ethylenediaminetetraacetic acid
kg	kilogram
λ_z	elimination rate constant
L	liter
LLC	Limited Liability Company
LC-MS	Liquid Chromatography – Mass Spectrometry
LDH	lactate dehydrogenase
LDL	low density lipoprotein
m	meter
MAD	multiple ascending dose (study)
MD	Medical Doctor (physician)
MDC	macrophage-derived chemokine
<i>mdx</i>	mouse model lacking dystrophin
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
min	minute

Abbreviation	Definition/Term
MIST	Metabolites in Safety Testing
mITT	modified Intention to Treat
mL	milliliter
MMP-3	matrix metalloproteinase-3
MMP-12	matrix metalloproteinase-12
MTD	maximum tolerated dose
mTPI	modified Toxicity Probability Interval
NADPH	nicotinamide adenine dinucleotide phosphate
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
ng	nanogram
No., n	number
nmol	nanomole
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
OTC	over-the-counter (non-prescription medication)
%CV	percentage coefficient of variation
PBL	peripheral blood leukocytes
PD	pharmacodynamic(s)
PHI	Protected Health Information
P1NP	serum aminoterminal propeptide of type I collagen
PK	pharmacokinetic(s)
PR [PQ]	time from onset of P wave to start of the QRS complex
Q	quarter
QMT	quantitative muscle testing
QRS	in electrocardiography, the complex consisting of Q, R, and S waves, corresponding to depolarization of ventricles [complex]
QSAR	quantitative structure-activity relationship
QT	in cardiology, the time between the start of the Q wave and end of the T wave
QT _c	corrected QT interval
6MWT	Six-minute Walk Test
RR	in electrocardiography, the interval between successive Rs (peaks of QRS complexes)
SAD	single ascending dose (study)
SAE	serious adverse event
SAP	statistical analysis plan

Abbreviation	Definition/Term
SCR	screening
SD	standard deviation
SOC	system organ class
SOP	standard operating procedures
SSL	secure socket layers
$t_{1/2}$	terminal half-life
TEAE	treatment-emergent adverse event
T_{max}	time to maximum observed plasma concentration
TPI	toxicity probability interval
TTCLIMB	Time to Climb (Test)
TTSTAND	Time to Stand (Test)
TTRW	Time to Run/Walk (Test)
ULN	upper limit of normal
US	United States
vs.	versus
V_{ss}	volume of distribution at steady state
V_z	terminal phase volume of distribution
WBC	white blood cell
WHO	World Health Organization

1 INTRODUCTION

1.1 Background and Unmet Need

Duchenne muscular dystrophy (DMD) is a rapidly progressive form of muscular dystrophy that occurs primarily in males and manifests prior to the age of six years. DMD affects approximately 1 in 3,600 to 9,300 male births worldwide.¹ Duchenne muscular dystrophy is caused by mutations in the dystrophin gene which codes for a protein that provides structural stability to the dystroglycan complex on muscle cell membranes.² The lack of dystrophin reduces plasma membrane stability. Membrane destabilization results in altered mechanical properties and aberrant signaling, which contribute to membrane fragility, necrosis, inflammation, and progressive muscle wasting.³

In addition to the significant contribution of membrane destabilization and mechanical injury in DMD, aberrant intracellular signaling cascades that regulate inflammatory and immune processes also contribute to DMD pathophysiology. Up-regulated inflammatory gene expression and activated immune cell infiltrates, at least partially mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activation, are evident during early disease stages and play a significant role in muscle wasting.³ NF- κ B has been shown to regulate the expression of numerous inflammatory genes in immune cells and muscle fibers,^{4,5,6,7} and the infiltration and activation of these cells can trigger muscle fiber death.^{8,9}

Although significant advances have been made in understanding the etiology of DMD, a cure has not been found, and current treatment options are all medications used “off-label” to alleviate the symptoms of DMD. Despite scientific advances, only glucocorticoids, such as prednisone, have consistently demonstrated efficacy in clinical trials.¹⁰ Further, many disease modifying technologies that are currently in development focus on subsets of dystrophin mutations and therefore do not address the unmet need in all persons with DMD. However, it is likely that glucocorticoids will need to be co-administered with many of these compounds for maximum effect and glucocorticoids have extensive side effect profiles, often limiting long-term administration. The current

goal of DMD research is to find a mutation-independent treatment that matches or exceeds the efficacy of glucocorticoids with a significantly lower side effect profile.

Vamorolone is a first generation delta-9, 11 chemical compound belonging to the structural class of synthetic steroidal drugs, which includes the glucocorticoids prednisone, methylprednisone, and dexamethasone.¹¹ The chemical structure of vamorolone has optimized four subactivities of traditional glucocorticoid drugs, namely transactivation, transrepression, physiochemical membrane properties, and mineralocorticoid receptor antagonism.¹¹ By reducing transactivation subproperties, retaining transrepression, imparting membrane stabilizing properties, and inhibiting the mineralocorticoid receptor pathway, vamorolone has favorable efficacy and side effect profiles relative to classic glucocorticoids in nonclinical models and is anticipated to be an attractive candidate for the treatment of DMD in pediatric patients.

In vitro and nonclinical data to date suggest that vamorolone may offer a much needed alternative to the current glucocorticoids which are standard of care for DMD,¹² with administration beginning around the age of 5 years in most developed countries, or even earlier in some cases.

The significant effects of glucocorticoids on growth and development, however, prevent their routine administration in infancy or ‘toddler’ years, despite evidence that the earlier the administration, the better the overall functional outcome.¹³ The cumulative side effects of glucocorticoids, including excess weight, delayed puberty, fragile skin, loss of bone mineral density, bruising, and Cushingoid appearance continue to negatively impact on the quality of life of the individual, leading to significant variations in clinical practice.¹⁴ Glucocorticoids also contribute to further muscle damage with long-term administration. Vamorolone has shown few if any of the side effects of traditional glucocorticoids in mouse models of DMD.^{11,15,16}

This study is targeted to explore whether vamorolone will show at least equal efficacy to glucocorticoids with a more favorable side effect profile, thereby improving the quality of life for DMD patients. This profile would enable use of vamorolone in DMD boys

who are at a younger age than when glucocorticoid treatment is currently initiated. In addition, vamorolone could be prescribed in later stage non-ambulant young men with DMD and for a longer period of time, where the risk:benefit balance of glucocorticoids is often less favorable.

Efficacy may also be improved over classic glucocorticoids in the longer term. In addition to the anti-inflammatory properties of vamorolone as a result of NF- κ B pathway inhibition, vamorolone may also improve efficacy over conventional glucocorticoids due to the lack of interference in the AKT1/FOXO pathway, a key feature of glucocorticoid therapy which leads in the long term to muscle wasting and atrophy.¹⁷ Further, vamorolone has been recently demonstrated to improve asynchronous remodeling, believed to be a component of progressive muscle weakness and wasting in DMD¹⁸ and may also prevent muscle membrane damage, thereby delaying progression of the disease further. Vamorolone is an antagonist to the mineralocorticoid receptor, whereas glucocorticoids are typically agonists. An antagonist for the mineralocorticoid receptor, epleronone, was recently shown to significantly improve DMD heart function.¹⁹ Finally, vamorolone imparts physical stability to myofiber plasma membranes, whereas prednisone destabilizes membranes. This property addresses the primary defect of membrane instability in dystrophin deficient myofibers in DMD.¹⁵

Potentially, the administration of vamorolone to a DMD patient may begin soon after birth to slow the dystrophic process of muscle, retaining regenerative capacity and substantially improving patient quality of life.

1.2 Nonclinical Experience

The safety pharmacology, pharmacokinetics (PK) and metabolism, and toxicology of vamorolone have been evaluated in multiple nonclinical studies *in vitro* and in mice, rats, beagle dogs, and cynomolgus monkeys *in vivo*.

1.2.1 Safety Pharmacology

Stunted growth is a significant side effect of chronic glucocorticoid use in children.^{20,21} Chronic treatment with glucocorticoids negatively affects bone growth and development and can cause osteoporosis.^{22,23}

The effect of vamorolone as compared to prednisolone on bone growth and development was evaluated in the *mdx* mouse model of DMD that lacks dystrophin due to a premature chain-terminating mutation in the mouse homologue of the dystrophin gene. In the pre-symptomatic *mdx* study, tibia length was measured to determine if vamorolone inhibited bone growth. Prednisolone significantly decreased tibia length whereas vamorolone did not affect tibia length at any concentration tested. Micro-computed tomography was performed on femurs to examine bone density and structure. Comparison of vehicle, prednisolone, and the highest vamorolone dose showed prednisolone to significantly reduce trabecular thickness compared to vehicle, while vamorolone did not.¹⁵

In normal, male CD-1[®] mice, these effects were reproduced. Unlike CD-1 mice treated with prednisolone, CD-1 mice receiving vamorolone did not experience tibia length shortening.¹⁶ However, at the highest vamorolone dose tested, mice did have significantly reduced body length, though to a lesser extent as compared to prednisolone.

Duchenne muscular dystrophy is associated with cardiomyopathy that can become life threatening, and increased fibrosis with prednisone treatment in heart muscle of the *mdx* mouse has been reported.²⁴ Histologically, clear fibrosis was evident in 50% of young (8-week) prednisolone-treated mouse hearts compared to no incidence of fibrosis identified in the other groups (wild type; *mdx* vehicle, and vamorolone -treated).

Pharmacologically, glucocorticoids show immunosuppressive and immunotoxic properties that limit therapeutic windows and long-term use. Vamorolone (5, 15, 30 mg/kg/day) was benchmarked against prednisolone (5 mg/kg/day) to determine if similar properties were observed.¹⁵ Untreated *mdx* mice showed increased numbers of peripheral blood leukocytes (PBL) and enlarged spleens resulting from ongoing muscle damage compared to wild type mice. Vamorolone treatment reduced spleen mass and PBL

counts in a dose-dependent manner. This finding is attributed to a reduction in muscle damage by vamorolone that decreases spleen size to levels resembling those in wild type mice. Prednisolone reduced these measures below wild type, suggesting immunosuppressive and/or immunotoxic properties. Further, prednisolone significantly decreased viable splenocytes per gram of tissue ($p < 0.005$), whereas this decrease was not observed for any vamorolone dose tested (ReveraGen Report No. MDX-RBP-VBP15-02).¹⁵

To further query the potential immune modulation, the effects of vamorolone and prednisolone on counts of splenic B and T-lymphocytes isolated from treated *mdx* mice were examined. CD4+ T-cell activation was assayed by stimulation of splenocytes with the T-cell mitogen, concanavalin A (ConA). Splenocytes obtained from prednisolone-treated mice displayed a significant reduction of the percentage of splenic activated CD4+CD25+ T-cells upon ConA stimulation while splenocytes derived from vamorolone-treated mice did not (ReveraGen Report No. MDX-RBP-VBP15-02).

Taken together, these findings suggest that while prednisolone treatment leads to a reduction in T-cell number and activation status, vamorolone modulates inflamed *mdx* immune systems towards a wild type state without compromising T-cell activation status.

1.2.2 Pharmacokinetics and Metabolism

1.2.2.1 Single Dose

Vamorolone PK profiles were determined in male CD-1 mice, Sprague Dawley rats and beagle dogs after a single intravenous injection of 10 mg/kg and after a single oral dose of 50 mg/kg in mice and rats and 30 mg/kg in dogs.

Pharmacokinetic results for vamorolone following a single intravenous administration of 10 mg/kg in Crl:CD1(ICR) mice demonstrated a clearance (CL) of 18.8 mL/min/kg. The terminal half-life ($t_{1/2}$) was 0.35 hours. Volume of distribution at steady state (V_{ss}) was 0.76 L/kg. Following oral administration of 50 mg/kg in mice, the maximum observed plasma concentration (C_{max}) of 6787 ng/mL was observed at 2 hours (time to maximum observed plasma concentration [T_{max}]) after drug administration, and percent

bioavailability (F%) was 74.5%. Following oral administration of 15 mg/kg via cherry syrup, the C_{\max} of 1527 ng/mL was observed at 2 hours after drug administration and bioavailability was 47.7% (ReveraGen Report No. PH-DPMK-VBP-10-004).

Pharmacokinetic results for vamorolone following a single intravenous administration of 50 mg/kg in Sprague Dawley rats indicated a CL of 20.2 mL/min/kg. The $t_{1/2}$ was 0.58 hours. V_{ss} was 0.77 L/kg, which was similar to that observed in mice. After oral administration of 50 mg/kg in rats, a C_{\max} of 2543 ng/mL was observed at 4 hours after dose administration, and bioavailability was 47.8% (ReveraGen Report No. PH-DPMK-VBP-10-007).

In beagle dogs, vamorolone had a CL of 24.7 mL/min/kg. The $t_{1/2}$ was 5.42 hours and V_{ss} was 1.93 L/kg. After oral administration of 30 mg/kg in dogs, a C_{\max} of 814 ng/mL was observed at 6 hours after dose administration and bioavailability was 53.2% (ReveraGen Report No. 48504-10-464).

Vamorolone clearance was therefore comparable in all 3 species studied (19-25 mL/min/kg). Bioavailability ranged from approximately 50% in mouse (cherry syrup), rat, and dog to 75% in the mouse (30% Labrafil) (ReveraGen Report Nos. PH-DPMK-VBP-10-004, PH-DPMK-VBP-10-007, 48504-10-464).

1.2.2.2 Multiple Dose

Cr1:CD1(ICR) mice were administered vamorolone or vehicle once daily for 28 consecutive days. Vamorolone exposure (as assessed by the C_{\max} and area under the concentration-time curve [AUC]) increased with increasing dose on Study Days 1 and 28. Repeated dosing of vamorolone over a 28-day duration was associated with decreases in mean vamorolone AUC_{last} values in the 30 and 100 mg/kg dose groups compared to Day 1, indicating possible enzyme induction. On Study Day 28, mean AUC_{last} values were 1.81-fold and 5.02-fold lower compared to Study Day 1 for the 30 and 100 mg/kg dose groups, respectively. The observed difference in exposure relative to Day 1 increased with the increase in administered dose of vamorolone (ReveraGen Report No. 1998-009).

Beagle dogs were either administered vamorolone or vehicle once daily for 28 consecutive days. Vamorolone exposure in dogs (as assessed by C_{max} and AUC_{last}) generally increased with increasing dose on Study Days 1 and 28. For the 2 and 10 mg/kg dose groups, exposure on Day 28 was generally higher than on Day 1, indicating possible inhibition of metabolism of vamorolone at these dose levels. On Day 28, mean AUC_{last} values were 2.35-fold and 2.43-fold (males) and 3.03-fold and 3.23-fold (females) higher compared to Study Day 1 for the 2 and 10 mg/kg/day dose groups, respectively. For the 50 mg/kg dose group, exposure on Day 28 was similar to that on Day 1. At the 50 mg/kg dose, AUC_{last} values in males were 1.71-fold lower whereas females were 1.22 higher on Day 28 compared to Day 1 (ReveraGen Report No. 031302).

Non-naïve cynomolgus monkeys were administered vamorolone (300 and 600 mg/kg/day) or vehicle once daily for 7 consecutive days. Vamorolone exposure (as assessed by C_{max} and AUC_{last}) generally increased with increasing dose on Study Days 1 and 7 with the exception of male monkeys on Day 7, which showed no clear increase in exposure between the 300 and 600 mg/kg/day dose levels. Repeated dosing over the 7-day study duration was associated with decreases in mean plasma vamorolone AUC_{last} values for female and male monkeys indicating possible metabolic induction. On Day 7, mean AUC_{last} values were 1.60-fold, 2.19-fold, and 2.02-fold lower in females and 1.20-fold, 2.09-fold, and 2.88-fold lower in males compared to Study Day 1 for the 100, 300 and 600 mg/kg/day dose groups, respectively (ReveraGen Report Nos. 1998-001, SW11-0418).

1.2.2.3 *Distribution*

In the plasma protein binding studies, percent bound was similar in human and mouse cells in culture (88.06% and 86.71%, respectively). In the blood partition experiment done *ex vivo*, the blood to plasma ratio was similar between human and mouse (0.87 and 0.68, respectively), but the red blood cell to plasma ratio for the mouse (0.33) was less than half that of the human (0.74). Human *in vivo* data are presented in [Section 1.3](#) (VBP15-001). In the blood/brain concentration mouse experiment *in vivo*, the plasma

concentrations of vamorolone were higher than brain concentrations with the AUC and C_{max} approximately 2-fold higher in plasma than in brain (ReveraGen Report Nos. ADME-NCG-PPB-NC135, ADME-VBP-PPB-V002, ADME-NCG-BP-NC134, NCATS 2013-38).

1.2.2.4 Metabolism

The *in vitro* and *in vivo* data demonstrate that vamorolone can be metabolized via multiple metabolic pathways, including glucuronidation, hydroxylation, and reduction. Glucuronidation appeared to be the major metabolic pathway in human cells *in vitro*. All metabolites observed in human *in vitro* were observed in monkey *in vitro*. Most human metabolites identified *in vitro* were also found in mouse and dog. Thus, there is no unique human metabolite identified for vamorolone.

The metabolic stability of vamorolone was assessed in non-Good Laboratory Practice (GLP) studies. Based on the data generated, vamorolone was highly stable for up to 60 minutes in human, monkey, dog, and mouse liver microsomes in the presence or absence of nicotinamide adenine dinucleotide phosphate (NADPH) and stable for up to 60 minutes in rat liver in the absence of NADPH. Moderate metabolism was apparent in rat liver microsomes in the presence of NADPH stimulation (35% remaining), suggesting that rat was a high metabolizer of vamorolone relative to other species (mouse, dog, human) (ReveraGen Report Nos. NIH-R2526, and ADME-VBP-LM-V003).

Vamorolone did not significantly inhibit any of the cytochrome P450 (CYP) enzyme isoforms tested (CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4). Vamorolone moderately induced CYP3A4 (24% to 42%), indicating that vamorolone is a potential inducer of CYP3A4 (ReveraGen Report Nos. ADME-VBP-Inhibition-V005, ADME-VBP-Induction-V006, ADME-VBP-Induction-V009).

1.2.2.5 Excretion

Vamorolone showed high plasma clearance in rats but, consistent with the extensive metabolism in hepatocytes from this species, the biliary and urinary excretion of the parent compound was low with an average of <0.05% of the dose recovered in bile and

approximately 0.1% in urine. Overall, vamorolone showed high plasma clearance and extremely low biliary and urinary excretion (ReveraGen Report No. NCATS 2013-44).

1.2.3 Toxicology

1.2.3.1 Single Dose

Crl:CD1(ICR) mice were administered vamorolone once via oral gavage at 50, 125, 250, and 500 mg/kg and observed for abnormalities. All animals survived to their scheduled termination, and there were no significant abnormalities observed. There was a slight decrease in body weight attributed to vamorolone in both males and females at doses above 125 mg/kg. A dose dependent decrease in food consumption related to vamorolone was also observed in males and females. No other abnormalities were observed in experimental mice (ReveraGen Report No. 1998-002).

Beagle dogs received single 60, 180, 360, and 750 mg/kg doses of vamorolone using an escalation study design with a 4-day washout period between doses. All animals survived dose escalation. Clinical signs attributed to vamorolone (750 mg/kg) included red discoloration of the ears and face. This effect occurred within a few hours of dosing and was transient. The highest dose also resulted in increased white blood cell count (increased neutrophils and monocytes [female only] and decreased lymphocytes and eosinophils [male and female]). At the 360 and 750 mg/kg dose levels, slight elevations in albumin were observed. A mild elevation in cholesterol at the 750 mg/kg (and possibly 360 mg/kg) dose level was also observed (ReveraGen Report No. 13788.01.01).

In cynomolgus monkeys, single oral doses of up to 500 mg/kg were well tolerated with no significant abnormalities observed (ReveraGen Report No. 1998-001).

1.2.3.2 Multiple Dose

Vamorolone or vehicle was administered to Crl:CD1(ICR) mice once daily for 28 consecutive days at doses of 10, 30 and 100 mg/kg/day. All animals survived to their scheduled necropsy with the exception of a female mouse (100 mg/kg/day dose group)

that was found dead on Day 16. The cause of death was considered incidental and attributed to a dosing injury based on the amount of red fluid in the thoracic cavity.

No effects attributable to vamorolone were observed in food consumption, ophthalmic examination, or urinalysis during the study. Dose-dependent decrease in body weight gain was observed at all doses; however, weight was fully regained during the recovery period. Adrenal gland weights were variable between groups and generally decreased, but without a dose response relationship, and correlated microscopically with minimal to moderate vacuolar degeneration and cortical atrophy. After the 2-week recovery period there was evidence of vacuolar degeneration of the adrenal gland. Liver weights were significantly increased at the 100 mg/kg/day dose level. Hepatocellular hypertrophy, increased vacuolation, and necrosis (single cell) were seen in a few male mice at 30 mg/kg/day. There was evidence of lipid and glycogen accumulation. Serum alanine aminotransferase and aspartate aminotransferase levels were higher with associated microscopic hypertrophy/vacuolation/necrosis at 100 mg/kg/day. Spleen weights decreased in a dose-dependent manner and correlated with a decreased number of lymphocytes in spleen. Thymus weights decreased in a dose dependent manner and were associated microscopically with lymphoid atrophy. Mice had dose-dependent reductions in serum lymphocytes which were significant in the 100 mg/kg dose group. After the recovery period, all parameters returned to normal (untreated) except for thymus weights, which were increased.

Based on the liver-related findings in this study, the no observed adverse effect level (NOAEL) for vamorolone in mice is 30 mg/kg/day (ReveraGen Report No. 1998-009).

A GLP-compliant study was carried out to evaluate the toxicity of the test article, vamorolone, in Crl:CD1[®] (ICR) mice after administration for 26 weeks, including evaluation of the reversibility, progression, or delayed appearance of any observed changes following a 4-week post-dose observation period. Assessment of toxicity was based on mortality, clinical observations, body weight, and food consumption; ophthalmoscopic examinations; and clinical and anatomic pathology. Toxicokinetic assessment was conducted for the test article.

There were no vamorolone-related effects on mortality, detailed clinical observations, food consumption, ophthalmology, sperm evaluations, or bone lengths (femur or tibia).

Five test article-treated mice were unscheduled deaths (euthanized *in extremis* or found dead) during the dosing phase. Three of these were considered to be potentially due to dosing injury based on microscopic findings in mediastinum, epicardium, or lung. One of these unscheduled deaths was attributed to moderate progressive nephropathy; a spontaneous background finding. The death of one male at the 5 mg/kg/day dose was undetermined since there were no major pathologic findings to explain the unscheduled death of this animal; there was no target organ toxicity in the mouse. Target organ toxicity was not considered a contributor to the death of these animals and there was no dose-relationship in incidence.

A vamorolone-related increase in body weight gain was observed relative to controls in males (+14%) and females (+23%) at the 45 mg/kg/day dose. Increases in body weights at 45 mg/kg/day were not considered to be adverse due to the general health of the animals overall. During the recovery phase, bodyweights in males returned to comparable levels as controls, however female body weights remained increased compared to female controls.

Evidence of a minimal to mild vamorolone-related hepatic effects were observed in males at doses ≥ 5 mg/kg/day and females at 45 mg/kg/day, indicated by mild to moderate increases in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase activities, and/or total bilirubin, as related to microscopic hepatocellular vacuolation, inflammation, and/or necrosis in males at ≥ 15 mg/kg/day and females at 45 mg/kg/day; these changes had generally resolved at recovery collections with the exception of minimal increases in alanine aminotransferase activity in females at 45 mg/kg/day, which may have correlated to microscopic liver pathology.

A mild vamorolone-related increase in neutrophil counts was observed in both sexes at 45 mg/kg/day with concurrent decreases in lymphocyte counts in females at 45 mg/kg/day consistent with a glucocorticoid-like effect, as related to microscopic

lymphoid depletion. However, an inflammatory stimulus may have contributed to increases in neutrophil counts, as related to microscopic liver inflammation; these changes had generally resolved at recovery collections.

A mild vamorolone -related decrease in chloride was observed in males at doses ≥ 5 mg/kg/day and females at doses ≥ 15 mg/kg/day that lacked correlative findings among other study endpoints; resolution for this observation could not be determined.

A mild vamorolone -related increase in albumin was observed in males at doses ≥ 5 mg/kg/day and females at 45 mg/kg/day with concurrent mild increases in globulin in females at 45 mg/kg/day; these changes had resolved at recovery collections.

A minor vamorolone -related alteration in lipid metabolism was observed in both sexes at the 45 mg/kg/day dose and females at the 15 mg/kg/day dose indicated by increases in triglyceride and/or cholesterol; these changes had resolved at recovery collections.

Vamorolone-related macroscopic findings occurred in the liver of mice at the 45 mg/kg/day dose. Tan discoloration occurred in one female and four males at this dose in the dosing phase. This correlated with microvesicular/macrovesicular hepatocyte vacuolation. There were no test article-related macroscopic findings in recovery animals.

Test article-related microscopic findings occurred in adrenal gland (cortical atrophy with correlating decreases in adrenal weights in females), liver (increased severity of centrilobular hypertrophy; hepatocyte vacuolation; and inflammation/necrosis), lymphoid tissues (thymus, spleen, mandibular lymph node, mesenteric lymph node, and gut associated lymphoid tissue [GALT]) skin, and pancreatic islets (minimal to mild hypertrophy). Observed changes in these tissues are considered pharmacologically-mediated and not adverse.

An increased incidence of decreased anagen hair follicles occurred in mice at the 45 mg/kg/day dose. Decreased anagen hair follicles were documented for individual animals when there were no anagen hair follicles in the section of skin. Incidence in controls and mice at the 5 and 15 mg/kg/day doses were similar. A severity score was

not given to the decrease as this may have been somewhat dependent on size of skin sample. This change is not considered adverse.

There was full reversibility of lymphoid changes in thymus, spleen, mesenteric lymph node, mandibular lymph node, and GALT. There no meaningful differences between treated animals and controls at the end of the recovery tissues for these lymphoid tissues. There was recovery of adrenal gland findings in females and partial recovery of adrenal gland findings in males. In addition, there was partial reversibility of liver findings for males and females. Minor changes persisted in the pancreas and skin.

Systemic exposure to vamorolone appeared to be sex-dependent on Day 1 (males > females) and appeared to be independent of sex on Day 179. Following daily administration of vamorolone in females and males, systemic exposure (AUC_{0-24hr}) and C_{max} values of vamorolone increased with increasing dose in a greater than dose-proportional manner on Day 1 and in an approximately dose-proportional manner on Day 179. Systemic exposure to vamorolone in females appeared to increase following repeated administration of vamorolone at 5 mg/kg/day, did not appear to change following repeated administration of vamorolone at 15 mg/kg/day, and appeared to decrease following repeated administration of vamorolone at 45 mg/kg/day. Systemic exposure to vamorolone in males appeared to decrease following repeated administration of vamorolone.

The once daily administration of vamorolone via oral gavage to mice for 26 weeks at 5, 15, and 45 mg/kg/day did not produce any adverse effects. Therefore, the NOAEL is considered to be 45 mg/kg/day under the conditions of this study.

Vamorolone or vehicle was administered to beagle dogs once daily for 28 consecutive days at doses of 2, 10 and 50 mg/kg/day. All animals survived to their scheduled termination and no effect of vamorolone was noted on gross visual inspection, body weight, body temperature, food consumption, ophthalmology, electrocardiography or urinalysis parameters at necropsy. A dose-dependent decrease in the expected normal

body weight gain was observed at all doses but weights generally increased to a normal level during the recovery period.

Adrenal gland weights decreased with vamorolone treatment, which correlated with mild or moderate diffuse bilateral atrophy of the adrenal cortex, mild multifocal bilateral vacuolation of the adrenal cortex, increased white blood cell and neutrophil counts, and decreased eosinophil counts. Liver weights increased in the 50 mg/kg/day dose group, which correlated with diffuse hypertrophy and vacuolation and increased levels of alkaline phosphatase and gamma glutamyltransferase. Spleen weights decreased, which correlated with lymphoid depletion. Thymus weights decreased, which corresponded to diffuse lymphoid depletion. With the exception of diffuse depletion of lymphocytes in thymus in the 50 mg/kg group, all abnormal parameters returned to normal during the recovery period.

The NOAEL was considered by the study director to be 10 mg/kg/day. Although reversible, the liver changes were considered adverse at 50 mg/kg/day because the severity score was moderate and the changes were diffuse in nature in all animals treated at the high dose. This determination is in contrast to the conclusion drawn by the study pathologist, who considered the NOAEL to be 50 mg/kg/day due to reversibility following cessation of dosing (ReveraGen Report No. 31302).

Vamorolone or vehicle was administered to beagle dogs once daily for 39 weeks at doses of 2, 10 and 50 mg/kg/day. Six dogs of each sex received each dose or placebo, and two of the six dogs of each sex at each dose or placebo were followed for an additional 4 weeks to evaluate reversibility, progression, or delayed appearance of any observed changes. One male dog who received 50 mg/kg/day was euthanized in extremis on Day 273 due to paraphimosis (an extended penis). All other animals survived to their scheduled termination.

Detailed clinical observations considered test article-related at 50 mg/kg/day, and reversible included decreased activity (considered adverse), struggling during dosing, feces soft, limb function impaired, interdigital cysts, and unkempt appearance

(considered adverse). Test article-related, dose-dependent increases in body weight gains correlating with increases in food consumption were observed relative to controls in males at all dose levels and in females at 10 and 50 mg/kg/day. Test article-related, reversible increases in average mean food consumption, relative to controls, over the course of the 39-week dose phase were observed in both sexes at 10 and 50 mg/kg/day.

No test article-related ophthalmological effects were noted. No test-article-related changes were noted in respiratory rates or rectal temperatures. There may have been a mild dose related reversible increase in the heart rate at the terminal post-dose interval that was significantly different from vehicle in both sexes following the 50 mg/kg/day dose. Semen analysis/evaluation for test article effects could not be conducted as there were not enough viable samples collected.

Test article-related effects on clinical pathology endpoints with microscopic correlates included the following:

- A hepatocellular and hepatobiliary effect in males at 10 mg/kg/day and both sexes at 50 mg/kg/day, which included increased alkaline phosphatase, gamma glutamyltransferase, alanine aminotransferase and aspartate aminotransferase activity. These changes correlated with microscopic changes in the liver, bile duct and gall bladder. This spectrum of changes was considered adverse in both sexes at 50 mg/kg/day.
- There was also evidence of an inflammatory response in both sexes at 50 mg/kg/day, which included increased total leukocyte, neutrophil and monocyte counts, and increased fibrinogen and/or globulin concentrations. The inflammatory response was likely secondary to inflammation in the liver associated with hepatocellular necrosis. Platelet counts were also increased in both sexes at 50 mg/kg/day and may have been secondary to the inflammatory response.

Following a 4-week recovery period, all noted clinical pathological changes resolved, with the exceptions of increased alanine aminotransferase activity in both sexes at 50 mg/kg/day, and increased globulin in males at 50 mg/kg/day.

Reversible, test article-related macroscopic findings included mildly to moderately enlarged livers in males and females at 50 mg/kg/day, which correlated microscopically with panlobular hepatocellular hypertrophy and/or hepatocellular vacuolation; hemorrhage in the gall bladder of one 50 mg/kg/day female, that was associated with moderate acute inflammation and mild vascular necrosis, and considered to be adverse; red focus/foci within the pylorus of the stomach of one 50 mg/kg/day female and one male at 10 mg/kg/day, which correlated microscopically with mild acute inflammation in the female.

Test article-related organ weight changes at the terminal necropsy included decreases in adrenal gland weights in both sexes at ≥ 2 mg/kg/day (microscopic correlate of bilateral cortical atrophy); increases in liver weights in both sexes at ≥ 10 mg/kg/day (microscopic correlates of panlobular hepatocellular hypertrophy and/or hepatocellular vacuolation); increases in kidney weights in females at ≥ 10 mg/kg/day and males at 50 mg/kg/day (microscopic correlate of bilateral tubular vacuolation); decreases in prostate gland weights in males at 50 mg/kg/day (microscopic correlate of decreased secretory product). These organ weight changes were all reversible, except for the decreases in the prostate gland. Microscopic evaluation revealed the following test article-related changes: adrenal glands (atrophy of the zona fasciculata and zona reticularis and hypertrophy/hyperplasia of the zona glomerulosa in both sexes at ≥ 10 mg/kg/day and atrophy was considered adverse); esophagus and pylorus of the stomach (erosion/ulceration in a few animals of both sexes at 50 mg/kg/day); gallbladder (hypertrophy/hyperplasia of the mucosal epithelium in both sexes at ≥ 10 mg/kg/day and cytoplasmic vacuolation of the mucosal epithelium in males at ≥ 10 mg/kg/day and females at ≥ 2 mg/kg/day); liver (hepatocellular vacuolation in males at ≥ 10 mg/kg/day and females at ≥ 2 mg/kg/day, panlobular hypertrophy in males at 50 mg/kg/day and females at ≥ 10 mg/kg/day, and inflammation/necrosis in both sexes at 50 mg/kg/day and considered adverse, bile duct hyperplasia in both sexes at 50 mg/kg/day, bile duct hypertrophy in males at 50 mg/kg/day and females at ≥ 10 mg/kg/day, and cytoplasmic vacuolation of the bile duct epithelium in both sexes at ≥ 10 mg/kg/day); kidneys (bilateral tubular vacuolation in males at 50 mg/kg/day and females at ≥ 10 mg/kg/day

and an increased incidence of bilateral basophilic tubules in males and females at 50 mg/kg/day); lymphoid depletion in both sexes at 50 mg/kg/day in mandibular and mesenteric lymph nodes, thymus and spleen (with extramedullary hematopoiesis in 50 mg/kg/day females); bone marrow in the sternum (increased adipocytes in males at ≥ 2 mg/kg/day and females at 50 mg/kg/day); testes (spermatocyte/spermatid degeneration in males at 50 mg/kg/day); epididymides (oligospermia/germ cell debris in males at 50 mg/kg/day); ovaries (absent corpora lutea in females at ≥ 2 mg/kg/day and considered adverse); the mammary gland and other tissues in the female reproductive tract (uterus, cervix, and vagina) of these animals were consistent with animals that have not ovulated; vacuolation in the epithelium of the mammary gland duct in females at 50 mg/kg/day; parotid salivary gland (cytoplasmic alteration in both sexes at ≥ 10 mg/kg/day); biceps femoris (atrophy of the skeletal muscle in both sexes at 50 mg/kg/day); skin (atrophy and alopecia/hypotrichosis in males at 50 mg/kg/day and females at ≥ 10 mg/kg/day); prostate gland (decreased secretory product in males at 50 mg/kg/day); thyroid glands (bilateral increased colloid in males at ≥ 10 mg/kg/day).

Many of the findings were felt by the Study Director to be consistent with the pharmacology of the test article including cortical atrophy of the adrenal glands (affecting the zona fasciculata and reticularis), generalized lymphoid depletion in lymphoid tissues (thymus, spleen, and lymph nodes), increased adipocytes in the bone marrow, atrophy of the skeletal muscle, alopecia/hypotrichosis and atrophy of the skin (thinning of the dermal collagen and atrophy of hair follicles and adnexa), an absence of corpora lutea in the ovary (likely indicative of delayed puberty), decreased secretory product in the prostate gland, and multiple changes in the liver. The liver had panlobular hypertrophy and vacuolation of hepatocytes consistent with glycogen accumulation. Due to the magnitude of hypertrophy and vacuolation, there were (likely secondary) foci of hepatocellular necrosis and inflammation.

Test article-related microscopic findings at the recovery necropsy were present in the adrenal glands, liver, gallbladder, kidneys, stomach (pylorus), female reproductive tract (ovaries), male reproductive tract (testes, epididymides, prostate gland), mesenteric lymph node, skeletal muscle (biceps femoris), and parotid salivary gland.

The No Observed Adverse Effect Level was 2 mg/kg/day for males; a No Observed Adverse Effect Level was not observed for females (ReveraGen Report No. 1998-014).

Non-naive cynomolgus monkeys were administered vamorolone or vehicle once daily for 7 consecutive days at doses of 100, 300, and 600 mg/kg. All animals survived until the end of the study period. There were effects on clinical observations, food consumption, and urinalysis attributable to vamorolone that are described below.

There was a dose proportional decrease in body weight gain observed in males and females at each dose (up to 11% and 9% respectively) related to vamorolone. A cessation of the body weight loss in treatment was observed during the recovery phase but no recovery of body weight lost during the 7 days of dosing was observed.

At termination there were nonsignificant increases in red cell mass and decreases in lymphocytes (up to 56%) in the 600 mg/kg/day dose group. However, most individual animals, including controls, had decreases in lymphocytes (up to 81%) at termination relative to their respective pretest. They had resolved by the recovery interval in both sexes.

In both sexes receiving ≥ 300 mg/kg/day, there was increased urea nitrogen (up to 141%), creatinine (up to 58%), total protein (up to 15%), albumin (up to 11%), globulin (up to 25%), and/or potassium (up to 39%) with concurrent decreases in sodium (up to 10%) and chloride (up to 10%) relative to controls. At the recovery interval, the majority of these effects had resolved (ReveraGen Report No. 1998-001).

1.2.3.3 Genotoxicity

The mutagenic and genotoxic potential of vamorolone was assessed in several assays. A non-GLP Ames screen was negative for bacterial mutations (ReveraGen Report No. BIO-VBP-001-AMES). In a GLP Ames test, no background lawn toxicity was observed; however, a reduction in revertant counts was observed (ReveraGen Report No. AD79DT.502ICH.BTL). Vamorolone was negative for inducing chromosomal aberrations in cultured mouse lymphocytes without and with metabolic activation (ReveraGen Report No. AD79DT.704.BTL).

Femoral bone marrow was microscopically evaluated for the presence of polychromatic erythrocytes (PCEs) containing micronuclei. No significant reductions in the PCEs/EC (total erythrocytes) ratio were observed in the vamorolone groups compared to the vehicle control group. Although statistically significant increases in the incidence of micronucleated PCEs in the vamorolone treated groups were observed, no dose response was observed with respect to other groups and the values of micronuclei for the individual animals were within the historical range. Therefore, the statistically significant increase was considered as biologically insignificant (ReveraGen Report No. AD76BK.123012ICH.BTL).

A study was performed to evaluate the potential mutagenicity of two theoretical epoxide impurities related to the drug substance vamorolone (formerly VBP15), which is a steroid-like structure containing a delta 9,11 double bond. The delta 9,11 epoxide structures evaluated were VBP15-B-3, which is structurally similar to vamorolone except for the epoxide moiety, and VBP15-B-2, which has a 21-acetate substitution (vamorolone and VBP15-B-3 contain a 21-hydroxy moiety). Two validated and complementary *in silico* prediction methodologies were used for assessing mutagenic potential. The statistics-based quantitative structure-activity relationship (QSAR) program MultiCASE CASE Ultra was used, employing four different modules (GT1_A7B, GT1_AT_ECOLI, PHARM_ECOLI, and PHARM_SAL) designed to cover a wide range of molecular substructures collected from both proprietary and public compounds. In addition, the expert rule-based SAR program Derek Nexus was used to determine if the theoretical impurities contained structural alerts associated with known genotoxicants. CASE Ultra predicted both VBP15-B-2 and VBP15-B-3 as negative for mutagenicity [ReveraGen Report “In Silico Mutagenicity Evaluation of Delta 9,11 Epoxide Structures of VBP15: VBP15-B-2 (21-Acetate) and VBP15-B-3 (21-Hydroxy)”].

Taken together, these data indicate vamorolone has not generated a mutagenic signal based on these simulations.

1.3 Clinical Experience

1.3.1 Phase I Study in Healthy Adult Volunteers

Clinical experience is limited to a single Phase I clinical trial of vamorolone in healthy adult volunteers (VBP15-001). This study evaluated the safety, tolerability, and PK of vamorolone in a Phase I randomized, placebo-controlled, double-blind, single ascending dose (SAD) and multiple ascending dose (MAD) study. In the SAD portion of the study, Cohorts 1 through 5 and Cohort 7 were comprised of eight subjects each; six subjects in each cohort received a single oral dose of vamorolone (0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg, 8.0 mg/kg, and 20 mg/kg, respectively) and two subjects in each cohort received placebo under fasted conditions. In Cohort 6, six subjects received a single oral dose of 8.0 mg/kg vamorolone within 30 minutes of beginning a high fat/high calorie meal. The MAD portion of the study had four cohorts (6 drug, 2 placebo in each) receiving 14 daily doses of vamorolone (1.0, 3.0, 9.0 and 20.0 mg/kg/day). The clinical conduct for all seven SAD cohorts, and all four MAD cohorts has been completed; data analysis is ongoing.

The primary objectives of the Phase I study were to evaluate the safety and tolerability of single and multiple oral doses of vamorolone, and to evaluate the PK of single doses and multiple doses of vamorolone. A secondary objective was to evaluate the effect of food on the absorption and PK of vamorolone. Other objectives were to obtain samples from subjects on Day 1 (pre-dose) and Day 14 of the MAD cohorts for use in Metabolites in Safety Testing (MIST) assessments, and to test back-up PK samples from a subset of MAD subjects for pharmacodynamic (PD) biomarkers.

1.3.1.1 SAD Cohorts

1.3.1.1.1 SAD Cohorts – Pharmacokinetics Fasted

Vamorolone PK data shows strong adherence to dose linearity and dose proportionality, with relatively little subject-subject variation (**Figure 1**, **Table 1**, **Figure 2**). The half-life was about 2 hours for doses 0.1-1.0 mg/kg. Doses at 3.0, 8.0 and 20.0 mg/kg showed an extended tail, increasing half-life to 2.5, 3.8 and 3.8 hours, respectively (**Figure 1**).

Figure 1: Arithmetic mean \pm standard error plasma concentrations of vamorolone (VBP15) after oral administration of single doses of 0.1, 0.3, 1, 3, 8, and 20 mg/kg to healthy subjects under fasted conditions - linear plot (top); semi-logarithmic plot (bottom)

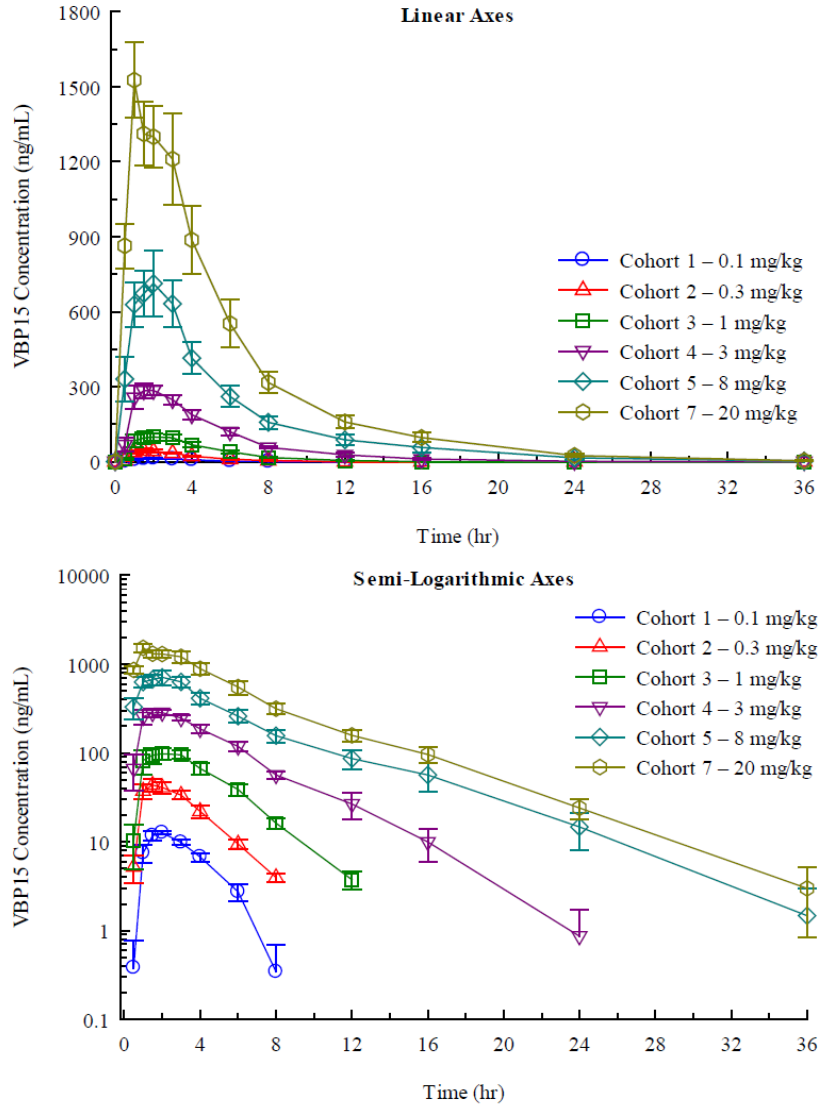
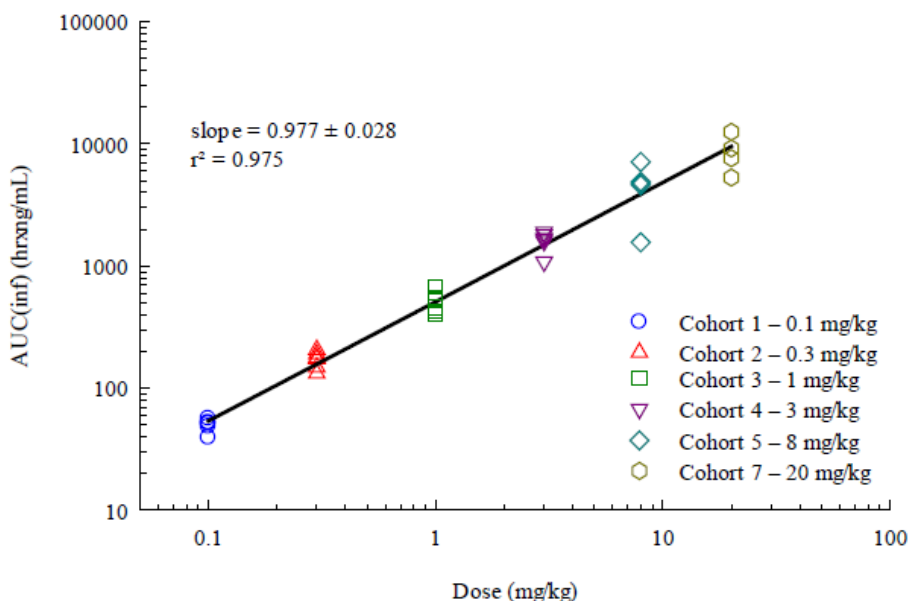


Table 1: Summary of pharmacokinetic parameters for vamorolone after oral administration of single doses of 0.1, 0.3, 1.0, 3.0, 8.0, and 20.0 mg/kg to healthy subjects under fasted conditions

Parameter*	Dose					
	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg	8 mg/kg	20 mg/kg
C _{max} (ng/mL)	13.1 (12.8) (6)	50.8 (16.5) (6)	122 (32.8) (6)	305 (24.4) (6)	718 (42.5) (6)	1,648 (16.7) (6)
T _{max} (hr)	1.50 (6) [1.50 – 2.03]	1.50 (6) [1.00 – 3.00]	1.75 (6) [1.00 – 3.00]	1.75 (6) [1.00 – 2.00]	1.78 (6) [1.00 – 2.00]	1.50 (6) [1.00 – 3.03]
AUC _(0-t) (hr×ng/mL)	41.9 (16.8) (6)	161 (15.9) (6)	486 (19.7) (6)	1,578 (20.7) (6)	3,997 (55.0) (6)	8,545 (29.5) (6)
AUC _(inf) (hr×ng/mL)	49.5 (12.5) (6)	170 (16.5) (6)	500 (19.2) (6)	1,600 (20.3) (6)	4,137 (62.1) (5)	8,277 (37.2) (4)
λ _z (1/hr)	0.4064 (12.4) (6)	0.4376 (16.5) (6)	0.3829 (18.0) (6)	0.2792 (18.5) (6)	0.1823 (52.3) (5)	0.1827 (11.9) (4)
t _{1/2} (hr)	1.71 (12.4) (6)	1.58 (16.5) (6)	1.81 (18.0) (6)	2.48 (18.5) (6)	3.80 (52.3) (5)	3.79 (11.9) (4)
CL/F (L/hr/kg)	2.02 (12.5) (6)	1.76 (16.5) (6)	2.00 (19.2) (6)	1.88 (20.3) (6)	1.93 (62.1) (5)	2.42 (37.2) (4)
V _z /F (L/kg)	4.97 (6.06) (6)	4.03 (19.5) (6)	5.22 (19.1) (6)	6.72 (30.5) (6)	10.6 (57.8) (5)	13.2 (32.4) (4)

C_{max} = maximum observed plasma concentration; T_{max} = time to maximum observed plasma concentration; AUC_(0-t) = area under concentration-time curve from time 0 to time t; AUC_(inf) = area under concentration-time curve from time 0 to infinity; λ_z = elimination rate constant; t_{1/2} = terminal half-life; CL/F = apparent total clearance from plasma; V_z/F = apparent volume of distribution during terminal phase.

Figure 2: Relationship between individual subject vamorolone AUC_(inf) and dose after oral administration of single doses of 0.1, 0.3, 1, 3, 8, and 20 mg/kg to healthy subjects under fasted conditions



1.3.1.1.2 SAD Cohorts – Pharmacokinetics Fed

For the food effect group, a high fat meal (45 grams fat) was given to a cohort of Phase I SAD volunteers with the 8.0 mg/kg dose of vamorolone. These data were then compared to the fasted 8.0 mg/kg cohort data. This showed that absorption was increased by 2.5-fold by the high fat meal, consistent with the lipophilic character of vamorolone (steroidal compound) (Figure 3, Table 2).

Figure 3: Arithmetic mean \pm standard error plasma concentrations of vamorolone (VBP15) after single dose oral administration of 8 mg/kg to healthy subjects under fed and fasted conditions - linear (top panel) and semi-logarithmic (bottom panel) axes

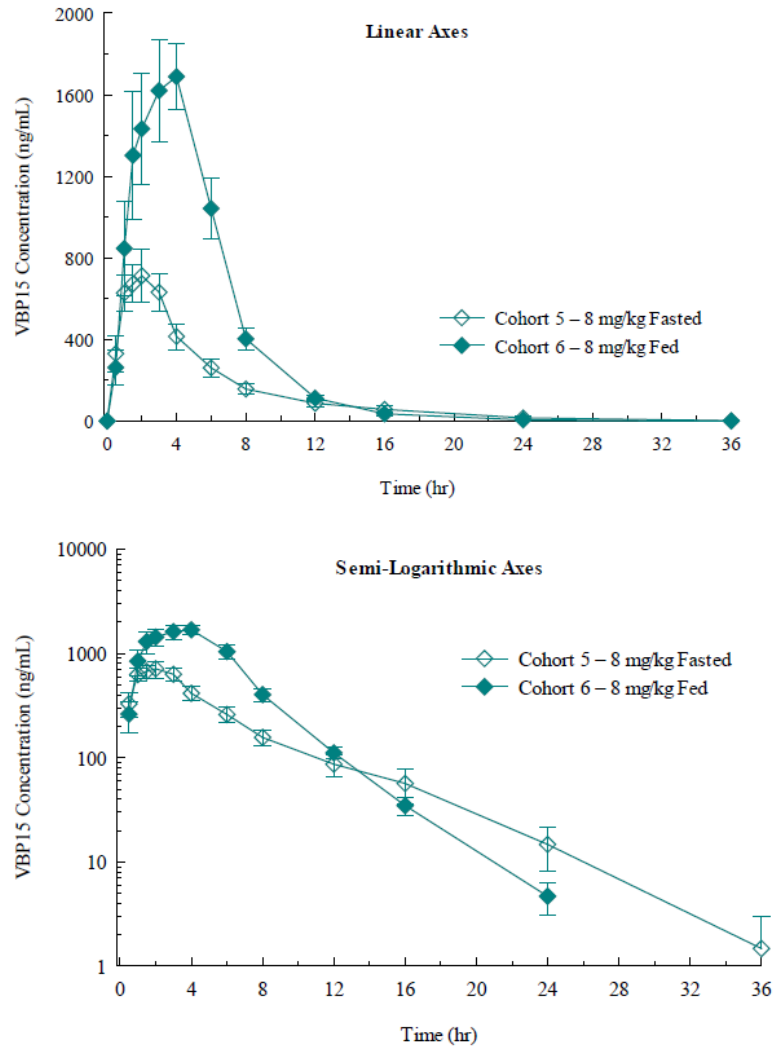


Table 2: Summary of pharmacokinetic parameters for vamorolone after single dose oral administration of 8 mg/kg to healthy subjects under fed and fasted conditions

Parameter*	8 mg/kg		Ratio†
	Fasted	Fed	
C _{max} (ng/mL)	718 (42.5) (6)	1,817 (31.4) (6)	2.53
T _{max} (hr)	1.78 (6) [1.00 – 2.00]	4.00 (6) [2.00 – 6.00]	
AUC _(0-t) (hr×ng/mL)	3,997 (55.0) (6)	10,139 (25.1) (6)	2.54
AUC _(inf) (hr×ng/mL)	4,137 (62.1) (5)	10,170 (24.9) (6)	2.46
λ _z (1/hr)	0.1823 (52.3) (5)	0.2950 (18.9) (6)	
t _{1/2} (hr)	3.80 (52.3) (5)	2.35 (18.9) (6)	
CL/F (L/hr/kg)	1.93 (62.1) (5)	0.79 (24.9) (6)	
V _z /F (L/kg)	10.6 (57.8) (5)	2.67 (23.4) (6)	

*Geometric mean (%CV) (N) except T_{max} for which the median (N) is reported.

†Ratio of the geometric means.

C_{max} = maximum observed plasma concentration; T_{max} = time to maximum observed plasma concentration; AUC_(0-t) = area under concentration-time curve from time 0 to time t; AUC_(inf) = area under concentration-time curve from time 0 to infinity; λ_z = elimination rate constant; t_{1/2} = terminal half-life; CL/F = apparent total clearance from plasma; V_z/F = apparent volume of distribution during terminal phase.

1.3.1.1.3 SAD Cohorts – Adverse Events

One subject in the SAD 8.0 mg/kg showed a delayed mild elevation of liver enzymes. This was not felt to be drug-related due to timing of the elevations, and it is not known if this was a drug-treated or placebo subject (study remains blinded). There were no other adverse events (AEs) seen in any dose group.

1.3.1.2 MAD Cohorts

The Phase I MAD treatment plan was discussed in light of the initial PK data. The relatively short half-life of vamorolone (2-4 hours), coupled with the planned daily dose schedule, would be expected to give PK data on each single dose, not cumulative dose, as the dosing interval was $> 5 \times t_{1/2}$. Thus, the MAD component would be a study of individual daily doses, rather than dose-related accumulation and pharmacodistribution related to cumulative drug exposure. In other words, a typical goal of a MAD study is to determine steady state drug levels after multiple doses; yet with the short half-life of vamorolone, useful information would not be expected to be gained with the current daily

dosing schedule. Safety and tolerability are additional goals of the MAD study, and these remain important endpoints independent of the PK studies.

1.3.1.2.1 MAD Cohorts – Pharmacokinetics Fasted

The original design for the Phase I MAD was modified to remove the two lowest doses (0.1, 0.3 mg/kg/day), and to begin dosing at 1.0 mg/kg/day. The clinical conduct of all four cohorts has been completed (1.0 mg/kg/day, 3.0 mg/kg/day, 9.0 mg/kg/day, 20.0 mg/kg/day) for the MAD study (**Table 3**).

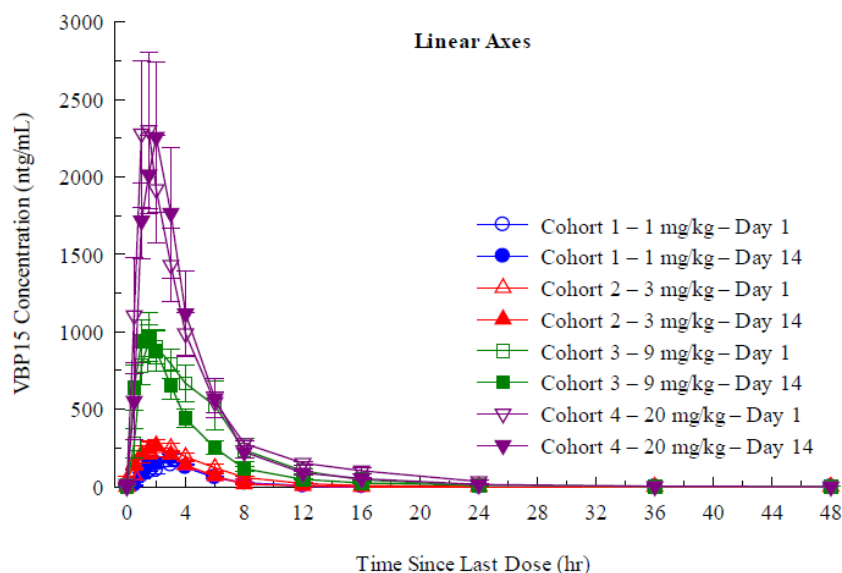
Table 3: Summary of pharmacokinetic parameters for vamorolone during oral administration of 1, 3, 9, and 20 mg/kg doses once daily for 14 days to healthy subjects under fasted conditions

	Vamorolone Dose			
	1 mg/kg	3 mg/kg	9 mg/kg	20 mg/kg
Day 1 C _{max} (ng/mL)	153 (15.9)	281 (36.9)	1,082 (23.3)	2,416 (51.1)
T _{max} (hr)	3.04 [1.50 – 4.00]	2.01 [1.00 – 3.00]	1.75 [1.00 – 6.00]	1.00 [0.50 – 3.00]
AUC _(0-t) (hr _x ng/mL)	686 (22.4)	1,471 (23.6)	5,709 (29.9)	10,182 (28.1)
AUC ₍₀₋₂₄₎ (hr _x ng/mL)	686 (22.4)	1,471 (23.6)	5,709 (29.9)	10,182 (28.1)
AUC _(inf) (hr _x ng/mL)	695 (22.1)	1,487 (23.7)	5,745 (29.5)	10,190 (27.0)
λ _z (1/hr)	0.3848 (10.9)	0.2918 (18.1)	0.2317 (22.6)	0.1747 (44.3)
t _{1/2} (hr)	1.80 (10.9)	2.38 (18.1)	2.99 (22.6)	3.97 (44.3)
CL/F (L/hr/kg)	1.44 (22.1)	2.02 (23.7)	1.57 (29.5)	1.96 (27.0)
V _z /F (L/kg)	3.74 (16.9)	6.91 (34.8)	6.76 (46.9)	11.2 (77.6)
Day 14 C _{max} (ng/mL)	203 (30.1)	276 (35.6)	935 (48.3)	2,491 (27.9)
T _{max} (hr)	2.96 [1.50 – 3.00]	2.50 [1.00 – 4.00]	1.25 [0.55 – 3.00]	2.00 [1.00 – 2.00]
AUC ₍₀₋₂₄₎ (hr _x ng/mL)	794 (22.3)	1,494 (18.6)	4,366 (20.2)	9,309 (38.8)
λ _z (1/hr)	0.3993 (20.4)	0.3273 (25.2)	0.1629 (63.5)	0.1879 (31.6)
t _{1/2} (hr)	1.74 (20.4)	2.12 (25.2)	4.25 (63.5)	3.69 (31.6)
CL/F (L/hr/kg)	1.26 (22.3)	2.01 (18.6)	2.06 (20.2)	2.15 (38.8)
V _z /F (L/kg)	3.15 (20.6)	6.14 (39.7)	12.7 (79.9)	11.4 (49.1)

C_{max} = maximum observed plasma concentration; T_{max} = time to maximum observed plasma concentration; AUC_(0-t) = area under concentration-time curve from time 0 to time t; AUC₍₀₋₂₄₎ = area under concentration-time curve from time 0 to 24 hours; AUC_(inf) = area under concentration-time curve from time 0 to infinity; λ_z = elimination rate constant; t_{1/2} = terminal half-life; CL/F = apparent total clearance from plasma; V_z/F = apparent volume of distribution during terminal phase.

Taking into account the small numbers and different subjects, the geometric mean values for C_{\max} , $AUC_{(0-t)}$, and $AUC_{(inf)}$ are not different for the SAD and MAD cohorts. Within the MAD, there is good agreement between Days 1 and 14 at all dose groups. There is no accumulation — the geometric mean C_{\max} and $AUC_{(0-24)}$ on Days 1 and 14 are not different, consistent with the $t_{1/2}$ (~2 hour) and dosing interval (24 hours) (**Figure 4**; **Table 3**).

Figure 4: Arithmetic mean \pm standard error plasma concentrations of vamorolone (VBP15) on Days 1 and 14 during oral administration of 1, 3, 9, and 20 mg/kg doses once daily for 14 days to healthy subjects under fasted conditions (linear axes)



1.3.1.2.2 MAD Cohorts – Adverse Events

Three subjects have discontinued dosing; one subject (1.0 mg/kg/day group) withdrew consent on Day 10 so that he could receive care for an exacerbation of a previously existing dental condition. Dosing was discontinued in a second subject on Day 9 (on placebo in the 1.0 mg/kg/day cohort after completing 8 days of dosing) due to an increase in alanine aminotransferase (ALT). The subject's baseline value was 40 (normal \leq 50); on Days 7, 8, and 9, it was 70, 86, and 106, respectively. Following cessation of dosing, the ALT has slowly declined and is continuing to be followed. Aspartate

aminotransferase (AST) was within normal limits with the exception of a value of 47 on Day 9 (normal ≤ 45), which was again normal the following day. Alkaline phosphatase, bilirubin, and coagulation parameters have remained within normal limits and the subject has remained asymptomatic. A third subject who was on active drug in the 20 mg/kg dose group was discontinued after 9 days of dosing due to an increase in ALT. The subject's baseline value was 43 (normal ≤ 50) at screening; on Days 7, 8, and 9, it was 56, 60, and 73, respectively. Following cessation of dosing, the ALT increased to 96 and 104 on Days 10 and 12; on Day 17, it had declined to 85. The subject continues to be followed and has remained asymptomatic. Aspartate aminotransferase was within normal limits with the exception of a value of 47 on Day 12 (normal ≤ 45); total bilirubin was 0.7 at screening (normal 0.2-1.2); it was increased at 1.4, 1.9, and 1.4 on Days 1, 2, and 3, respectively, and within normal limits for the remainder of dosing and follow-up. No subject in the 3.0 or 9.0 mg/kg/day cohorts had to discontinue dosing for an AE.

1.3.1.2.3 Pharmacodynamic Safety Biomarkers

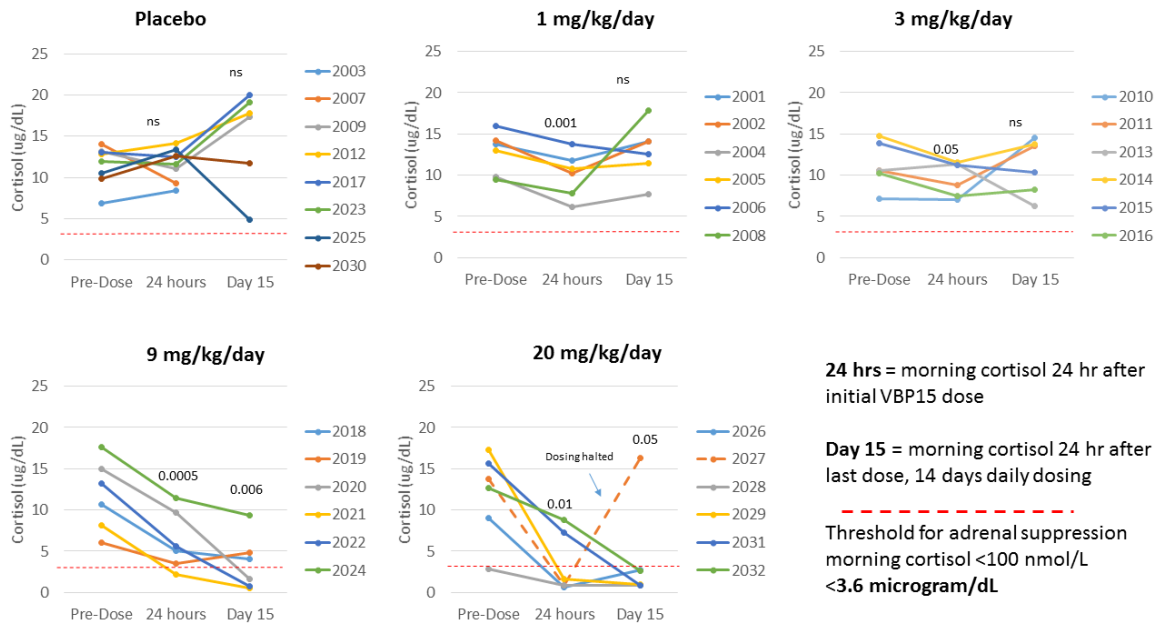
Vamorolone has shown improved safety profiles relative to prednisone in pre-clinical testing, both *in vitro* and *in vivo*.^{15,17} Safety concerns with glucocorticoids include suppression of the adrenal axis and insulin resistance. Pharmacodynamic biomarker assays of suppression of the adrenal axis (serum cortisol) and insulin resistance (serum glucose) were measured in the Phase I MAD studies of vamorolone.

Suppression of the adrenal axis. Prednisone directly impinges on cortisol regulatory pathways (adrenal axis) both acutely and chronically. Acute suppression of adrenal function is seen within hours of doses of a single 0.1 mg/kg/day (approximate) dose of prednisone, as evidenced by reductions in adrenocorticotrophic hormone (ACTH) levels in normal volunteers.²⁵ More chronic suppression of the adrenal axis, characterized as severe, is typically diagnosed when morning cortisol is < 100 nmol/L (< 3.6 microgram/dL) when drawn > 24 hrs after the last dose of pharmacological steroids.

Morning serum cortisol levels were measured in the vamorolone Phase I MAD cohorts, at baseline (prior to drug administration), 24 hours after the first dose (Day 1), and 24 hours after the 14-day dose (Day 15) (**Figure 5**). Active substance volunteers at four MAD

dose levels are shown (1.0 mg/kg/day; 3.0 mg/kg/day; 9.0 mg/kg/day; 20.0 mg/kg/day); all subjects were treated for 14 days with daily dosing. The red hatched line on each graph shows a typical threshold for adrenal axis suppression (< 100 nmol/L, or < 3.6 µg/dL). P values shown are for paired T test, indicating significance of the consistency of longitudinal changes of subjects relative to their own individual baseline values. Acute adrenal axis suppression is measured at 24 hours (after first dose), whereas chronic adrenal axis suppression is measured after 14 days of daily dosing (24 hours after last dose).

Figure 5: Morning cortisol measures in the vamorolone Phase I MAD volunteers.*



* Placebo subjects from each of the four MAD cohorts are graphed together.

Vamorolone showed little evidence of either acute (24 hour data), or chronic (Day 15 data) suppression of the adrenal axis at doses of either 1.0 mg/kg/day or 3.0 mg/kg/day. The data suggest that vamorolone induces variable, mild, acute and chronic suppression of the adrenal axis at 9.0 mg/kg/day, and stronger evidence of both acute and chronic adrenal axis suppression at 20.0 mg/kg/day. Prednisone typically shows both acute and chronic adrenal axis suppression approximately at 0.1 mg/kg/day,²⁵ suggesting that vamorolone has an improved safety window regarding adrenal axis suppression.

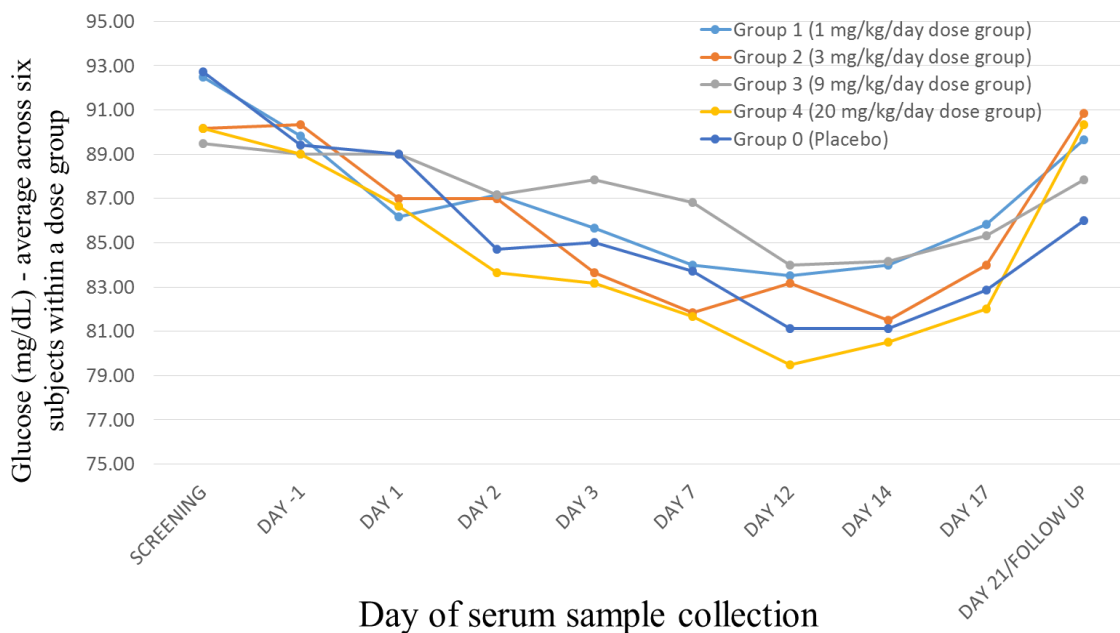
Vamorolone thus shows approximately a 100-fold improvement in safety window compared to prednisone on a mg/kg comparative basis. These data are consistent with *in vitro* and *ex vivo* pre-clinical mouse data comparing VBP15/vamorolone to prednisone for adrenal suppression.¹⁵

Insulin resistance. Prednisone induces the safety signal of insulin resistance, where glucose is not efficiently taken up from the blood by target tissues, such as muscle and liver, leading to hyperglycemia.²⁵ Insulin resistance may be an important safety signal for dystrophic muscle, where the dysfunctional myofibers have been shown to have inadequate energy stores,^{18,26} and insulin resistance likely limits availability of glycogen substrates for glycolysis. The hyperglycemia, in turn, leads to chronic increases in insulin levels (hyperinsulinemia).

Levels of fasting glucose and insulin are reasonably sensitive and reliable measures of insulin resistance in non-diabetic individuals. Glucose is acutely (single dose) and chronically (multiple doses) elevated after treatment with pharmacological glucocorticoids. Glucose is elevated 24 hours after a single administration of glucocorticoids (2.0 mg/kg).^{27,28}

In the Phase I MAD of vamorolone, fasting serum glucose was measured at 10 time points during the 2-week study; each sample was taken 24 hours after the previous dose of vamorolone (**Figure 6**).

Figure 6: Fasting serum glucose during the Phase I MAD period (two weeks daily treatment)



Glucose levels for all vamorolone dose groups were similar to those of the placebo group. There was no evidence of elevations of glucose levels at any time point or any dose of vamorolone, suggesting that the side effect of insulin resistance was not seen with vamorolone. These data are consistent with a pre-clinical study in a dystrophin-deficient mouse model, where chronic treatment of prednisolone (5 mg/kg/day) versus vamorolone (15 mg/kg/day; 30 mg/kg/day) showed development of insulin resistance with prednisolone, but not vamorolone.²⁹

1.3.1.3 Summary of Phase I data

In summary:

- Vamorolone PK data show strong adherence to dose linearity and dose proportionality, with relatively little subject-subject variation (both SAD and MAD).
- The half-life was about 2 hours for doses 0.1-1.0 mg/kg. Doses at 3.0, 8.0, and 20.0 mg/kg showed an extended tail, increasing half-life to 2.5, 3.8, and 3.8 hours, respectively. The PK for the MAD cohorts was very similar to the SAD cohorts,

showing little if any drug accumulation, consistent with the short half-life and daily dosing schedule.

- For the food effect group, a high fat meal was given to a cohort of Phase I SAD volunteers with the 8.0 mg/kg dose of vamorolone. These data were then compared to the fasted 8.0 mg/kg cohort data. The comparison showed that absorption was increased by 2.5-fold by the high fat meal, consistent with the lipophilic character of vamorolone (steroidal compound).
- For the MAD cohorts, there were no AEs precluding further escalations in dosing.
- Regarding the primary target organ, liver, one subject on placebo in the 1.0 mg/kg/day cohort and one subject on vamorolone in the 20 mg/kg/day cohort showed mild elevations of liver enzymes, and drug dosing was halted. No subjects in the 3.0 or 9.0 mg/kg/day MAD cohorts showed elevations of liver enzymes.
- Safety PD biomarker studies showed that vamorolone had an improved safety window for adrenal axis suppression (100-fold increase in therapeutic window), and no evidence of insulin resistance, compared to prednisone studies reported in the literature.

The results show that the half-life of vamorolone is very similar to glucocorticoids, such as prednisone. Despite the short half-life, prednisone is typically given once per day for most indications (including DMD), and once daily dosing is also proposed for vamorolone. Previous studies of increasing the frequency of drug administration of glucocorticoids have shown that this has increased side effect profiles without a significant gain in efficacy. Thus, the mechanism of action of glucocorticoids may be related to the short-term (pulsed) daily drug exposure. As vamorolone is thought to share a similar anti-inflammatory mechanism of action as glucocorticoids (NF- κ B inhibition), it is felt that the daily pulsed exposure in the Phase I MAD is most relevant to the planned clinical trials in DMD including the Phase II MAD and Phase II efficacy/safety studies, where daily dosing schedules will be followed. The option of increasing the dosing regimen in the Phase I MAD to better study drug accumulation and steady state PD was considered, but this was felt to be unrelated to planned Phase II trials, and drug mechanism of action.

1.4 Rationale for Study Design

Vamorolone is under development for the treatment of DMD. The first-in-human clinical assessment of vamorolone is in the SAD/MAD study (VBP15-001) and is providing an initial clinical assessment and assessment of the PK characteristics of vamorolone following administration of an oral suspension of vamorolone in healthy adult subjects. While there are likely to be some differences between adults and children, and between healthy and DMD subjects, the data from VBP15-001 have established the 2-4 hour half-life of vamorolone, food effect, dose proportionality and variability between subjects.

The current study is designed to determine the safe and tolerable dose(s) to enable future studies with chronic administration in DMD subjects, ages 4-< 7 years. The narrow age window for this study is driven by several factors: 1) the average age of diagnosis is still approximately 4.8 years due to lack of screening programs; 2) the current standards of clinical care in Western countries, where glucocorticoid therapy is typically initiated between 5 and 7 years of age for DMD patients; and 3) the observation that peak function for DMD patients occurs at around 7 years (after which time most affected males with DMD will begin to decline), all of which result in a narrow age window to identify steroid-naïve males with DMD.

The starting dose level of 0.25 mg in this study is one quarter of the dose of 1 mg, which was administered to healthy adult males in the MAD, resulting in an AUC_{0-24hr} of 794 ng x hr/mL on Day 14. The maximum proposed dose in this study of 6 mg/kg is lower than the 9 mg/kg dose administered to healthy adult males in the MAD which resulted in an AUC_{0-24hr} of 4366 ng x hr/mL, which is the lowest dose at which possible adrenal suppression was seen.. Thus, this range of doses seems appropriate to test in the first-in-patient study of vamorolone.²⁹

Key safety parameters in conjunction with PK data will be evaluated during the course of the study to assess vamorolone safety and tolerability.

Placebo has not been included in this initial study in DMD for the following reasons:

1) AE incidence versus placebo will have already been assessed for both single and

multiple doses in the prior SAD/MAD study VBP15-001; 2) the rarity of this disease makes it difficult to recruit large numbers of patients across the entire program; 3) as specified above, the patient pool is reduced further due to the narrow window of opportunity to enroll DMD boys not yet receiving glucocorticoids between the ages of 4 and 7 years; and 4) there are increasing numbers of DMD clinical studies using drugs of different modalities, all competing for the same population, i.e., those boys most likely to benefit and show a response.

The trial design includes the study of acute and chronic responses of PD biomarkers bench-marked against historical prednisone PD biomarker data. Acute responses in the Phase I study (hours after a single dose) will be studied in back-up PK samples on Day 1, to generate data on 17-hydroxyprogesterone and ACTH (adrenal axis suppression), insulin and glucose (insulin resistance), and osteocalcin (bone turn over), and data compared to prednisone-treated subjects in Kauh et al. (2012).²⁵ Chronic responses of PD biomarkers will be studied after 2 weeks of vamorolone treatment, and 2 weeks of wash out. Samples will be studied for glucocorticoid-responsive biomarkers in DMD patients previously defined in the Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study.³⁰ Exploratory PD biomarkers examining tissue breakdown and repair will also be evaluated.

The rationale for assessing acutely-responsive glucocorticoid PD biomarkers (back-up PK samples) is as follows. Kauh et al. (2012)²⁵ reported acutely-responsive serum glucocorticoid biomarkers after a single morning fasted dose of prednisone (dose groups: 0, 0.13 mg/kg, 0.33 mg/kg, 0.8 mg/kg) in adult volunteers.

They found that established biomarkers for known safety signals for glucocorticoids showed marked changes in blood levels within a few hours of a single dose of prednisone. These included:

- Reductions in ACTH with all doses, reflecting adrenal axis suppression
- Increased insulin and glucose with all doses, reflecting impaired insulin suppression of hepatic glucose production, and insulin resistance

- Decreased osteocalcin (bone turnover marker) at all doses, reflecting perturbations of bone remodeling

Nonclinical studies of vamorolone treatment of mice have shown that vamorolone does not show the glucocorticoid PD signals of adrenal axis suppression, insulin resistance, and increased bone turnover. Thus, we anticipate that DMD patients will similarly not show these same PD signals. To monitor the glucocorticoid PD biomarkers reflective of adrenal axis suppression, insulin resistance, and bone turnover, serum samples from this Phase II study of vamorolone in young DMD boys will be assayed for these same biomarkers as in the studies by Kauh et al. (2012).²⁵

In addition to these acutely-responsive glucocorticoid biomarkers, a panel of biomarkers in DMD patient sera that show response to chronic (~4 month) treatment with glucocorticoids has been identified. These chronic prednisone PD biomarkers were discovered through study of serum samples of DMD patients enrolled in the CINRG Duchenne Natural History Study. SomaScan aptamer panels testing 1,200 serum proteins were used to discover a candidate set of prednisone-responsive biomarkers; a subset of these were validated in a longitudinal sample set (individual DMD patients pre/post steroid treatment). These PD biomarkers were assigned to a safety panel or efficacy panel based on comparison to normal controls and information concerning the function of each protein (**Table 4**). All safety biomarkers were validated in a separate cohort of pediatric inflammatory disease patients (longitudinal pre/post steroids). The same SomaScan assay will be used on the chronic samples from the vamorolone Phase IIa trial (entry, 2 weeks treatment, 2 weeks washout), with initial data analyses limited to those validated biomarkers shown (**Table 4**).

Table 4: Biomarkers previously defined to be responsive to chronic doses of glucocorticoids in DMD boys

Chronic safety	Assay method	Chronic efficacy	Assay method
MMP-3	SomaScan	CD23	SomaScan
Leptin	SomaScan	MDC	SomaScan
Insulin	SomaScan	IL-22BP	SomaScan
IGFBP-5	SomaScan	Lymphotoxin a1/b2	SomaScan
Angiotensinogen	SomaScan	IGFBP-2	SomaScan
Afamin	SomaScan	Integrin a1b1; CD49a	SomaScan
17-hydroxyprogesterone	LC-MS	MMP-12	SomaScan
Corticosterone	LC-MS		
Testosterone	LC-MS		
11-deoxycortisol	LC-MS		

Adrenal axis suppression is a well-documented safety signal with chronic use of glucocorticoids. As noted above, pre-clinical data suggest that vamorolone may not cause adrenal axis suppression. To test if two weeks of vamorolone treatment causes chronic adrenal axis suppression, four steroid hormones will be tested by liquid chromatography-mass spectrometry (LC-MS) in the vamorolone-treated DMD boys (17-hydroxyprogesterone, corticosterone, testosterone, 11-deoxycortisol) (**Table 4**). All four steroid hormones showed significant reductions after glucocorticoid treatment in DMD boys in the cross-sectional and longitudinal CINRG Duchenne Natural History Study.

It is obligatory that the Investigator become familiar with all sections of the Vamorolone Investigator’s Brochure.²⁹

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 *Primary Objective*

The primary objective of this study is:

To evaluate the safety and tolerability of multiple ascending oral doses of vamorolone in ambulant boys ages 4-< 7 years with DMD.

2.1.2 *Secondary Objectives*

The secondary objectives of this study are:

1. To investigate the single-dose and multiple-dose PK of oral vamorolone at multiple dose levels in ambulant boys ages 4-< 7 years with DMD;
2. To investigate the effects of single and multiple oral doses of vamorolone on serum PD biomarkers in ambulant boys ages 4-< 7 years with DMD;
3. To evaluate metabolites of vamorolone in Metabolites in Safety Testing (MIST) assessments following administration of multiple ascending oral doses.

2.1.3 *Exploratory Objective*

The exploratory objective of this study is:

1. To investigate the effect of multiple oral doses of vamorolone on muscle strength, mobility, and functional exercise capacity, as measured by Quantitative Muscle Testing (QMT), Time to run/walk 10 meters Test (TTRW), Time to Stand Test (TTSTAND), Time to Climb Test (TTCLIMB), North Star Ambulatory Assessment (NSAA), and Six-minute Walk Test (6MWT) in ambulant boys ages 4-< 7 years with DMD.

2.2 Study Endpoints

2.2.1 Safety Endpoints

1. Treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs), by system organ class (SOC): overall by treatment, by treatment and relationship, by treatment and outcome, and by treatment and intensity (Common Terminology Criteria for Adverse Events [CTCAE] grade) (see [Section 7.2.6](#));
2. Vital sign [supine blood pressure (BP), heart rate, respiratory rate, oral temperature] values: change from Pretreatment to each of the scheduled on-treatment and post-treatment assessment time points;
3. Body weight: change from Pretreatment to each of the scheduled on-treatment and post-treatment assessment time points;
4. Clinical laboratory values: change from Pretreatment to each of the scheduled on-treatment and post-treatment assessment time points in:
 - Hematology and biochemistry
 - Lipid profile (triglycerides, total cholesterol, low density lipoprotein [LDL], high density lipoprotein [HDL])
 - Urinalysis (urine protein and glucose);
5. Concentrations of serum metabolites of vamorolone;
6. 12-lead electrocardiogram (ECG) results: change from Pretreatment to Day 14 and Day 28; and
7. Physical examination findings of abnormality.

2.2.2 Pharmacodynamic Endpoints

1. Concentration of acute and chronic serum PD biomarkers (change from Pretreatment to on-treatment and post-treatment time points): osteocalcin, serum aminoterminal propeptide of type I collagen (P1NP), carboxy-terminal telopeptide (CTX), cortisol, ACTH, 17-hydroxyprogesterone, testosterone, corticosterone, and 11-deoxycortisol, insulin, and glucose.

Samples will also be collected for additional SomaScan and proteomics profiling.

2.2.3 Exploratory Clinical Efficacy Endpoints

All exploratory clinical efficacy measures are assessed as change from Pretreatment to each of the scheduled on-treatment and post-treatment assessment time points:

1. Quantitative Muscle Testing (QMT): unilateral elbow and knee muscle flexion and extension;
2. Time to Stand Test (TTSTAND) from a supine position;
3. Time to Climb 4 Steps Test (TTCLIMB);
4. Time to Run/Walk 10 meters Test (TTRW);
5. North Star Ambulatory Assessment (NSAA); and
6. Six-minute Walk Test (6MWT).

2.2.4 Endpoints for Subject Reported Outcomes

Safety endpoints based on subject reports of adverse experiences are listed in [Section 2.2.1](#). Additionally, subjects will be asked to assess acceptability of vamorolone by a 5-point hedonic scale. No other subject reported outcomes are planned.

2.2.5 Pharmacokinetic Endpoints

Plasma PK parameters derived from serial blood samples collected 0.5 hour pre-dose and 1, 2, 4, 6, and 8 hours after the first and final doses of study drug on Day 1 and Day 14 (see [Section 7.5](#)).

3 STUDY DESIGN

3.1 Overall Study Design

This is a Phase IIa, multiple site, open-label, multiple ascending dose study to evaluate the safety, tolerability, PK, PD biomarker responsiveness, and exploratory clinical efficacy of vamorolone administered once daily by liquid oral suspension over a Treatment Period of 14 days to ambulant boys ages 4-< 7 years with DMD.

The study is comprised of a Pretreatment Screening Period of up to 26 days duration, a 1-day Pretreatment Baseline Period, a 14-day Treatment Period, and a 14-day Follow-up Period. Four dose level groups of approximately 12 subjects each will receive vamorolone once daily for 14 days (**Table 5**). The planned dose levels are 0.25 mg/kg (Dose Level Group 1), 0.75 mg/kg (Dose Level Group 2), 2.0 mg/kg (Dose Level Group 3), and 6.0 mg/kg (Dose Level Group 4).

Table 5: Dose level group composition

Planned Dose Level Group	No. Subjects in Dose Level Group	Vamorolone Dose	Treatment Duration
1	12	0.25 mg/kg	14 days
2	12	0.75 mg/kg	14 days
3	12	2.0 mg/kg	14 days
4	12	6.0 mg/kg	14 days

Subjects will be enrolled into this study only after completion of all Pretreatment Screening and Baseline assessments.

3.2 Dose Escalation Plan

Treatment of the approximately 12-subjects in each dose level group will be completed and safety results evaluated prior to enrollment of subjects in each subsequent dose level group. The process of dose escalation will be based on the appearance of dose-limiting toxicities. See **Section 8.3** for definitions of dose-limiting toxicities.

Subjects may be screened for eligibility up to 26 days in advance of the anticipated start of dosing. The first two subjects in Dose Level Group 1 may be entered immediately upon completion of screening. The first two subjects in subsequent dose level groups may be entered immediately upon completion of screening and the decision of escalation to the next dose. Screening for next subjects may occur when at least 11 of the approximately 12 enrolled and treated subjects have completed the 2-week Treatment Period and are being monitored in the 14-day Follow-up Period. If fewer than two of the approximately 12 subjects at any dose level experience a dose-limiting toxicity, following review of the available safety data for the Treatment and Follow-up Periods for all

subjects at a given dose level by the Study Chair and Medical Monitor, the Study Chair and Medical Monitor will jointly decide whether to recommend dose escalation to the next dose level. If two or more subjects at any dose level experience a dose-limiting toxicity, the Medical Monitor and Study Chair will be notified, and enrollment and dosing at that dose level will be halted; the MTD will be defined as the previous dose level, unless the decision is made to study lower intermediate dose level(s). The DSMB will be informed promptly of any dose decisions which are a result of dose-limiting toxicities.

If dose escalation is terminated due to dose-limiting toxicities, the remaining dose level groups may be enrolled to evaluate the safety and PK effects of vamorolone at lower intermediate dose level(s). Once safety data from additional dose level group(s) are assessed, the MTD will be defined as the dose level below the dose at which the DSMB determines there to be an unacceptable risk to subjects. This plan allows the maximum number of safe dose level groups to have sufficient numbers for overall estimates of safety, PK, PD and baseline assessments of preliminary efficacy outcomes to be followed via an extension study (VBP15-003).

Each dose level group of approximately 12 subjects will be assessed for safety and tolerability, PK, clinical efficacy, and PD during the 14-day Treatment Period and a 14-day Follow-up Period. Treatment Period study visits will occur on Day 1, Week 1 (Day 7), and Week 2 (Days 13 and 14). During the Follow-up Period, a scheduled telephone contact will be made by site study staff on Week 3 (Day 21), and a final study site visit will occur on Week 4 (Day 28). Subjects will receive their first and final doses of study medication on Day 1 and Week 2 (Day 14), respectively, at the study site; blood will be drawn for PK analysis 0.5 hours pre-dose, and 1, 2, 4, 6, and 8 hours post-dose on Day 1 and Week 2 (Day 14). Blood will be collected for MIST assessment 0.5 hours pre-dose and 1, 2, 4, 6, and 8 hours post-dose at Week 2 (Day 14). Once-daily study drug dosing on Days 2-13 will occur at home. Additional blood and urine samples for clinical laboratory tests and serum PD biomarkers will be collected at scheduled visits throughout the study (see [Section 6.3](#) for detailed assessment schedule). A physical examination and

12-lead ECG will be recorded at Screening, Week 2 (Day 14), and the final Week 4 (Day 28) Follow-up Visit. Exploratory clinical efficacy will be assessed by QMT, TTRW, TTSTAND, TTCLIMB, NSAA, and 6MWT at Screening and Baseline, Week 2 (Day 13), and the final Week 4 (Day 28) Follow-up Visit. Vital signs will be recorded at each study visit. Adverse events, including SAEs, and concomitant medications will be recorded throughout the study. Subjects will be discharged from the study following completion of all Week 4 (Day 28) Follow-up assessments. In the event that any clinical or laboratory parameters remain abnormal at the time of discharge from the study, the subject will be followed medically as clinically indicated.

Subjects who have completed the 2-week Follow-up Period may choose to receive vamorolone in an open-label extension study under a separate clinical protocol (VBP15-003) or receive standard of care treatment (including glucocorticoids) for DMD. Subjects choosing to enroll in the vamorolone extension study will receive the same dose of vamorolone in the extension study as they received in the current study VBP15-002.

3.3 Study Summary

This Phase IIa study is an open-label, multiple ascending dose study to evaluate the safety, tolerability, PK, PD, and exploratory clinical efficacy of vamorolone 0.25 mg/kg, 0.75 mg/kg, 2.0 mg/kg, or 6.0 mg/kg administered daily by liquid oral suspension over a Treatment Period of 14 days to ambulant boys ages 4-< 7 years with DMD.

The study is comprised of a Pretreatment Screening Period of up to 26 days duration, a 1-day Pretreatment Baseline Period, a 14-day Treatment Period, and a 14-day Follow-up Period.

4 SELECTION AND WITHDRAWAL OF STUDY SUBJECTS

4.1 Subject Screening and Enrollment

Subjects will be recruited through the clinics of participating site investigators and other mechanisms including, but not limited to: posting on www.clinicaltrials.gov, posting on the Sponsor and/or designee website, and patient foundations. After identification of a

possible subject, the site investigator will discuss the study with the subject's parent or legal guardian. The subject's parent or guardian will be provided with a copy of the informed consent document and allowed time to consider participation prior to signing. Individuals interested in participating will be asked to come to one of the participating study sites to complete the informed consent process with a site investigator or designee prior to initiation of screening procedures. Subjects will not be excluded on the basis of race, ethnicity, or age, except that the target population for the trial is 4- < 7 years of age.

A subject screening log will be maintained at each investigational site for all subjects who are considered for the study, including those not enrolled. Limited data will be collected for these subjects, including initials, date of birth, and reason for exclusion from the study. Subject screening and enrollment logs will be maintained for all subjects enrolled in the study. These logs will be reviewed during routine monitoring calls and/or visits.

4.2 Inclusion Criteria

To qualify for enrollment in this study, the subject must satisfy the following inclusion criteria:

1. Subject's parent or legal guardian has provided written informed consent/Health Insurance Portability and Accountability Act (HIPAA) authorization prior to any study-related procedures;
2. Subject has a confirmed (by Central Genetic Counselor) diagnosis of DMD as defined as:
 - a. Dystrophin immunofluorescence and/or immunoblot showing complete dystrophin deficiency, and clinical picture consistent with typical DMD, OR
 - b. Identifiable mutation within the *DMD* gene (deletion/duplication of one or more exons) where reading frame can be predicted as 'out-of-frame', and clinical picture consistent with typical DMD, OR
 - c. Complete dystrophin gene sequencing showing an alteration (point mutation, duplication, other) that is expected to preclude production of the dystrophin

- protein (i.e. nonsense mutation, deletion/duplication leading to a downstream stop codon), with a typical clinical picture of DMD;
3. Subject is ≥ 4 years and < 7 years of age at time of enrollment in the study;
 4. Subject is able to complete the Time to Stand Test (TTSTAND) without assistance, as assessed at the Screening and Baseline Visits;
 5. Clinical laboratory test results are within the normal range at the Screening Visit, or if abnormal, are not clinically significant, in the opinion of the Investigator. (Note: Serum gamma glutamyl transferase [GGT], creatinine, and total bilirubin all must be \leq upper limit of the normal range at the Screening Visit);
 6. Subject has evidence of chicken pox immunity as determined by presence of IgG antibodies to varicella, as documented by a positive test result from the testing laboratory at the Screening Visit; and
 7. Subject and parent/guardian are willing and able to comply with scheduled visits, study drug administration plan, and study procedures.

4.3 Exclusion Criteria

A subject will be excluded from enrollment in this study if the subject meets any of the following exclusion criteria:

1. Subject has current or history of major renal or hepatic impairment, diabetes mellitus or immunosuppression;
2. Subject has current or history of chronic systemic fungal or viral infections;
3. Subject has had an acute illness within 4 weeks prior to the first dose of study medication;
4. Subject has used mineralocorticoid receptor agents, such as spironolactone, eplerenone, canrenone (canrenoate potassium), prorenone (prorenoate potassium), mexrenone (mexrenoate potassium) within 4 weeks prior to the first dose of study medication;
5. Subject has evidence of symptomatic cardiomyopathy. [Note: Asymptomatic cardiac abnormality on investigation would not be exclusionary];

6. Subject is currently being treated or has received previous treatment with oral glucocorticoids or other immunosuppressive agents. [Notes: Past transient use of oral glucocorticoids or other oral immunosuppressive agents for no longer than 3 months cumulative, with last use at least 3 months prior to first dose of study medication, will be considered for eligibility on a case-by-case basis. Inhaled and/or topical corticosteroids prescribed for an indication other than DMD are permitted but must be administered at stable dose for at least 3 months prior to study drug administration];
7. Subject has used idebenone within 4 weeks prior to the first dose of study medication;
8. Subject has an allergy or hypersensitivity to the study medication or to any of its constituents;
9. Subject has severe behavioral or cognitive problems that preclude participation in the study, in the opinion of the Investigator;
10. Subject has previous or ongoing medical condition, medical history, physical findings or laboratory abnormalities that could affect safety, make it unlikely that treatment and follow-up will be correctly completed or impair the assessment of study results, in the opinion of the Investigator;
11. Subject is taking any other investigational drug currently or has taken any other investigational drug within 3 months prior to the start of study treatment; or
12. Subject has previously been enrolled in the study.

Note: Any parameter/test may be repeated at the Investigator's discretion during Screening and/or Day -1 to determine sustainability and reproducibility. In addition, subjects may be rescreened if ineligible due to a transient condition which would prevent the subject from participating, such as an upper respiratory tract infection, or if ineligible due to negative anti-varicella IgG antibody test result (see [Section 7.2.4](#) for vaccination and retest parameters).

5 STUDY TREATMENT

5.1 Study Medication Administered

Vamorolone will be administered to all subjects as an oral liquid suspension. The suspension formulation utilized for this study is the same as that utilized in the Phase I clinical trial in adult volunteers.

Planned vamorolone dose levels: 0.25 mg/kg, 0.75 mg/kg, 2.0 mg/kg, and 6.0 mg/kg.

For each Dose Level Group 1-4, vamorolone will be administered once daily for 14 days, from Study Day 1 through Study Day 14.

5.2 Identity of Investigational Product

The Study Sponsor will supply the following investigational study medication:

Name:	Vamorolone
Active Substance:	VBP15
Strength:	4% by weight
Dosage Form:	Oral suspension
Manufacturer:	Velesco Pharma

5.3 Dosage Schedule and Administration of Study Medication

Subjects who meet all eligibility criteria (see [Sections 4.2](#) and [4.3](#)) and complete all Baseline assessments will be enrolled into the study. The unique subject study number assigned to the subject during the Screening Period will carry forward with the subject after enrollment. Subjects must meet all eligibility criteria to qualify for initial dosing in the study. Subjects will be enrolled just prior to receiving the initial dose of study medication.

The site pharmacist or designated site study staff will dispense study medication in 100 mL bottles sufficient for 16 days of oral dosing to each subject enrolled in the study after completion of all Baseline assessments and enrollment into the study. Bottles will be fitted with adaptors by the site pharmacist or designated site study staff prior to

dispensing. The number of 100 mL bottles to be dispensed to each subject depends on subject weight and the dose level group; see [Appendix 15.1](#) for complete instructions on calculating dose volume. Clinical supplies dispensed by the study site staff and ready for administration to subjects will be labeled with subject study number, dispense date, protocol number, dose level, volume to dispense per dose, and cautionary statement.

Enrolled subjects will receive all doses of vamorolone under the supervision of trained study staff or parent or legal guardian. Day 1 and Day 14 doses will be administered at the participating study site; all other doses will be administered at home. Study drug will be administered by mouth using a volumetric syringe. Following administration of the dose of study drug, the syringe will be filled once with water and the water will be administered by mouth using the volumetric syringe. The subject will then drink approximately 50 mL (approximately 2 ounces) of water to ensure the full dose has been ingested. Subjects should receive each dose of study medication at approximately the same time of day.

On Days 1 and 14 only, the subject will be asked to consume an 8 ounce glass of whole milk (or equivalent high fat food portion) and 1 cup of cereal at the study site 30 minutes prior to administration of the dose of study medication. Subjects will be asked to consume an 8 ounce glass of whole milk (or equivalent high fat food portion) each day prior to dosing during Study Days 2-13. There are no other food or drink restrictions during the study.

The dose per subject (in mg) throughout the Treatment Period will be calculated based on the recorded Screening weight of the subject (in kg) (see [Appendix 15.1](#) for dose calculation worksheet). All missed or incomplete doses will be documented. The dispensed study medication bottle(s) will be returned to the study site at the Week 2 Visit.

5.4 Rationale for Dose Selection

Escalating doses of 0.25 mg/kg, 0.75 mg/kg, 2.0 mg/kg, and 6.0 mg/kg of vamorolone were chosen for this study to ensure the safety of subjects enrolled in the study, to allow demonstration of PD effects, and to ensure adequate safety in future clinical studies. All

dosing will be done in the morning with a glass of whole milk (8 ounces or 230 mL) or similar fat equivalent meal (approximately 8 grams fat). Dose escalations are 2- to 3-fold increases for each subsequent dose level group.

The rationale for the starting dose of 0.25 mg/kg/day with a glass of whole milk is as follows. As Phase II dosing in 4- < 7 year old DMD children will be done at home in the morning, challenges were considered with participant and parent compliance with either fasting in the morning (prior to school or other activities), or a rigid prescribed diet of known fat content. To balance compliance with appropriate assessments of bioavailability and monitoring of safety, it was decided to administer morning doses at home with a glass of whole milk (approximately 8 grams of fat) or similar fat content meal.

From the Phase I study in adult volunteers (VBP15-001), a high fat diet (40 grams) increased bioavailability of vamorolone (8.0 mg/kg/day) by 250% compared to the fasted cohort at this dose. Because a formal food effect study has not been done, it must be assumed that any fat intake with drug delivery has the potential to have a 250% increase in bioavailability vs. the fasted state. Thus, a conservative approximation of vamorolone drug exposure in DMD children when taken with a whole glass of milk is 250% of the fasted bioavailability. A starting dose of 0.25 mg/kg/day with a glass of milk would be equivalent to 0.625 mg/kg/day fasted, and this starting dose is 3.1% of the highest safe dose tested in adults (20.0 mg/kg/day fasted).

A starting dose of 0.25 mg/kg/day, with four dose groups increasing by 2 to 3-fold increments, leads to dose groups of 0.25, 0.75, 2.0, and 6.0 mg/kg/day. The highest dose tested, 6.0 mg/kg/day, will similarly be given with a glass of milk, with an expected increased bioavailability of 250%, or equivalent to 15.0 mg/kg/day fasted. As 20.0 mg/kg/day fasted in adult volunteers was shown to be safe in the Phase I adult volunteer study, the proposed highest dose in 4- < 7 year old children is approximately 75% of the highest safe adult dose. Based on the Phase I PD biomarker safety data presented in [Section 1.3](#), safety signals reflective of insulin resistance are not anticipated at any of the planned Phase IIa doses. Also based on the Phase I data, regarding the safety signal of

suppression of the adrenal axis, no evidence of adrenal axis suppression is anticipated at the 0.25 mg/kg/day, 0.75 mg/kg/day and 2.0 mg/kg/day doses planned in the Phase IIa study, but suppression of the adrenal axis may be observed in the 6.0 mg/kg/day Phase IIa dose level group.

5.5 Treatment Compliance

Subject compliance with the dosing schedule will be assessed by site maintenance of accurate study drug dispensing and return records, and accurate recording of administered doses on all at-home administration days (Study Days 2-13) by completion of a diary by the subject's parent or guardian. Food and drink consumed prior to dosing on Study Days 2-13 will also be recorded in subject diaries. The Investigator is responsible for ensuring that dosing is administered in compliance with the protocol. The Investigator or designee will instruct the subject's parent or guardian with regard to proper dosing of study medication and completion of subject diaries, and will reinforce the importance of taking all study medication per protocol instructions. Doses of study drug on Day 1 and Day 14 will be administered at the participating study site by a trained investigational staff member. All complete, incomplete, or missed doses are to be documented in the source document and in the appropriate electronic case report form (eCRF).

5.6 Study Medication Management

5.6.1 Packaging and Labeling of Study Medication

The site pharmacist or designated study staff will receive clinical trial material (CTM) when all regulatory requirements have been completed by the site. Additional CTM will be available upon request. The Study Sponsor through the designated central pharmacy will provide CTM in bulk quantities sufficient to satisfy the protocol requirement. Vamorolone will be shipped in bulk to the study site's registered pharmacist or designated study staff in suitably labeled study cartons. Cartons will contain study medication packaged in sterile 100 mL glass bottles; each bottle will contain 4 grams of vamorolone as a 4% suspension in sterile water. Bulk drug supplies will be labeled with the name of Sponsor, protocol number, lot number, expiration or retest date, and other

appropriate study information. Carton and bottle labels will include the following statement: “Caution: New Drug – Limited by Federal Law to Investigational Use.”

5.6.2 Storage of Study Medication

All CTM for use in the trial must be stored in a locked container/cabinet free from environmental extremes, under the responsibility of the study site Investigator or site pharmacist. Bulk CTM should be stored at refrigerated temperature (2°C-8°C; 36°F-46°F). Excursions to ambient temperature are allowed. Access to CTM stored at participating study sites must be limited to authorized research personnel.

5.6.3 Study Medication Shipping and Handling

Clinical trial material will be shipped to the study sites only after receipt of required documents in accordance with applicable regulatory requirements and Sponsor procedures. Study sites will receive CTM in bulk in sufficient quantity for one subject. After a subject is enrolled, the site will be sent CTM in bulk in sufficient quantity for one additional subject until the study has completed enrollment.

Clinical trial material will only be dispensed to the subject once the subject’s parent/guardian has (1) a signed informed consent form (ICF) and HIPAA (as applicable) authorization on file, (2) met all eligibility criteria for entry into the study, (3) completed all screening requirements, and (4) been enrolled.

It is essential to this study that all CTM be accounted for during the study period. All unused study medication will be retained at the study center for reconciliation and collection by the Sponsor's study monitors (or designee) during routine monitoring visits. Study site personnel should not dispose of any CTM. Final disposition of all unused CTM will be coordinated by the Sponsor's study monitor (or designee) at the end of the study (see [Section 5.6.4](#)).

Clinical trial material must be dispensed and administered according to the procedures described in this protocol. Only subjects enrolled in the study may receive study medication, in accordance with all applicable regulatory requirements. Only authorized

study personnel may supply or administer CTM. Authorized study personnel refers to the Investigator (or designee), in accordance with all applicable regulatory requirements and the Site Signature Log/Delegation of Authority.

5.6.4 Study Medication Accountability

The site Investigator is responsible for the control of drugs under investigation. Adequate records of the receipt (e.g., Drug Receipt Record) and disposition (e.g., Drug Dispensing Log) of the study drug must be maintained. The Drug Dispensing Log must be kept current and should contain the following information:

- The identification of the subject to whom the study drug was dispensed.
- The date(s) and quantity of the study drug dispensed to the subject.
- The date(s) and quantity of the study drug returned by the subject.

All records and drug supplies must be available for inspection by the Monitor at every monitoring visit. Unused medication will be returned to the Study Sponsor or its designee at the end of the study. The completed Drug Dispensing Log and Drug Return Record(s) will be returned to the Study Sponsor or its designee. The Investigator's copy of the Drug Return Record(s) must accurately document the return of all study drug supplies to the Study Sponsor or its designee.

5.7 Procedures for Assigning Subject Study Numbers

Following the signing of the written ICF, subjects will be assigned a unique site-specific 6-digit subject study number in sequential order of screening into the study. The subject study number will be assigned by the site at the time of submission of the de-identified genetic test and/or muscle biopsy report to the Central Genetic Counselor to confirm that the subject meets the diagnostic eligibility criteria. If the de-identified genetic test and/or muscle biopsy report are submitted to the Central Genetic Counselor prior to signing of the ICF (only if acceptable per local Institutional Review Board [IRB]/Independent Ethics Committee [IEC]), then a screening number will be assigned to the potential subject by the Coordinating Center. Upon signing of the ICF, the site will assign the

subject a 6-digit subject study number and notify the Coordinating Center of the newly assigned subject number.

All data will be identified using the unique 6-digit subject study number. These subject study numbers assigned upon signing of the written ICF will be retained through enrollment and throughout participation in the study. Subject study numbers assigned to subjects who fail screening may not be used again.

The Investigator will keep a record relating the names of the subjects to their subject study numbers (Subject Identification Log) to permit efficient verification of data subject files, when required. This record will also include the dates of subject enrollment and completion/termination.

6 STUDY PROCEDURES

6.1 Time and Events Schedule

The study procedures to be conducted for each subject are divided into the following study periods:

- **Pretreatment Screening Period:** From subject's parent or guardian signing of the Informed Consent/HIPAA authorization until completion of all designated Screening procedures, 48 hours prior to the first dose of study medication.
- **Pretreatment Baseline Period:** The 24-hour period immediately prior to administration of the first dose of study medication, which includes completion of all Baseline assessments and enrollment into the study.
- **Treatment Period:** The 14-day interval starting with administration of the first dose of study medication on Day 1 and continuing through the time of administration of the final dose of study medication on Week 2 (Day 14) and completion of Week 2 safety, PK, PD, and clinical efficacy assessments.
- **Post-treatment Follow-up Period:** The 14-day interval beginning following completion of all Week 2 (Day 14) study assessments and continuing through the final Week 4 (Day 28) Follow-up Visit. Telephone contact to assess safety will

be made on Week 3 (Day 21), and final safety, PD, and clinical efficacy assessments will be performed at the final Week 4 (Day 28) Visit. Subjects will be discharged from the study following completion of all Week 4 (Day 28) safety, PD, and clinical efficacy assessments.

The procedures to be completed during each study period are presented as the Schedule of Study Activities in **Table 6** and in the sections that follow. Detailed descriptions of the assessments and the definition of study endpoints are provided in **Sections 7** and **2.2**. Any deviation from study procedures should be noted in the source documents and in the eCRF, and significant deviations should be reported immediately to the Sponsor or their designee.

Overall, approximately 8 weeks are allocated for each subject to complete the study, including a 26-day Pretreatment Screening Period, a 1-day Pretreatment Baseline Period, a 2-week Treatment Period, and a 2-week Post-treatment Follow-up Period.

Table 6: Schedule of Study Activities

Visit/Contact	Pretreatment Period		Treatment Period			Post-treatment Follow-up	
	SCR	BL					
	Day		Day	Week		Week	
	-28 to -3	-1	1	1 (7±1d)	2 ^a (14±1d)	3 (21±2d)	4 ^b (28±3d)
Informed Consent ^c	X						
Medical History	X						
Medication History	X	X					
Blood for Chicken Pox Immunity	X ^d						
Inclusion/Exclusion Criteria	X	X					
Physical Examination	X				X		X
Height	X						
Weight	X	X			X		X
Vital Signs ^e	X	X ^f	X ^f	X	X ^f		X
Blood for Clinical Labs ^g	X				X		X
Urinalysis ^g	X				X		X
12-lead ECG	X ^h				X ^h		X ^h
Enrollment		X					
Dispense Study Medication			X				
Return Study Medication					X		
Study Medication Dosing ⁱ			X	→	X		
Blood for Plasma PK		X ^j	X ^j		X ^j		
Blood for Metabolites in Safety Testing (MIST) ^q					X ^q		
Blood for Serum PD Biomarker Panel	X ^k	X ^l	X ^m		X ^{l,n}		X ⁿ
Quantitative Muscle Testing (QMT)	X	X			X ^o		X
Time to Run/Walk 10 Meters Test (TTRW)	X	X			X ^o		X
Time to Stand Test (TTSTAND)	X	X			X ^o		X
Time to Climb Test (TTCLIMB)	X	X			X ^o		X
North Star Ambulatory Assessment (NSAA)	X	X			X ^o		X
Six-minute Walk Test (6MWT)	X	X			X ^o		X
Study Medication Acceptability Assessment			X ^p		X ^p		
Study Medication Accountability					X		
Safety Monitoring			X	X	X	X	X
AE/SAE Recording	X	X	X	X	X	X	X
Concomitant Medications			X	X	X	X	X
Telephone Contact						X	
Discharge from Study							X

SCR=Screening Period; BL=Baseline Period, within 24 hours prior to administration of the first dose of study drug.

- Subjects who prematurely discontinue from the study prior to Week 2 (Day 14) should complete the Week 2 (Days 13 and 14) procedures at the time of early discontinuation and enter the 14-day Follow-up Period.
- Subjects who prematurely discontinue from the study after Week 2 (Day 14) but prior to the scheduled Week 4 (Day 28) Follow-up Visit should return to the study site for Week 4 (Day 28) procedures.
- Informed Consent must be obtained prior to any study-related procedures.
- All subjects will have blood collected at the Screening Visit for testing for IgG antibodies to varicella. Eligibility for study enrollment is dependent upon availability of positive result prior to enrollment.

- e. Supine blood pressure, oral temperature, respiratory rate, and heart rate. At visits where blood is also drawn, vital signs must be recorded prior to blood draws.
- f. On Days 1 and 14, vital signs will be recorded at 0.5 hour pre-dose and 0.5, 1, 2, 4, 6, and 8 hours post-dose, prior to PK blood draws where time points coincide.
- g. Blood for chemistry, hematology, lipids; urinalysis by dipstick and microscopic analysis.
- h. ECG must be conducted prior to vital signs.
- i. The first dose of study medication on Day 1 and the final dose of study medication on Day 14 will be administered in the study clinic; all other doses will be taken at home.
- j. Blood collected for plasma PK analysis on Day -1 (0.5 hour pre-dose) and 1, 2, 4, 6, and 8 hours post-dose on Day 1; and 0.5 hour pre-dose and 1, 2, 4, 6, and 8 hours post-dose on Day 14. Subjects must be fasting at the time of collection of Day 1 and Day 14 pre-dose samples.
- k. cortisol, ACTH, P1NP, osteocalcin, CTX, 17- hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, hemoglobin A1c (HbA1c), SomaScan, and proteomics testing.
- l. Blood collected for insulin and glucose on Day -1 (0.5 hour pre-dose) and on Day 14 (0.5 hour pre-dose). Subjects must be fasting at the time of blood collection.
- m. Day 1, 6 hours post dose: cortisol, ACTH, P1NP, osteocalcin, CTX, 17- hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol.
- n. Day 14, 6 hours post-dose and Week 4 (Day 28): cortisol, ACTH, P1NP, osteocalcin, CTX, 17- hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, SomaScan, and proteomics testing.
- o. Week 2 clinical efficacy assessments to be completed on Day 13.
- p. Study medication acceptability assessment performed immediately pre-dose (smell) and post-dose (taste).
- q. MIST assessment will be performed on portions of the blood samples collected for PK analysis; no additional blood will be collected.

6.2 Informed Consent/HIPAA Authorization

Each subject's parent or guardian will receive an explanation of the nature and purposes of the study from the Investigator or designee. The Investigator or designee will ensure the study is appropriate for the subject. Consent must be obtained in accordance with the principles outlined in the current version of the Declaration of Helsinki. The subject's parent or guardian will confirm that s/he understands that the study is for research purposes only and that it may not provide any therapeutic benefit to the individual. Each subject's parent or guardian will confirm that s/he understands that the subject is free to withdraw from the study at any time without prejudice. The Investigator or designee will review the elements of the HIPAA and Protected Health Information (PHI) with each subject's parent or guardian and each subject's parent or guardian will confirm that s/he understands HIPAA authorization and PHI. The Investigator (or designated staff) will obtain the written informed consent and HIPAA authorization on the approved ICF by the appropriate IRB/IEC at each site, from the subject's parent or guardian prior to any study-related procedures, including agreement to discontinuation of any prohibited medications, prior to the start of the study. The written assent of children will be obtained per individual site guidelines.

The ICF must be dated and signed by the Investigator or designee and the subject's legal representative and the original signed consent form must be kept by the Investigator in the study subject's file. "Legal representative" means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective study subject to the subject's participation in the procedure(s) involved in the research. The study subject's legal representative will receive a copy of the signed consent form.

6.3 Visit Schedule and Procedures

During the study, there will be a total of 7 study visits: 1) Pretreatment Screening Visit (Day -28 to Day -3); 2) Pretreatment Baseline Visit (Day -1); 3) Treatment Period Day 1 Visit; 4) Treatment Period Week 1 (Day 7) Visit (7 ± 1 day after the first dose of study medication); 5) Treatment Period Week 2 (Day 13) Visit (13 ± 1 day after the first dose of study medication); 6) Treatment Period Week 2 (Day 14) Visit (14 ± 1 day after the first dose of study medication); and 7) a Post-treatment Follow-up Visit at Week 4 (Day 28) (28 ± 3 days after the first dose of study medication, and approximately 14 days after the final dose of study medication). Additionally, a telephone contact will be made at Week 3 (Day 21) (21 ± 2 days after the first dose of study medication). Each subject will receive the investigational product for a period of 14 days. The Baseline Day -1 and Treatment Day 1 procedures may be performed at a single study visit, at the discretion of the Investigator and according to study site resources. The Week 2 assessments will be divided over a 2-day period (Days 13 and 14), with all exploratory clinical efficacy assessments performed on Day 13 and the remainder of the scheduled Week 2 assessments performed on Day 14. See [Section 7](#) for a detailed description of the safety, clinical efficacy, PD, and PK assessments to be performed in this study.

6.3.1 Screening Period (Day -28 to -3)

The Investigator or study staff will discuss with each subject and the subject's parent or legal guardian, the nature and purpose of the study and the required study procedures. The subject's medical history and medication history will be reviewed to determine initial eligibility for participation in the study and the subject's de-identified dystrophin genetic test report and/or muscle biopsy report will be sent to the Central Genetic Counselor(s)

for confirmation that the subject meets the DMD diagnostic inclusion criteria. Following the signing of the written ICF, subjects will be assigned a unique site-specific 6-digit subject study number that will be comprised of protocol, site, and subject numbers in sequential order of screening into the study. All data will be identified using the unique subject study number. The site Investigator will keep a record relating the names of the subjects to their subject study numbers (Subject Screening Log) to permit efficient verification of data subject files, when required. This record will also include the dates of subject enrollment and completion/termination. The Coordinating Center will not collect names or other identifiers except dates (of birth, diagnosis, study visits) and the subject study number.

Subjects will undergo the following procedures (the procedures may be completed over the course of several visits, if necessary, but all scheduled Screening procedures must be completed within the 26-day timeframe designated for the Pretreatment Screening Period and the actual date each procedure is performed must be recorded in the source document and eCRF). Any parameter/test may be repeated at the Investigator's discretion during Pretreatment Screening and/or Baseline Day -1 to determine sustainability and reproducibility. In addition, subjects may be rescreened if ineligible due to a transient condition which would prevent the subject from participating, such as an upper respiratory tract infection. Any subject who is ineligible due to a negative test result at Screening for IgG antibodies to varicella may be vaccinated or revaccinated and return for rescreening approximately 30 days after vaccination.

- Review of the Inclusion and Exclusion Criteria (see [Section 4.2](#) and [4.3](#))
- Recording of the medical history, including any toxicities or allergy-related events to prior treatments (see [Section 7.2.1](#))
- Recording of Medication History (see [Section 6.4](#))
- Complete physical examination, including weight (in kilograms) and height (see [Section 7.2.2](#))
- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) (see [Section 7.2.3](#))

- Testing for chicken pox immunity (see [Section 7.2.4](#))
- Clinical labs including hematology, clinical chemistry, lipids, and urinalysis tests (see [Section 7.2.4](#))
- Blood drawn for PD biomarkers including cortisol, ACTH, P1NP, osteocalcin, CTX, 17- hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, and hemoglobin A1c (HbA1c). Blood will also be collected and stored for future proteomics profiling and SomaScan studies (see [Section 7.3](#))
- 12-lead ECG (see [Section 7.2.5](#))
- Quantitative Muscle Testing (QMT) (see [Section 7.4.1](#))
- Time to Run/Walk Test (TTRW) (see [Section 7.4.2](#))
- Time to Stand Test (TTSTAND) (see [Section 7.4.3](#))
- Time to Climb Test (TTCLIMB) (see [Section 7.4.4](#))
- North Star Ambulatory Assessment (NSAA) (see [Section 7.4.5](#))
- Six-minute Walk Test (6MWT) (see [Section 7.4.6](#))
- Recording of AEs and SAEs beginning at the time written informed consent is obtained (see [Section 7.2.6](#)).

6.3.2 Baseline Visit (Day -1)

Following availability of the results of all Screening eligibility tests, subjects meeting all Screening eligibility criteria will return to the study site during the Pretreatment Baseline Period (Day -1, the 24-hour interval immediately preceding administration of the first dose of study medication) for baseline assessments and study enrollment. Subjects will retain their 6-digit study identification number which was assigned during the Screening Period.

The following procedures will be completed at the Pretreatment Baseline Visit:

- Recording of Medication History (see [Section 6.4](#))
- Recording of weight (in kilograms) (see [Section 7.2.2](#))
- Quantitative Muscle Testing (QMT) (see [Section 7.4.1](#))
- Time to Run/Walk Test (TTRW) (see [Section 7.4.2](#))

- Time to Stand Test (TTSTAND) (see [Section 7.4.3](#))
- Time to Climb Test (TTCLIMB) (see [Section 7.4.4](#))
- North Star Ambulatory Assessment (NSAA) (see [Section 7.4.5](#))
- Six-minute Walk Test (6MWT) (see [Section 7.4.6](#))
- Enrollment
- Recording of AEs and SAEs; review of all AEs for resolution status and date (see [Section 7.2.6](#))

6.3.3 Treatment Period Day 1 Visit

Treatment Period Day 1 begins with administration of the first dose of study medication. The Day 1 Visit occurs on the day following the Baseline Visit. However, the 0.5 hour pre-dose vital signs and PK and PD blood draws must occur at the same visit as the Day 1 procedures, and therefore are listed below as part of the Day 1 Visit procedures.

Subjects must have fasted ≥ 6 hours prior to arrival at the study site for Day 1 procedures and assessments. The pre-dose blood draws for PK and PD (insulin and glucose) determination must be collected after subjects have fasted for ≥ 6 hours. Breakfast will be served at the study site, prior to administration of the first dose of study medication.

The following procedures will be completed at the Day 1 Visit:

Pre-dose Assessments:

- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) at 0.5 hour prior to first dose of study medication (see [Section 7.2.3](#))
- Blood collected for plasma PK analysis at 0.5 hour prior to first dose of study medication; subjects must have fasted for ≥ 6 hours prior to blood draw (see [Section 7.5](#))
- Blood samples for PD biomarkers (i.e., insulin and glucose only) at 0.5 hour prior to first dose of study medication; subjects must have fasted for ≥ 6 hours prior to blood draw (see [Section 7.3](#))

- Consumption of an 8 ounce glass of whole milk (or equivalent high fat food portion) and 1 cup of cereal 30 minutes prior to administration of the dose of study medication.
- Dispense study medication and subject diaries (see [Sections 5.3, 5.6.1, and 5.5](#))
- Study Medication Acceptability Assessment (smell) (see [Section 7.2.7](#))
- Administration of the first dose of study medication (see [Section 5.3](#))

Post-dose Assessments:

- Study Medication Acceptability Assessment (taste) (see [Section 7.2.7](#))
- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) at 0.5, 1, 2, 4, 6, and 8 hours post-dose (see [Section 7.2.3](#))
- Blood samples for PK at 1, 2, 4, 6, and 8 hours post-dose (see [Section 7.5](#))
- Blood samples for PD biomarkers including cortisol, ACTH, P1NP, osteocalcin, CTX, 17- hydroxyprogesterone, testosterone, corticosterone, and 11-deoxycortisol at 6 hours post-dose. (see [Section 7.3](#))
- Safety monitoring at the participating study site for at least 8 hours after dosing
- Recording of AEs and SAEs; review of all AEs for resolution status and date (see [Section 7.2.6](#))
- Recording of concomitant medications administered after study medication dosing (see [Section 6.4](#))

The subject will remain at the participating study site for at least 8 hours after administration of the first dose of study medication to accommodate PK and PD blood sampling and to allow safety monitoring. Following the 8-hour post-dose assessments, the subject, when stable, may leave the center.

6.3.4 Treatment Period Week 1 (Day 7) Visit (Day 7 ± 1 day)

Subjects will return to the study site for the Week 1 (Day 7) safety assessments 7 days ± 1 day after receiving the first dose of study medication.

The following procedures will be completed at the Week 1 Visit:

- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) (see [Section 7.2.3](#))
- Recording of AEs and SAEs; review of all AEs for resolution status and date (see [Section 7.2.6](#))
- Recording of concomitant medications (see [Section 6.4](#))
- Return of subject diaries and distribution of new subject diaries (see [Section 5.5](#))

The subject may leave the center following completion of all scheduled Week 1 assessments.

6.3.5 Treatment Period Week 2 Visits

6.3.5.1 Treatment Period Week 2 (Day 13) Visit (Day 13 ± 1 day)

Subjects will return to the study site on Day 13 (± 1 day after receiving the first dose of study medication) for the scheduled Week 2 exploratory clinical efficacy assessments.

The following assessments will be performed on Day 13:

- Quantitative Muscle Testing (QMT) (see [Section 7.4.1](#))
- Time to Run/Walk Test (TTRW) (see [Section 7.4.2](#))
- Time to Stand Test (TTSTAND) (see [Section 7.4.3](#))
- Time to Climb Test (TTCLIMB) (see [Section 7.4.4](#))
- North Star Ambulatory Assessment (NSAA) (see [Section 7.4.5](#))
- Six-minute Walk Test (6MWT) (see [Section 7.4.6](#))

The subject may leave the center following completion of all scheduled Day 13 assessments.

6.3.5.2 Treatment Period Week 2 (Day 14) Visit (Day 14 ± 1 day)

Subjects will return to the study site for the Week 2 (Day 14) safety, PK, and PD assessments 14 days ± 1 day after receiving the first dose of study medication. The final dose of study medication will be administered at the Week 2 (Day 14) Visit following pre-dose PK and PD blood draws.

Subjects must have fasted ≥ 6 hours prior to arrival at the study site for Week 2 (Day 14) procedures and assessments. The pre-dose blood draws for PK and PD (insulin and glucose) determination must be collected after subjects have fasted for ≥ 6 hours. Breakfast will be served at the study site, prior to administration of the final dose of study medication.

The following procedures will be completed during the Week 2 Visit:

Pre-dose Assessments:

- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) at 0.5 hour prior to final dose of study medication (see [Section 7.2.3](#))
- Blood samples for PK at 0.5 hour prior to final dose of study medication; subjects must have fasted for ≥ 6 hours prior to blood draw (see [Section 7.5](#)). Sample will also be used for MIST assessment (see [Section 7.2.8](#)).
- Blood samples for PD biomarkers (i.e., insulin and glucose only) at 0.5 hour prior to final dose of study medication; subjects must have fasted for ≥ 6 hours prior to blood draw (see [Section 7.3](#))
- Consumption of an 8 ounce glass of whole milk (or equivalent high fat food portion) and 1 cup of cereal 30 minutes prior to administration of the dose of study medication.
- Study Medication Acceptability Assessment (smell) (see [Section 7.2.7](#))
- Administration of the final dose of study medication (see [Section 5.3](#))

Post-dose Assessments:

- Study Medication Acceptability Assessment (taste) (see [Section 7.2.7](#))
- Complete physical examination, including weight (in kilograms) (see [Section 7.2.2](#))
- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) at 0.5, 1, 2, 4, 6, and 8 hours post-dose (see [Section 7.2.3](#))

- Blood samples for PK at 1, 2, 4, 6, and 8 hours post-dose (see [Section 7.5](#)). Samples will also be used for MIST assessment (see [Section 7.2.8](#)).
- Clinical labs including hematology, clinical chemistry, lipids, and urinalysis at 8 hours post-dose (see [Section 7.2.4](#))
- Blood samples for PD biomarkers including cortisol, ACTH, P1NP, osteocalcin, CTX, 17-hydroxyprogesterone, testosterone, corticosterone, and 11-deoxycortisol (6 hours post-dose). Blood will also be collected and stored for future proteomics profiling and SomaScan studies (see [Section 7.3](#)).
- 12-lead ECG (see [Section 7.2.5](#))
- Recording of AEs and SAEs; review of all AEs for resolution status and date (see [Section 7.2.6](#))
- Recording of any concomitant medications administered to the subject (see [Section 6.4](#))
- Return of study medication and subject diaries (see [Sections 5.3 and 5.5](#))

The subject will remain at the study site for at least 8 hours after administration of the final dose of study medication to accommodate post-dose PK/MIST blood sampling and safety assessments. The subject, if stable, may leave the center following completion of all scheduled Week 2 assessments.

6.3.6 Post-treatment Follow-up Telephone Contact (Day 21 ± 2 days)

Study staff will contact the subject's parent or guardian by telephone at Week 3 (Day 21) (Day 21 ± 2 days after administration of the first dose of study medication, and approximately 7 days after receiving the final dose of study medication) to query the parent or guardian regarding any AEs or SAEs experienced by the subject and any concomitant medications taken. Any AEs or SAEs that meet the criteria for dose-limiting toxicities (see [Section 8.3](#)) must be reported immediately to the Study Chair and Medical Monitor and/or PRA safety management team (SAEs only). Adverse events and SAEs will be recorded in the source document and in the eCRF.

6.3.7 Post-treatment Follow-up Week 4 (Day 28) Visit (Day 28 ± 3 days)

Subjects will return to the study site for the Post-treatment Follow-up safety, PD, and exploratory clinical efficacy assessments at Week 4 (Day 28) (Day 28 ± 3 days after administration of the first dose of study medication, and approximately 14 days after receiving the final dose of study medication).

The following procedures will be completed during the Post-treatment Follow-up Visit:

- Complete physical examination, including weight (in kilograms) (see [Section 7.2.2](#))
- Recording of vital signs (supine blood pressure, heart rate, oral temperature, respiratory rate) (see [Section 7.2.3](#))
- Clinical laboratory evaluation including hematology, clinical chemistry, lipids, and urinalysis tests (see [Section 7.2.4](#))
- Blood samples for PD biomarkers including cortisol, ACTH, P1NP, osteocalcin, CTX, 17-hydroxyprogesterone, testosterone, corticosterone, and 11-deoxycortisol. Blood will also be collected and stored for future proteomics profiling and SomaScan studies (see [Section 7.3](#)).
- 12-lead ECG (see [Section 7.2.5](#))
- Quantitative Muscle Testing (QMT) (see [Section 7.4.1](#))
- Time to Run/Walk Test (TTRW) (see [Section 7.4.2](#))
- Time to Stand Test (TTSTAND) (see [Section 7.4.3](#))
- Time to Climb Test (TTCLIMB) (see [Section 7.4.4](#))
- North Star Ambulatory Assessment (NSAA) (see [Section 7.4.5](#))
- Six-minute Walk Test (6MWT) (see [Section 7.4.6](#))
- Recording of AEs and SAEs; review of all AEs for resolution status and date (see [Section 7.2.6](#))
- Recording of any concomitant medications administered to the subject (see [Section 6.4](#))

Following completion of the Post-treatment Week 4 Follow-up assessments, the subject may be discharged from the study and offered enrollment in the extension study.

6.4 Prior and Concomitant Medications and Therapies

All medications (prescription and over-the-counter [OTC]) taken within 30 days prior to the Screening Visit and during the study must be recorded in the source document and in the eCRF, including the name of the medication (or device or procedure), the dosage and regimen, the indication, and the treatment start and stop dates. All past (lifetime) steroid use will be recorded. Furthermore, each change in concomitant medications (e.g., new treatment, discontinuation of treatment, or change in dosage/regimen) during the study must be documented in the same manner. Details of any non-pharmacological therapies (e.g., devices, procedures), including name and dates of therapy, will also be recorded. Site personnel will review the information with the subject or his parent or guardian, if applicable, for completeness and accuracy at each study visit.

6.4.1 Prohibited Therapies

Subjects must discontinue use of the following medications prior to participation in the study, as indicated, and refrain from using these medications throughout the duration of the study:

- Mineralocorticoid receptor agents, such as spironolactone, eplerenone, canrenone (canrenoate potassium), prorenone (prorenoate potassium), mexrenone (mexrenoate potassium); use must be discontinued at least 4 weeks prior to the first dose of study medication;
- Oral glucocorticosteroids or other oral immunosuppressive agents. Subjects who have received more than 3 months cumulative treatment with oral immunosuppressive agents or last treatment within 3 months prior to first dose of study medication are ineligible for study entry. [Note: Inhaled and/or topical corticosteroids prescribed for an indication other than DMD are permitted but must be administered at stable dose for at least 3 months prior to study drug administration];

- Idebenone (use must be discontinued at least 4 weeks prior to first dose of study medication);
- Live attenuated vaccines (use must be avoided for the duration of participation in the study);
- Any investigational medications other than vamorolone (use must be discontinued at least 3 months prior to the first dose of study medication).

The Investigator should contact the Study Chair and Medical Monitor concerning individual medications or therapies not listed that may be of concern.

6.4.2 Permitted Therapies

Every effort should be made NOT to take any new prescription or OTC medications during the study, but particularly on Day 1 and Week 2 (Day14) Visit days. Concomitant medications should be maintained at the same dose and regimen throughout the study whenever possible. However, all other medications other than those specifically prohibited above may be taken during the study, if clinically indicated, provided they are recorded in the source document and in the eCRF.

6.5 Subject Discontinuation

In the event that a subject withdraws early from the study at any time, the reason for discontinuation must be fully documented in the source documents and the eCRF. The site personnel will document any AEs and other assessments in the source documents and will make every effort to complete all applicable Week 2 (Days 13 and 14) assessments (for subjects who withdraw prior to the scheduled Week 2 [Day 14] Visit - see [Section 6.3.5](#)) and Post-treatment Follow-up Week 4 (Day 28) assessments (for all subjects who withdraw early - see [Section 6.3.7](#)).

6.6 Study Completion

A completed subject is defined as a subject who has completed the 14-day Treatment Period and the 14-day Post-treatment Follow-up Period, and has not prematurely withdrawn from the study for any reason.

7 STUDY ASSESSMENTS AND MEASUREMENTS

7.1 Demographic Assessments

Demographic information (birth date, race, and ethnicity) will be collected during the Pretreatment Screening Period and will be recorded on the appropriate eCRF.

7.2 Safety and Tolerability Assessments

7.2.1 *Medical History*

The medical history will be recorded at the Screening Visit and will include significant past medical or surgical procedures as well as previous and current co-existent diseases. It should include the date (month/year) the subject was diagnosed with DMD, initial symptoms of DMD and the age at which they were first identified, and any toxicities or allergies to prior treatments. It should include relevant medical history for the following body systems: head, eyes, ears, nose and throat (HEENT), respiratory, cardiovascular, gastrointestinal, endocrine, hematological, dermatological, genital-urinary, neurological, musculoskeletal, psychological/psychiatric, and any other history of medical significance. The medical history will be recorded on the appropriate eCRF.

7.2.2 *Physical Examination with Weight*

A complete physical examination including height (in cm) and weight (in kg) will be performed at the Screening, Week 2 (Day 14), and Week 4 (Day 28) Visits, and will include examination of the following: head, eyes, ears, nose, and throat, neck (*including an examination of the thyroid*), heart, lungs, abdomen (*including an examination of the liver and spleen*), lymph nodes, extremities, nervous system, and skin. Height will be recorded at the Screening Visit only. Weight recorded at the Screening Visit will be used to calculate the vamorolone dose volume to be administered daily throughout the 14-day Treatment Period (see [Appendix 15.1](#)). Weight will also be recorded at the Baseline Day -1 Visit. Additional unscheduled symptom-directed physical examinations may be conducted at any time per the Investigator's discretion. Results will be recorded on the appropriate eCRF.

7.2.3 Vital Signs

Vital signs (supine blood pressure, heart rate, respiration rate, and oral temperature) will be recorded at each study visit. On Days 1 and 14, vital signs will be recorded at 0.5 hour pre-dose and 0.5, 1, 2, 4, 6, and 8 hours post-dose. At time points which coincide with PK blood draws, vital signs will be recorded prior to the PK blood draw.

Vital signs will be recorded after the subject has been resting for at least 5 minutes. Results will be recorded on the appropriate eCRF.

If vital signs assessment is performed at the same study visit as blood sampling and ECG recording, the following sequence should be followed: 1) ECG recording; 2) vital signs assessment; and 3) blood sampling.

7.2.4 Clinical Laboratory Tests

Each subject will have blood drawn and urine collected for the hematology, chemistry, lipids, and urinalysis clinical laboratory tests listed in [Table 7](#) and [Table 8](#). Clinical laboratory testing will be performed at the Screening, Week 2 (Day 14), and Week 4 (Day 28) Visits. In addition, all subjects will have blood drawn at the Screening Visit for determination of anti-varicella IgG antibodies; a positive test result, as assessed by the testing laboratory, must be recorded prior to enrollment in the study. All blood and urine samples will be sent to the designated central laboratory for testing.

Any subject deemed ineligible for enrollment due to a negative test result at Screening for anti-varicella IgG antibodies may be vaccinated and return for rescreening and retest approximately 30 days following vaccination.

For the hematology, chemistry, and lipids laboratory tests, blood will be collected by direct venipuncture of peripheral veins. Approximately 4.5 mL of blood will be obtained for clinical laboratory tests (hematology, chemistry, and lipids) at each scheduled visit. An additional 2.5 mL of blood will be collected by direct venipuncture of peripheral veins for anti-varicella IgG antibody testing. A total of approximately 16.0 mL of blood

will be collected over the course of this study for clinical laboratory evaluation (see **Section 7.6** for details of blood volumes to be collected during the study).

If blood sampling is performed at the same study visit as vital signs assessment and ECG recording, the following sequence should be followed: 1) ECG recording; 2) vital signs assessment; and 3) blood sampling. If PK and PD or clinical laboratory blood samples are to be collected at the same time point, the PK blood sample should be collected prior to the PD blood sample(s), which in turn should be collected prior to the clinical laboratory blood samples.

Any abnormal hematology, chemistry, lipid, or urinalysis test result deemed clinically significant by the Investigator or medically qualified sub-investigator may be repeated, including test results obtained on the final study day. Clinically significant results obtained at Screening should elicit a referral to the subject's personal healthcare provider.

Any treatment-emergent abnormal laboratory test result that is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE section of the eCRF:

- Accompanied by clinical symptoms
- Requiring a change in concomitant therapy (e.g., addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy, or treatment)
- Is otherwise considered clinically significant by the Investigator

Any clinically significant test abnormality as defined above should be recorded as an AE (unless it was considered spurious), and repeat analysis performed until resolution or until the Investigator or medically qualified sub-investigator determines that resolution of the abnormality is not expected.

Table 7: Hematology, Chemistry, and Lipids Clinical Laboratory Tests

Hematology	
Red Blood Cell Count	Numerical platelet count (estimate not acceptable)
Hemoglobin	White Blood Cells (WBC) with differential (percent)
Hematocrit	
Chemistry	
Sodium	Total Bilirubin ^a
Potassium	Uric Acid
Chloride	Glucose
Calcium	Alkaline phosphatase (ALP)
Inorganic Phosphorus	Gamma Glutamyl Transferase (GGT)
Blood Urea Nitrogen (BUN)	Aspartate aminotransferase (AST)
Creatinine	Alanine aminotransferase (ALT)
Total Protein	Creatine kinase (CK)
Albumin	Lipase
Bicarbonate	Amylase
Lactate Dehydrogenase (LDH)	
Lipids	
Triglycerides	Low Density Lipoprotein (LDL)
Total cholesterol	High density Lipoprotein (HDL)

^aIf outside normal range, direct bilirubin will be measured and reported.

Urine will be collected for routine analysis, by dipstick and microscopic analysis, for the tests described in **Table 8**. Urine will be analyzed by dipstick and microscopic analysis by the central laboratory at all assessment time points.

Table 8: Urinalysis Clinical Laboratory Tests

Urinalysis (including microscopic examination)	
Dipstick^a	Microscopic Analysis
Protein	WBC/hpf
Glucose	RBC/hpf
Ketones	Casts
pH	Bacteria
Leukocyte esterase	
Blood	

^aA midstream clean-catch urine specimen will be collected for dipstick analysis.

Clinical laboratory tests will be performed by a central laboratory; results will be reported to the study site and transferred electronically into the clinical study database.

The procedures for the collection, handling, and shipping of laboratory samples will be specified in the Laboratory Manual(s) provided to the study site.

7.2.4.1 Follow-up of Abnormal Laboratory Test Results

In the event of a medically significant, unexplained, or abnormal clinical laboratory test value, the test(s) may be repeated, evaluated by the Investigator for sustainability and reproducibility to determine if the abnormality represents an AE, and followed-up until the results have returned to the normal range, stabilized, and/or an adequate explanation for the abnormality is found. If a clear explanation is established, it should be recorded in the source document and eCRF. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range and the site Investigator will indicate which of these deviations are clinically significant. These clinically significant deviating laboratory results will then be further described as AEs, and the relationship to the treatment will be indicated (see [Section 7.2.6](#)).

7.2.5 12-Lead ECG

A 12-lead ECG will be recorded at the Screening, Week 2 (Day 14), and Week 4 (Day 28) Visits. 12-lead ECGs must be performed using a standard high-quality, high-fidelity machine equipped with computer-based interval measurements. Digital ECG recording is recommended. Automated ECG intervals (QRS duration, PR [PQ] interval, RR interval [interbeat interval], QT interval, QTc, and heart rate) will be captured or calculated.

12-lead ECGs will be obtained over a 3 to 5-minute period after the subject has been resting quietly in a supine position for at least 5 minutes. 12-lead ECGs should be recorded prior to vital signs at each assessment time point.

12-lead ECG results will be read locally. Results must be interpreted and recorded on the appropriate eCRF.

7.2.6 Adverse Events and Serious Adverse Events

An AE is any untoward medical occurrence in a subject which does not necessarily have to have a causal relationship with the intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the product. Pre-existing conditions that worsen during a study are to be reported as AEs.

Adverse events will be recorded from the date of informed consent and through the time of the subject's last study visit (study completion or early discontinuation). Serious adverse events will be recorded from the date of informed consent, throughout the clinical trial, and for up to 30 days after the final administration of study drug. Subjects who enroll in the VBP15-003 extension study will have any AEs which initiated during VBP15-002 participation and which are ongoing at the time of enrollment into VBP15-003 recorded in the VBP15-003 eCRF and followed during VBP15-003 participation. In addition, subjects (and their parent or legal guardian) will be questioned by study staff at each study visit for any new signs or symptoms or changes in existing signs or symptoms.

All AEs and SAEs that are spontaneously reported, identified during questioning, or are apparent from a participant's physical appearance will be recorded in the source document and in the subject's eCRF. The date and time of onset will be recorded. Any laboratory abnormality that is outside the normal range and is considered an AE (see [Section 7.2.4](#)) should be recorded as an AE on the appropriate eCRF. The details recorded shall include the nature, date of onset, final outcome and its date, intensity assessment (CTCAE grade), and a determination of the relationship of the event to administration of the study drug (i.e., causality). All AEs will be graded by CTCAE, Version 4.03. Details of any medications given to the subject to abate the AE should be recorded in the appropriate eCRF.

Intensity

All clinical AEs encountered during the clinical study will be recorded in the eCRF. Intensity of AEs will be graded using the most current version of the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, 5-point scale, and reported in detail as indicated on the eCRF. A description of the intensity scales can be found below:

Mild (Grade 1): Asymptomatic or mild symptoms: clinical or diagnostic observations only; intervention not indicated.

Moderate (Grade 2): Minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).

Severe (Grade 3): Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL; incapacitating with inability to work or perform normal daily activity.

Life-Threatening (Grade 4): Urgent intervention indicated.

Death (Grade 5): Death related to AE.

Relationship

Relationship to study drug will be graded on a 5-point scale (definite, probable, possible, remote, or unrelated). A description of the relationship scale can be found below:

Definite: This category applies to an AE that meets at least criteria 1, 2, and 4 of the “Probable” category.

Probable: This category applies to those AEs that are considered, with a high degree of certainty, to be related to the study drug. An AE may be considered probable, if (must include first 3):

1. It follows a reasonable temporal sequence from administration of the study drug.

2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases after dosing is complete. (There are important exceptions when an AE does not disappear upon discontinuation of study drug, yet drug relatedness clearly exists, e.g., [1] bone marrow depression and [2] tardive dyskinesia.)
4. It follows a known pattern of response to the suspected study drug.

Possible: This category applies to those AEs for which the connection with study drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related to study drug if or when (must include first 2):

1. It follows a reasonable temporal sequence from administration of the study drug.
2. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It follows a known pattern of response to the suspected study drug.

Remote: In general, this category is applicable to an AE that meets the following criteria (must include the first 2):

1. It does not follow a reasonable temporal sequence from administration of the study drug.
2. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It does not follow a known pattern of response to the suspected study drug.

Unrelated: This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.) and do not meet the criteria for study drug relationship listed under remote, possible, or probable.

Clinical Laboratory Test Abnormalities

Clinical laboratory test results will be recorded in the designated eCRF. The intensity of abnormal clinical laboratory test results that are AEs will also be graded using the most current version of the CTCAE, version 4.03, 5-point scale and reported in detail as indicated in the eCRF. A description of the intensity scale can be found above.

Any treatment-emergent abnormal clinical laboratory test result that is clinically significant, i.e., meeting one or more of the following conditions, should be recorded as a single diagnosis on the AE section of the eCRF:

- Accompanied by clinical symptoms
- Requiring a change in concomitant therapy (e.g., addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy, or treatment)
- Is otherwise considered clinically significant by the Investigator

This applies to any protocol and non-protocol-specified safety laboratory result from tests performed after the first dose of study drug, which falls outside the laboratory reference range and meets the clinical significance criteria per Investigator standard operating procedures (SOPs).

This does not apply to any abnormal laboratory result that falls outside the laboratory reference range, but does not meet the clinical significance criteria (which will be analyzed and reported as laboratory abnormalities); those that are considered AEs of the type explicitly exempted by the protocol; or those that are the result of an AE which has already been reported.

Please Note: any clinical laboratory abnormality fulfilling the criteria for an SAE should be reported as such, in addition to being recorded as an AE in the eCRF.

Follow-Up of Adverse Events

Adverse events will be followed until they have returned to baseline status, stabilized, or the Investigator, Study Chair, Medical Monitor and Sponsor agree that follow-up is no longer needed. If a clear explanation of cause is established, it should be recorded in the source document and eCRF. In the event of unexplained abnormal laboratory test values, the tests may be repeated as soon as possible and followed up until they have returned to the normal range or baseline value and/or an adequate explanation of the abnormality is found. In case of ongoing AEs at the time of database closure, the data obtained at the time of database closure will be used in the statistical analysis. The follow-up of AEs will be documented in the source documents and will be described in the final report only if considered relevant by the Investigator, the Study Chair, the Medical Monitor and/or the Sponsor.

In addition, the Medical Monitor may request additional blood tests, diagnostic imaging studies, or specialist physician consultations in order to further evaluate any AE or test abnormality considered to be clinically significant by the Study Sponsor.

Dosing Error

For the purposes of this study, a dosing error is defined as a dose exceeding or less than the scheduled dose of 0.25 mg/kg, 0.75 mg/kg, 2.0 mg/kg, or 6.0 mg/kg specified for each dose level group. Such occurrences should be reported and recorded in the dosing page of the eCRF and as follows:

- Use of study medication in doses in excess of that specified in the protocol should not be recorded as an AE unless there are associated signs or symptoms.
- A dosing error with associated non-serious AEs should be recorded as AEs on the relevant AE forms in the eCRF.
- A dosing error with an associated SAE should be recorded as an SAE.

- Details of all dosing errors, including actual dose administered, should be documented in the source document and recorded on the Protocol Deviations eCRF.

7.2.6.1 *Serious Adverse Events*

For treatment-eligible subjects, SAEs will be collected and reported during the study from the time informed consent is obtained through 30 days after the final dose of study medication, according to the protocol and applicable regulations.

All SAEs, including those that continue beyond the normal AE collection period (i.e., are ongoing at the subject's last study visit), will be followed until resolution or until stabilized without sequelae. Serious adverse events that begin after the subject's participation in the study is complete but that the Investigator considers to be related to study drug will be reported to the Sponsor within 24 hours of discovery by the Investigator.

During the SAE collection period, the Investigator or clinical site personnel should notify the PRA safety management team of all SAEs, regardless of relationship to the investigational drug, within 24 hours of clinical staff becoming aware of the event; notification to the PRA safety management team will trigger alerts to the Coordinating Center, Study Chair, the Sponsor, and the Medical Monitor. The Investigator will provide the initial notification by completing the SAE eCRF in the electronic data capture (EDC), which must include the Investigator's assessment of the relationship of the event to investigational drug. In the unlikely event that the electronic study database is inaccessible and the Investigator is unable to complete the SAE eCRF within 24 hours, the SAE Notification Form (pdf) should be completed and emailed or printed/faxed to the PRA safety management team.

In addition, notification is sent by the Investigator to the IRB and the subject's Primary Care Physician.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the PRA safety management team within 24 hours of knowledge of the new or follow-up information, which will forward the information to the Coordinating Center, Study Chair, the Sponsor, and the Medical Monitor.

All SAE reports should be completed within the EDC.

An AE or suspected adverse reaction is considered serious if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Is fatal (results in the outcome of death)
- Is life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly or birth defect
- Is an important medical event that may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

The term “sudden death” should only be used when the cause is of a cardiac origin as per standard definition.

The terms death and sudden death are clearly distinct and must not be used interchangeably.

Any AE or clinically significant abnormal laboratory test value, as determined by the Investigator, that is serious and which occurs during the course of the study (as defined above) must be reported to the PRA safety management team, who will notify the Coordinating Center, Study Chair, the Sponsor, and the Medical Monitor within 24 hours of the Investigator becoming aware of the event. Additional information that becomes available for an SAE after the initial report is submitted will be reported to the PRA safety management team, who will notify the Coordinating Center, Study Chair, the

Sponsor, and the Medical Monitor within 24 hours of the Investigator becoming aware of the new information.

Related SAEs MUST be collected and reported regardless of the time elapsed from the last administration of study drug, even if the study has been closed. Unrelated SAEs must be collected and reported during the study from the time of informed consent through 30 days after the final dose of study medication.

If, at any time during the study, a subject experiences an SAE, appropriate care should be instituted.

In the event of an SAE, the Investigator will complete the SAE eCRF within 24 hours of first awareness of the event. In the unlikely event that the electronic study database is inaccessible and the Investigator is unable to complete the SAE eCRF within 24 hours, the SAE Notification Form (pdf) should be completed and emailed or printed/faxed to the PRA safety management team within 24 hours, using the contact information below:

Email CHOSafety@prahs.com

Drug Safety Fax: 1-888-772-6919 or 1-434-951-3482

SAE Questions: Drug Safety Hotline: 1-800-772-2215 or 1-434-951-3489

Serious Adverse Events will be recorded from the time the subject's written informed consent is obtained. Serious adverse events that occur within 30 days of study drug dosing must continue to be recorded and reported to the Study Sponsor or its designee. Should there be an SAE that occurs that suggests an increased risk to the participants, the following steps will be considered, depending on the number and severity of the SAE(s): modification of the protocol, investigation of the relationship of the SAE(s) to study drug, suspension of the study, and/or discontinuation of the study.

7.2.7 Study Medication Acceptability Assessment

Acceptability of the study medication will be assessed using a 5-point hedonic scale immediately before (to assess smell) and after (to assess taste) dosing on Study Days 1

and 14. The assessments will be administered by trained study staff. Results will be recorded in the source documents and eCRF.

7.2.8 *Metabolites in Safety Testing (MIST)*

A portion of each blood sample collected for PK testing (see [Section 7.5](#)) at each of the Week 2 (Day 14) assessment time points will be used for analysis of vamorolone metabolites. No additional blood volume will be collected for MIST assessment (see [Table 10](#)).

7.3 Pharmacodynamic Biomarker Panel

The effect of vamorolone on biomarkers of muscle cellular pathology and biomarkers associated with acute and chronic glucocorticoid treatment, as listed in [Table 9](#), will be explored through periodic blood sampling. Samples for analysis of PD biomarkers response will be collected at Screening, Baseline Day -1 (0.5 hours pre-dose), Day 1 (6 hours post-dose), Week 2 (Day 14) (0.5 hours pre-dose and 6 hours post-dose), and Week 4 (Day 28). The biomarker analysis will include cortisol, ACTH, P1NP, osteocalcin, CTX, 17-hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, glucose, and insulin. In addition, HbA1c will be drawn at screening as a baseline. An additional assay by Immulite technology will be done to assess levels of these PD biomarkers from leftover blood samples collected for the PK sampling done on Day 1 and Day 14. Blood will also be collected at Screening, Week 2 (Day 14), and Week 4 (Day 28) and stored at these time points for future proteomics profiling and SomaScan studies.

Subjects must have fasted ≥ 6 hours prior to the pre-dose blood draws for insulin and glucose PD determination on Day 1 and Day 14.

A total of 68 mL of blood will be collected for PD biomarkers over the course of the 8-week study.

Table 9: Pharmacodynamic Biomarkers

Adrenal Axis Suppression
Cortisol
17- hydroxyprogesterone
ACTH
Testosterone
Corticosterone
11- deoxycortisol
Insulin Resistance
Glucose
Insulin
HbA1c
Bone Turnover
Osteocalcin
P1NP
CTX
Exploratory Biomarkers
SomaScan and Proteomics Profiling

If blood sampling is performed at the same study visit as vital signs assessment and ECG recording, the following sequence should be followed: 1) ECG recording; 2) vital signs assessment; and 3) blood sampling. If PK and PD or clinical laboratory blood samples are to be collected at the same time point, the PK blood sample should be collected prior to the PD blood sample(s), which in turn should be collected prior to the clinical laboratory blood samples. The exact times of blood sampling will be recorded in the source document and eCRF. Tests for PD biomarkers will be performed by a central laboratory; results will be reported to the study site and transferred electronically into the clinical study database. The procedures for the collection, handling, and shipping of laboratory samples will be specified in the Laboratory Manual(s) provided to the clinical study sites.

7.4 Exploratory Clinical Efficacy Endpoints

Exploratory clinical efficacy parameters will be assessed at the Screening and Baseline Visits, and at the Week 2 (Day 13) and Week 4 (Day 28) Visits.

7.4.1 Quantitative Muscle Testing (QMT)

Quantitative Muscle Testing (QMT) will be administered to all subjects using the CINRG Quantitative Measurement System (CQMS).³¹ The QMT will measure unilateral strength of four muscle groups: elbow flexion/extension, and knee flexion/extension.³¹ Complete instructions for administering the QMT are given in the study manual to be supplied to the sites prior to study start.

Results will be collected using the CQMS3 software and transferred into the eCRF.

7.4.2 Time to Run/Walk Test (TTRW)

The Time to Run/Walk Test (TTRW) measures the time (in seconds) that it takes the subject to run or walk 10 meters and is administered as part of the NSAA (see [Section 7.4.5](#)). Complete instructions for administering and scoring the TTRW are given in the study manual to be supplied to sites prior to study start.

Results will be recorded in the source document and in the eCRF.

7.4.3 Time to Stand Test (TTSTAND)

The Time to Stand Test (TTSTAND) measures the time (in seconds) required for the subject to stand to an erect position from supine (floor), and is administered as part of the NSAA (see [Section 7.4.5](#)). The TTSTAND test must be performed without assistance at the Screening and Baseline Visits to qualify for enrollment into the study. Complete instructions for administering and scoring the TTSTAND are given in the study manual to be supplied to the sites prior to study start.

Results will be recorded in the source document and in the eCRF.

7.4.4 Time to Climb Test (TTCLIMB)

The Time to Climb Test (TTCLIMB) measures the time (in seconds) required for the subject to climb 4 standard stairs, beginning and ending in a standing position with arms at the sides.³² Complete instructions for administering the TTCLIMB are given in the study manual to be supplied to the sites prior to study start.

Results will be recorded in the source document and in the eCRF.

7.4.5 North Star Ambulatory Assessment (NSAA)

The North Star Ambulatory Assessment (NSAA) is a clinical assessment scale specifically designed to measure functional ability in ambulant boys with DMD.³³ The NSAA consists of 17 scored items and 2 timed tests, including the TTRW and the TTSTAND (see [Section 7.4.3](#)).

Subjects should be barefoot and wear comfortable clothing. Complete instructions for administering and scoring the NSAA are given in the study manual to be supplied to the sites prior to study start.

The NSAA should be administered BEFORE the 6MWT at study visits where both tests are administered.

Results will be recorded in the source document and in the eCRF.

7.4.6 Six-Minute Walk Test (6MWT)

Functional exercise capacity and mobility will be assessed in all subjects by means of the modified version of the Six-Minute Walk Test (6MWT), adapted for use in DMD subjects.³⁴

The total distance traveled, in meters, should be recorded along with the validity of the test as assessed by the test administrator. If a subject cannot complete 6 minutes of walking, the total meters and the time until discontinuation of the test should be recorded. Complete instructions for administering the 6MWT are given in the study manual to be supplied to the sites prior to study start.

The 6MWT should be administered AFTER the NSAA at study visits where both tests are administered.

Results will be recorded in the source document and in the eCRF.

7.5 Pharmacokinetic (PK) Assessments

On Treatment Period Day 1 and Day 14, all subjects will have blood collected for PK assessments at 0.5 hour pre-dose and at 1, 2, 4, 6, and 8 hours post-dose. Approximately 2 mL of blood will be collected into K₂-EDTA tubes at each assessment time point. A total of approximately 24 mL of blood will be collected for PK analysis over the course of the 8-week study.

Subjects should have fasted ≥ 6 hours prior to collection of PK blood samples at the 0.5 hour pre-dose time points on Days 1 and 14.

Plasma concentrations of vamorolone will be measured using a specific and validated liquid chromatography tandem mass spectrometry assay. PK assessments will be performed by a central laboratory. The procedures for the collection, handling, and shipping of laboratory samples will be specified in the Laboratory Manual(s) provided to the study sites.

The exact times of blood sampling will be recorded in the source document and eCRF.

If PK and PD or clinical laboratory blood samples are to be collected at the same time point, the PK blood sample should be collected prior to the PD blood sample(s), which in turn should be collected prior to the clinical laboratory blood samples.

7.6 Total Blood Volume Required

The number and volume of blood samples and total volume of blood to be collected from each subject throughout the duration of the 8-week study are summarized in [Table 10](#).

Table 10: Blood Sample Volume by Visit

Test	Total mL of Blood					Total Volume
	SCR	Day -1	Day 1	Week 2 (Day 14)	Week 4 (Day 28)	
Clinical Safety Labs ^a	7.0			4.5	4.5	16.0
PD Biomarker Panel	17 ^b		11 ^c	15 ^d	15 ^d	58
PD Insulin/Glucose		5 ^e		5 ^e		10
PK		2 ^f	10 ^g	12 ^{f,g}		24
Total Volume by Visit	24	7	21	36.5	19.5	
Total Volume: 108.0 mL						

SCR = Screening Period.

^a Hematology, Chemistry, Lipids. Volume collected at Screening includes 2.5 mL blood for determination of anti-varicella IgG antibodies.

^b cortisol, ACTH, P1NP, osteocalcin, CTX, 17-hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, HbA1c, SomaScan, and proteomics testing.

^c Day 1, 6 hours post dose: cortisol, ACTH, P1NP, osteocalcin, CTX, 17-hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol.

^d Day 14, 6 hours post-dose, and Week 4 (Day 28): cortisol, ACTH, P1NP, osteocalcin, CTX, 17-hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, SomaScan, and proteomics testing.

^e Insulin and glucose measured at 0.5 hour pre-dose on Day 1 and Day 14. Subjects must have fasted \geq 6 hours prior to blood draws.

^f Blood drawn for PK at 0.5 hour pre-dose on Day 1 and Day 14. Subjects must have fasted \geq 6 hours prior to blood draws. Volume collected on Day 14 includes blood for MIST assessment.

^g 1, 2, 4, 6, and 8 hours post-dose. Volume collected on Day 14 includes blood for MIST assessment. Back-up samples used for immunoassay panel.

8 SAFETY PROCEDURES AND PROCESSES

8.1 Overall Benefit/Risk

The current study is a Phase IIa study in young boys with DMD. It is anticipated that the side effect profile of the investigational product will be more favorable than standard of care glucocorticoids in the long term. The short-term side effects over the 14-day treatment period are not currently known but are predicted to be benign based on Phase I 14-day treatment in adult volunteers. In the Phase I study, no SAEs were observed in any cohort. Cohorts tested in the MAD portion of the Phase I study were 1.0, 3.0, 9.0 and 20.0 mg/kg/day for 14 days. In the Phase I clinical trial in adult volunteers, vamorolone has shown suppression of the adrenal axis at higher doses (9.0 mg/kg/day and 20.0 mg/kg/day in the fasted state).²⁹

Subjects will not receive any known health benefit from participating in the study. It is not anticipated that two weeks of treatment will fundamentally alter the course of their

disease, but minor, temporary cellular benefits within the muscle may result from vamorolone treatment. In view of the initial clinical evidence of safety and the ability to monitor key nonclinical toxicological findings, the available data support an acceptable risk profile for vamorolone.

8.2 Medical Monitor and Data and Safety Monitoring Board

The Sponsor will appoint a Medical Monitor for the study. The Medical Monitor will review all SAE reports and is responsible for identifying any safety concerns with the study. The Medical Monitor will also review all AEs that are dose-limiting in an individual subject. The Medical Monitor will review all summaries of safety data and monitor the conduct of the study on behalf of the Sponsor. The Medical Monitor will be available to the Coordinating Center for any reported SAEs. The Medical Monitor will work closely with the Study Chair on the evaluation of any dose-limiting toxicities and dose escalation decisions.

The independent Data and Safety Monitoring Board (DSMB) will be consulted to review the safety data from each dose level group. The DSMB will receive reports when it is assessed that a dose level group is completed and no more de-escalations to that dose are expected, but no less frequently than every 6 months. The DSMB will meet at regular intervals to review all pertinent safety data. The DSMB will also be notified at any point where a dose de-escalation occurs and the dose is deemed to have unacceptable toxicity. The DSMB may request summaries at other points in time. In addition, the Medical Monitor may request at any time that the DSMB review safety data if the Medical Monitor has specific concerns.

In all cases, data will be compiled by the Coordinating Center and presented to the DSMB in a format that allows for complete review of all compiled safety data. The DSMB can recommend to the Sponsor altering or terminating the trial for safety or other study integrity-related issues.

8.3 Study Drug Dose Interruption or Discontinuation (Dose-Limiting Toxicities)

Subjects will be monitored for clinical signs and symptoms throughout the duration of the study. Subjects will remain at the participating study site for at least 8 hours following administration of the first dose of study medication on Day 1 for safety monitoring of AEs and SAEs, and at least 8 hours after administration of the last dose on Day 14. Clinical AEs and clinical laboratory AEs will be graded according to the CTCAE, version 4.03.

Administration of study drug to individual subjects either at the study site or at home should be discontinued and the case reported to the Coordinating Center and discussed with the Medical Monitor within 24 hours under any of the following circumstances which constitute a dose-limiting toxicity:

1. Any CTCAE Grade ≥ 3 AE considered to be probably or definitely related to study medication, in the opinion of the site Investigator;
2. The presence of a CTCAE Grade ≥ 3 clinical laboratory AE considered to be probably or definitely related to study drug, in the opinion of the Investigator; or
3. Deterioration of the muscle condition, unexpected for the natural course of DMD and without other clear cause, in the opinion of the site Investigator. The possibility to consider the event as a dose-limiting toxicity will be discussed by the Medical Monitor and the DSMB. The SAE data collection tool must be completed if the event also meets the criteria for an SAE.

The Coordinating Center information is below:

Coordinating Center

Study Management Team

Phone: (412) 383-7207 or (412) 224-2030

Email: info@cinrgresearch.org

The Coordinating Center will notify the Medical Monitor, the Study Chair, and the Sponsor of any dose-limiting toxicity reported, whether it is an SAE or not.

All results will be retained in the source document, and results will be recorded in the eCRF.

8.4 Study Stopping Rules

If a dose-limiting toxicity occurs in two or more subjects at any dose level during the two-week Treatment Period or during the Follow up Period, the Study Chair and Medical Monitor will be immediately notified, and enrollment and dosing at that dosing level will be halted; the MTD will be defined as the previous dose level, unless the decision is made to study lower intermediate dose level(s). The DSMB will be informed promptly of any dose decisions which are a result of dose-limiting toxicities.

If dose escalation is terminated due to dose-limiting toxicities, the remaining dose level groups may be enrolled to evaluate the safety and PK effects of vamorolone at lower intermediate dose level(s). Once safety data from additional dose level group(s) are assessed, the MTD will be defined as the dose level below the dose at which the DSMB determines there to be an unacceptable risk to subjects.

If any death occurs, the study will be immediately suspended to new enrollment, the Sponsor will be notified (as well as the relevant IRB/IECs), in addition to the Study Chair, the Medical Monitor and the DSMB. Subjects currently receiving treatment will not be asked to stop their treatment unless the Medical Monitor or the DSMB requires that, but no new subjects will be enrolled until the DSMB finds it safe to do so.

The study will be considered to have completed accrual when approximately 12 subjects are enrolled at each of the safe dose levels.

8.5 Withdrawal of Subjects from Study

A subject may withdraw from the study, or may be withdrawn by his parent or guardian at any time without the need to justify the decision.

The Investigator has the right to terminate participation of a subject in the study for any of the following reasons:

- The subject's parent/legal guardian is uncooperative/noncompliant and does not adhere to study responsibilities, including failure to appear at study visits
- Difficulty in obtaining blood samples from the subject for safety monitoring
- The subject experiences an unmanageable or non-tolerable AE/SAE which is considered to be possibly, probably, or definitely related to study drug, in the opinion of the Investigator
- The Sponsor terminates the study
- Any other reason relating to subject safety or integrity of the study data.

In the event a subject is withdrawn from the study, the Coordinating Center will be informed within one business day. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until resolution of the event. The Coordinating Center will inform the Medical Monitor and the Study Chair.

All subjects who withdraw early from the study should enter the Post-Treatment Follow-up Period where possible and continue to be monitored for at least 14 days after the last dose of study drug or for as long as clinically indicated, assuming the subject has not withdrawn informed consent. Subjects who have received at least one dose of study medication and are withdrawn prior to Day 14, should undergo the Day 14 assessments at the time of early discontinuation prior to entering the 14-day Follow-up Period. Subjects who prematurely discontinue from the study after Day 14 but prior to the scheduled Day 28 Follow-up Visit should continue to be followed for at least 14 days after the last dose of study drug. In all cases, subjects should return to the study site for completion of the scheduled Day 28 assessment 14 days after the final dose of study drug, where possible. In the event a subject withdraws informed consent, no further study procedures should be performed and no additional data should be collected. Any data collected up to the point of withdrawal may be used by the Sponsor.

8.6 Replacement of Withdrawn Subjects

Subjects prematurely discontinued from the study for reasons other than safety may be replaced to ensure adequate numbers of evaluable subjects. The decision to replace a withdrawn subject will be made at the discretion of the Sponsor. The decision regarding the replacement of subjects will be documented.

8.7 Termination of Study

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. An example of a circumstance that may warrant termination is determination of unexpected, significant, or unacceptable risks to subjects.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Study Chair, the Coordinating Center and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The Coordinating Center will inform the study sites who will inform their IRB(s)/IEC(s) and provide the reason(s) for the termination or suspension.

Subject enrollment at a given site may be terminated by the Sponsor or the Study Chair. Possible reasons for termination of the study at a given site include, but are not limited to:

1. Unsatisfactory enrollment with respect to quantity or quality
2. Inaccurate or incomplete data collection
3. Falsification of records
4. Failure to adhere to the protocol.

9 DATA COLLECTION

9.1 Source Documents

Source documents are defined as original documents, data, and records. These documents may include hospital records, clinical and office charts, laboratory data/information, subjects' diaries or evaluation checklists, pharmacy dispensing and other records, recorded data from automated instruments, microfilm or magnetic media,

and/or x-rays. Data collected during this study must be recorded on the appropriate source documents.

A subject screening/enrollment log is to be completed at each study site. Data recorded on the screening/enrollment log are to include a subject identifier, the date of screening, and the reason the subject was not entered (if applicable). All subjects initially screened are to be recorded in this log.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data documents.

9.2 Electronic Case Report Form Completion

Subject data will be collected in this study using an EDC system. The EDC and database system will be OpenClinica by Akaza Research, LLC. OpenClinica is a web-based (<https://www.openclinica.com>) data entry system utilizing a high security environment. The underlying storage facility will be PostgreSQL, whose structure permits the linking of subject information across all tables in relational databases. OpenClinica uses secure socket layers (SSL) and in its Enterprise version used in this study is 21 Code of Federal Regulations (CFR) Part 11 compliant. Once an eCRF is created in the database, a data dictionary exists and the data team creates compatible paper source documentation.

The Coordinating Center will design an electronic data base in OpenClinica for this study. Access rights to the EDC system for the study site team members will need to be requested. Every user of the system will be made aware of the fact that user name and password should never be shared and their electronic signature constitutes the legally binding equivalent of a handwritten signature. Only trained personnel certified by the Coordinating Center will receive a user name and password.

All data will be directly entered or collected on a source document and then entered into OpenClinica or transferred electronically to the study database (e.g., clinical laboratory results, quantitative muscle testing). The Coordinating Center data management team will monitor the eCRFs for completeness and acceptability throughout the course of the

study. The study Sponsor or their designee will be allowed access to all source documents in order to verify eCRF entries.

9.3 Data Processing

A clinical study database will be constructed from the eCRFs and any data merged electronically, and the data will be validated both manually and electronically. Clarification of data will be requested from the study site as required. The database will be quality assured in accordance to the data management plan and will be available for statistical analysis according to the methods outlined in [Section 10.6](#) and the Statistical Analysis Plan (SAP).

10 STATISTICAL METHODS AND PLANNED ANALYSES

10.1 Statistical and Analytical Plan

Given the small sample size of this Phase IIa open-label study, no inferential statistical analyses are planned. However, all analyses will be based on modified Intention to Treat (mITT) in which all subjects who received at least one dose of vamorolone will be included in analyses. Detailed methods will be presented in the SAP, finalized prior to database lock.

10.2 Planned Sample Size

A sample of 12 in each cohort is large enough to have a confidence interval of no wider than 50% for the proportion of adverse events assessed or any other event outcome. For the continuous outcomes such as safety laboratory markers or pharmacodynamics markers, a two-sided 95.0% confidence interval for the mean will be no wider than 0.8 standard deviation (SD) from the observed mean, with 90.0% coverage probability, based on the t statistic; thus, the total confidence interval will be approximately 1.6 SDs wide for any continuous parameter if its underlying distribution is approximately normal.

10.3 Analysis Populations

Two populations will be defined for data analysis.

Safety Population:

All subjects who receive at least one dose of vamorolone study medication will be included in the Safety Population.

Pharmacokinetic Population:

All subjects who receive at least one dose of vamorolone study medication and have sufficient data for PK analysis will be included in the PK population.

10.4 Interim Analysis

No interim statistical evaluations of data will be conducted.

10.5 Statistical Considerations for Dose Escalation

There are no statistical considerations for dose escalation. The process of dose escalation will be based on the appearance of dose-limiting toxicities. See [Section 8.3](#) for definitions of dose-limiting toxicities.

10.6 Statistical Analysis

10.6.1 Analysis of Demographic and Baseline Characteristics

Subject demographics (age, race, and ethnicity) and baseline characteristics (height, weight, body mass index [BMI] and BMI percentile, and months/years since DMD diagnosis) will be summarized descriptively and the anthropometric measures plotted as box and whisker plots for all subjects combined and by dose level group. The summaries will be compared visually by dose level group and reviewed for any differences among the dose level groups.

10.6.2 Summary of Treatment Data and Subject Disposition

The number of subjects at each dose level, the determinations within the dose level of the mTPI algorithm and the compliance and completion rates of dosing at each dose level will be summarized. The number of discontinuations (if any) and whether they occurred before Day 14 or after Day 14 but before Day 28 and whether the Day 28 assessments were completed will be summarized by dose level group.

10.6.3 Safety Analyses

All subjects who received at least one dose of vamorolone (Safety Population) will be included in the safety analyses. In general, descriptive statistics for each safety endpoint will be presented by dose level, while individual subject listings of safety endpoints, sorted by dose level group, will be reviewed for any evidence of dose-related differences or trends in the safety profile of vamorolone.

All safety data will be listed.

10.6.3.1 Adverse Events Summaries

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 18.1. The incidence of TEAEs will be summarized by dose level, SOC and preferred term; dose level, SOC, and intensity (CTCAE grade; CTCAE version 4.03); dose level, SOC, and outcome; and dose level, SOC and relationship to study drug.

Clinical laboratory test results will be categorized relative to the laboratory's normal range as low, within range, or high, and shift tables comparing the distributions from Screening to Week 2 (Day 14) and Week 4 (Day 28) will be provided for each parameter.

Physical examination results will be listed only.

10.6.3.2 Vital Signs and Laboratory Outcomes

Vital signs, 12-lead ECG intervals, clinical laboratory test results, and other laboratory test results will be summarized by dose level using descriptive statistics and box and whisker plots. Descriptive statistics include median, interquartile range, and minimum and maximum. For those parameters that are either approximately normal, or can be transformed to achieve normality, a mean and 95% confidence interval (potentially back transformed) will be provided by dose level and overall.

10.6.4 Clinical Efficacy and Pharmacodynamic Analyses

The evaluations of exploratory clinical efficacy and PD will be performed using the Safety Population. The last non-missing value prior to the first dose of study medication will be used as the baseline value for analyses.

The QMT measurements will be done unilaterally using the dominant site, if known. There may also be a training session in which the subject has a practice QMT measurement prior to the corresponding study measurement. Once calculated, each muscle group (knee extension/flexion, elbow extension/flexion) will be summarized at each time point and dose assessed. Summaries will include as above descriptive statistics and box and whisker plots. Since change in strength is not expected from a two-week treatment period, all measurements will be used to obtain coefficients of variation and assess reliability in this age group. The coefficients of variation vs. age will be plotted to see whether within this developing age group, the reliability increases with growth and better comprehension of the test, or if within this narrow age group, there is no change in reliability.

The same approach will be taken for all timed function tests (TTRW, TTSTAND, TTCLIMB, 6MWT) and the NSAA.

Percentage change from Baseline in serum PD biomarker concentrations will be summarized. Descriptive statistics, including mean, standard deviation, median and range, will be provided.

Individual clinical efficacy and PD data will be listed. Individual subject changes in clinical efficacy and PD variables over time, together with their mean changes by dose group, will be presented and reviewed for any evidence of dose-related trends or any relations between the PD data and the individual clinical efficacy outcomes.

Acutely-responsive glucocorticoid PD biomarker (cortisol, ACTH, 17-hydroxyprogesterone, testosterone, corticosterone, 11-deoxycortisol, osteocalcin, CTX, P1NP) data will be listed, accompanied by indication of whether the parameter is

out of range. A summary listing of values outside the reference range will be presented and compared to published data on these same markers in glucocorticoid-treated subjects.

10.6.5 Pharmacokinetic Analyses

All PK analyses will be performed using the PK Population. Plasma concentrations and the derived plasma PK parameters will be summarized and listed for vamorolone, as appropriate. Individual and mean vamorolone concentrations versus time will be plotted on linear and semi-logarithmic scales by dose for vamorolone, as appropriate.

Summary statistics (number of subjects, mean, SD, minimum, median, maximum, geometric mean, and percentage coefficient of variation [%CV]) for the derived PK parameters of vamorolone will be presented by dose level for the PK Population.

Derived PK parameters for vamorolone will be listed by dose level. Plots of plasma concentration-time profiles will be provided by dose level. All available concentration-time data will be listed.

A (compartmental) population PK model will be developed based on data obtained from VBP15-001 (a study in healthy adults). As part of this procedure, the number of systemic compartments will be determined. In addition, linearity with respect to dose and time and appropriateness of a first-order absorption model will be evaluated. The parameters of this model, with systemic parameters scaled either by weight or allometrically (clearances scaled by weight raised to the 0.75 power), will be applied to the dosing history for pediatric subjects in VBP15-002 to predict the plasma concentration profile. The observed values will then be compared to the predicted concentration and the model adjusted as necessary.

10.6.6 Concurrent Medications

A summary of all concomitant medications taken during the course of the study will be presented in tabular form by therapeutic drug class and generic drug name using the World Health Organization (WHO) Drug classification (Version 4.3). All concomitant medications will be detailed in the subject data listings.

10.6.7 Study Deviations and Violations

A summary of study deviations and violations will be provided by dose level group and by study site.

11 STUDY MANAGEMENT AND ETHICAL AND REGULATORY REQUIREMENTS

11.1 Regulatory Approval and Good Clinical Practice

This study will be conducted in accordance with the principles of the 18th World Medical Assembly (Helsinki, June 1964), and amendments of the 29th (Tokyo, 1975), 35th (Venice, 1983), 41st (Hong Kong, 1989), 48th (Somerset West, 1996), 52nd (Edinburgh, 2000), 53rd (Washington, 2002), 55th (Tokyo, 2004), 59th (Seoul, 2008), and 64th (Fortaleza, 2013) World Medical Assemblies.

Further, the trial will be conducted in accordance with:

- International Conference on Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP)
- The United States (US) Food and Drug Administration (FDA) Code of Federal Regulations, Title 21 CFR Part 312 – IND Application, Part 50 – Protection of Human Patients with particular focus in SubPart D, and/or Part 56 – Institutional Review Boards
- US Health Insurance Portability and Accountability Act of 1996 (HIPAA)

11.2 Investigator Responsibilities

11.2.1 Subject Information and Informed Consent

Before being admitted to the clinical study, a parent/guardian for each subject must consent in writing for the subject to participate. Written or verbal assent will also be obtained from each subject as required per regulations. An approved ICF will be given to each parent/guardian, which will contain all United States federally required elements, all ICH-required elements, and HIPAA authorization information, if applicable, in language

that is understandable to the parent/guardian. The consent should note that the Investigator is receiving compensation for the expenses of conducting the study.

The process of obtaining the informed consent will be in compliance with all federal regulations, ICH requirements, and local laws.

The Investigator or designee will review the study with the parent/guardian of each subject. The review will include the nature, scope, procedures, and possible consequences of the subject's participation in the study. The consent, assent, and review must be in a form understandable to the parent/guardian of the subject. The Investigator or designee and the parent/guardian of the subject must both sign and date the ICF after review and before the subject can participate in the study. The parent/guardian of the subject will receive a copy of the signed and dated form, and the original will be retained in the site study files. The Investigator or designee must emphasize to the parent/guardian of the subject that study participation is entirely voluntary and that consent regarding study participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If the ICF is amended during the study, the Investigator must follow all applicable regulatory requirements pertaining to all new subjects and repeat the consent process with the amended ICF for any ongoing subjects.

11.2.2 Institutional Review Board/Independent Ethics Committee Approval and Other Institutional Requirements

Before the start of the study, the study protocol, ICF, and any other appropriate documents will be submitted to the IRB/IEC for review and approval. Per institutional requirements, the study protocol and any other appropriate documents will be submitted to scientific committees for approval.

The Investigator will forward to the Sponsor, or designee (Coordinating Center), a copy of the IRB/IEC's approval of this protocol, amendments, ICF and any changes to the ICF, based on the FDA regulations set forth in Part 56 of Title 21 of the CFR. The

Investigator will also keep documentation of study approval by internal scientific committees per institutional requirements.

Study medication can only be supplied to the Investigator after documentation of all ethical and legal requirements for starting the study has been received by the Sponsor or designee (Coordinating Center). This documentation must also include an IRB/IEC membership list that contains members' occupations and qualifications. If the IRB/IEC will not disclose the names of the committee members, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP. The IRB Assurance Number may be accepted as a substitute for the IRB membership list.

The Investigator will keep the IRB/IEC informed regarding the progress of the study, per institutional requirements. No changes will be made in the study without IRB/IEC approval, except when required to eliminate apparent immediate hazards to the subjects.

While the study is ongoing and at study completion/discontinuation, the Investigator must submit to the IRB/IEC the following information in accordance with US Federal regulatory requirements:

1. Information on serious or unexpected AEs, showing due diligence in providing this information as soon as possible
2. Periodic reports on the progress of the study
3. Final Study Summary upon study completion or closure.

Notification of the end of the trial will be sent to the IRB/IEC within 30 days after completion of the study close-out visit. In case the study is ended prematurely, the IRB/IEC will be notified within 15 days, including the reasons for the premature termination. The end of the trial is defined as the date of final analysis of the study data according to the SAP.

11.2.3 Study Documentation

11.2.3.1 Before the Start of the Study

The following study documentation will be in place at the study site prior to the first administration of study drug:

- Fully signed protocol and protocol-supporting manuals
- Investigator's Brochure²⁹
- Protocol Acceptance form signed by the Investigator
- IRB/IEC-approved copy of the ICF
- Curriculum vitae of the Investigator and all sub-investigators listed on the FDA Form 1572
- A letter of IRB/IEC approval for protocol
- A list of members of the IRB/IEC and their affiliations
- A copy of the Investigator-signed FDA 1572 form
- An Investigator-signed financial disclosure form

11.2.3.2 During the Study

The following documentation should be added to the site study file during study conduct:

- Any paper source forms completed and subsequently entered into the study database. An explanation should be given for all missing data and any protocol deviations documented in the site study file.
- Any changes to the documentation identified above in [Section 11.2.3.1](#)
- Shipping documents relating to shipment of medication (drug accountability) and bioanalytical samples
- Copies of relevant correspondence such as letters, emails, meeting notes, and telephone calls

11.2.3.3 After the Study

After completion or premature termination of the trial, all of the documents identified should be in the file, together with the following:

- Study drug accountability documents
- Audit certificates (if applicable)
- Investigator delegation of responsibilities log
- Site signature log
- Subject screening and enrollment logs
- Substantive correspondence with the Sponsor and IRB/IEC
- Notification of the end of the trial to the IRB/IEC

11.2.4 Delegation of Investigator Responsibilities

The Investigator must (a) ensure that any individual to whom a task is delegated is qualified by education, training, and experience (and licensure, if relevant) to perform the task; and (b) provide adequate supervision. The Investigator should maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant study-related duties.

11.3 Protocol Deviations and Violations

11.3.1 Protocol Deviation and Violation Definitions

11.3.1.1 Protocol Deviation

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the Investigator's control and that has not been approved by the IRB/IEC.

Changes or alterations in the conduct of the trial which do not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data are considered minor protocol deviations.

11.3.1.2 Protocol Violation

A protocol violation is a deviation from the IRB/IEC-approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. This includes examples such as inappropriate consent, errors in drug dosing, or lack of reporting of safety data.

11.3.2 Reporting Deviations/Violations

Upon discovery of a major protocol deviation or violation, the Investigator is responsible for reporting protocol deviations or violations to the IRB/IEC and Sponsor or designee (Coordinating Center) within 24 hours of discovery.

All other deviations must be reported in writing within 7 days of the event or its discovery.

11.4 Study Records Retention and Direct Access to Source Documents

Federal regulations require that, following completion of a clinical study, a copy of all records of that study be maintained by the Investigator for at least the shorter of the following two time periods:

- Two years after FDA approval of the drug for the indication for which it was investigated, or
- In situations where no application is to be submitted or an application is not approved for such indication, 2 years after the Investigational New Drug (IND) Application is discontinued and the FDA is notified.

The Investigator must maintain a copy of all data collected for each subject treated (including eCRFs and source data). In order to assure the accuracy of data collected in the eCRF, it is mandatory that representatives of the Sponsor, or designee, as well as representatives of the FDA or other health authorities have direct access to original source documents (e.g., subject records, subject charts, and laboratory reports). During the review of these documents, the anonymity of the subject will be respected with strict adherence to professional standards of confidentiality.

The Sponsor reserves the right to terminate the study for refusal of the Investigator to supply source documentation of work performed in this clinical study.

The following includes, but is not limited to, the records that must be retained by the Investigator:

1. Signed informed consent documents for all subjects
2. Subject screening log and enrollment log
3. Record of all relevant communications between the Investigator and the IRB/IEC.
4. Composition of the IRB/IEC
5. Record of all relevant communications between the Investigator and the Sponsor (or designee)
6. List of sub-investigators and other appropriately qualified persons to whom the Investigator has delegated significant study-related duties, together with their roles in the study and their signatures
7. Drug accountability records (see [Section 5.6.4](#))
8. Record of any body fluids or tissue samples retained
9. All other source documents (subject records, hospital records, laboratory records, etc.)
10. All other documents as listed in Section 8 of the ICH consolidated guideline on GCP (Essential Documents for the Conduct of a Clinical Trial).

11.5 Study Monitoring

In accordance with applicable regulations, GCP, and the procedures of the Sponsor or its designees, the Study Monitor will periodically contact the site and conduct on-site visits. The extent, nature, and frequency of on-site visits will be based on enrollment rate and data quality at the site. Through frequent communications (e.g., letter, e-mail, and telephone), the Study Monitor will ensure that the investigation is conducted according to protocol and regulatory requirements.

During these contacts, the monitoring activities will include:

1. Checking and assessing the progress of the study
2. Reviewing study data collected to date for completeness and accuracy
3. Reviewing compliance with protocol assessments
4. Conducting source document verification by reviewing eCRF database data against source documents when available (e.g., medical records, subject diaries, ICF [and assent, if applicable], laboratory result reports, raw data collection forms)
5. Identifying any issues and addressing resolutions.

These activities will be done in order to verify that the:

1. Data are authentic, accurate, and complete
2. Safety and rights of the subjects are being protected
3. Study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

The Investigator will allow the Study Monitor direct access to all relevant documents, and allocate his/her time and the time of his/her staff to the Study Monitor to discuss findings and any relevant issues.

In addition to contacts during the study, the Study Monitor will contact the site prior to the start of the study to discuss the protocol and data collection procedures with site personnel.

At study closure, Study Monitors will conduct all activities as indicated in [Section 11.7](#).

11.6 Quality Assurance

At its discretion, the Sponsor or its designee may conduct a quality assurance audit of this study. Auditing procedures of the Sponsor and/or its designee will be followed in order to comply with GCP guidelines and ensure acceptability of the study data for registration purposes. If such an audit occurs, the Investigator will give the auditor direct access to all relevant documents, and will allocate his/her time and the time of his/her staff to the auditor as may be required to discuss findings and any relevant issues.

In addition, regulatory authorities and/or the IRB/IEC may conduct an inspection of this study. If such an inspection occurs, the Investigator will allow the inspector direct access to all source documents, eCRFs, and other study documentation for source data check and/or on-site audit inspection. The Investigator must allocate his/her time and the time of his/her staff to the inspector to discuss findings of any relevant issues.

An explanation will be given for all missing, unused, and spurious data in the relevant section of the study report.

11.7 Study Termination and Site Closure

Upon completion of the study, the following activities, when applicable, must be conducted by the Study Monitor in conjunction with the Investigator, as appropriate:

1. Provision of all study data to the Sponsor
2. Data clarifications and/or resolutions
3. Accounting, reconciliation, and final disposition of used and unused study medication
4. Review of site study records for completeness.

In addition, the Sponsor reserves the right to temporarily suspend or prematurely terminate this study for any reason.

If the study is suspended or terminated for safety reason(s), the Sponsor will promptly inform the Investigator, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. The Investigator is responsible for promptly informing the IRB/IEC, and providing the reason(s) for the suspension or termination of the study.

If the study is prematurely terminated, all study data must be returned to the Sponsor. In addition, the site must conduct final disposition of all unused study medications in accordance with the Sponsor procedures for the study.

11.8 Site Termination

The Sponsor may at any time, at its sole discretion, terminate the study site for various reasons, including, without limitation, the following:

1. Failure of the Investigator to enroll subjects into the study at a reasonable rate
2. Failure of the Investigator to comply with applicable laws and/or pertinent FDA regulations
3. Submission of knowingly false information from the research facility to the Sponsor, Study Monitor, FDA, or other regulatory authorities
4. Insufficient adherence to protocol requirements.

If participation of a study center is terminated, the Sponsor and Study Chair will issue a written notice to the Investigator. The written notice will contain the reasons for taking such action. If the study site is terminated for noncompliance, appropriate regulatory authorities will also be notified by the Sponsor.

Study termination and follow-up will be performed in compliance with the conditions set forth in 21 CFR 312.50 and 21 CFR 312.56.

11.9 Discontinuation of Study

The Sponsor reserves the right to discontinue the study for any reason at any time. In addition, the study may be stopped at any time if, in the opinion of the Sponsor and Medical Monitor, safety data suggest that the medical safety of subjects is being or may become compromised. Dose escalation may be halted upon review of data by the Sponsor and DSMB, according to the criteria and process described in [Section 3.2](#).

12 DISCLOSURE OF DATA

12.1 Confidentiality

The rights and privacy of participants in this study will be protected at all times. All personal details of subjects will be treated as confidential by the Investigator and

handling of personal data will be in compliance with HIPAA. All applicable data protection laws in the relevant countries will be adhered to at all times.

Subject names will remain confidential and will not be included in the database. Only enrollment number and birth date will be recorded on the eCRF. If the subject's name appears on any other document collected (e.g., hospital discharge summary), the name must be obliterated before the document is transmitted to the Sponsor or its designee. All study findings will be stored in electronic databases. The subjects' parents or guardians will give explicit permission for representatives of the Sponsor, regulatory authorities, and the IRB/IEC to inspect the subjects' medical records to verify the information collected. The subjects' parents or guardians will be informed that all personal information made available for inspection will be handled in the strictest confidence and in accordance with all state, local, and federal data protection/privacy laws, including, without limitation, the HIPAA, as applicable.

The parents or guardians of all participants in the United States will provide written authorization to disclose private health information either as a part of the written ICF or as a separate authorization form. The authorization will contain all required elements specified by 21 CFR 50, and will contain a waiver of subject access to study-related private health information until the conclusion of the clinical study. The authorization will remain valid and in full force and effect until the first to occur of (1) the expiration of 2 years after the study medication is approved for the indication being studied, or (2) the expiration of 2 years after the research program is discontinued. Individual subject medical information obtained during this study is confidential, and its disclosure to third parties (other than those mentioned in this section) is strictly prohibited. In addition, medical information obtained during this study may be provided to the subject's personal physician or to other appropriate medical personnel when required in connection with the subject's continued health and welfare.

The study Investigator will maintain a subject identification log (enrollment numbers and corresponding subject names) to enable records to be identified.

12.2 Publication

The study Sponsor retains the ownership of all data and results collected during this study. Therefore, the Sponsor reserves the right to use the data from this present study, either in the form of eCRFs (or copies of these), or in the form of a report, with or without comments and analysis in order to submit them to the US FDA or the Health Authorities of any country.

Furthermore, in the event that the clinical research leads to patentable results, the Investigator (or entity acting on his/her behalf according to local requirements) shall refrain from filing patent application(s). Patent applications will be filed by the study Sponsor or another entity delegated by the Sponsor.

All information concerning the product as well as any information such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by the Sponsor or designee, and are unpublished, are confidential and must remain the sole property of the Sponsor. The Investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from the Sponsor is obtained. The Sponsor has full ownership of the eCRFs completed as part of the study.

By signing the study protocol, the Investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals by the Sponsor. If necessary, the authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement.

The Sponsor or designee will prepare a final report on the study. The Investigator may not publish or present any information on this study without the express written approval of the Sponsor. Additionally, the Sponsor, may, for any reason, withhold approval for publication or presentation.

13 INVESTIGATOR'S PROTOCOL AGREEMENT

The Investigator's Revised Protocol Agreement at the front of this document must be signed by the study site Investigator. The original *or a copy* must be kept on file by the Sponsor and the Investigator must retain the original *or a copy*. The completed Revised Protocol Agreement signifies review and acceptance of the revised protocol by the Investigator prior to initiation of the study.

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15 APPENDICES

Appendix 15.1 Vamorolone Dose Calculation Worksheet

The volume per dose is determined by the subject's dosing level (dose level group) and screening body weight (in kg):

$$\frac{\text{Subject Weight (kg)} \times \text{Dose Level (mg/kg)}}{40 \text{ mg/mL}} = \text{Subject Dose (in mL)}$$

Subject weight (in kg) should be rounded to the nearest whole integer for the calculation of volume per dose.

Calculated dose volume will be rounded to the nearest 0.01 mL (1 mL syringe), 0.1 mL (3 mL syringe), or 0.2 mL (5 mL syringe), depending upon the total dose volume and calibration of the volumetric syringe to be used for administration of dose, as shown in the examples below:

Example 1: Dose volume calculation for a subject enrolled in Dose Level Group 1 (0.25 mg/kg) with a body weight of 19 kg:

$$\frac{\text{Subject Weight (19 kg)} \times \text{Dose Level (0.25 mg/kg)}}{40 \text{ mg/mL}} = 0.119 \text{ mL}$$

The subject will receive a dose volume of 0.119 mL per dose throughout the 14-day Treatment Period. Dose will be administered using a 1 mL volumetric syringe rounded to the nearest 0.01 mL, or for this subject, 0.12 mL daily.

Example 2: Dose volume calculation for a subject enrolled in Dose Level Group 2 (0.75 mg/kg) with a body weight of 15 kg:

$$\frac{\text{Subject Weight (15 kg)} \times \text{Dose Level (0.75 mg/kg)}}{40 \text{ mg/mL}} = 0.281 \text{ mL}$$

The subject will receive a dose volume of 0.281 mL per dose throughout the 14-day Treatment Period. Dose will be administered using a 1 mL volumetric syringe rounded to the nearest 0.01 mL, or for this subject, 0.28 mL daily.

Example 3: Dose volume calculation for a subject enrolled in Dose Level Group 3 (2.0 mg/kg) with a body weight of 27 kg:

$$\frac{\text{Subject Weight (27 kg)} \times \text{Dose Level (2.0 mg/kg)}}{40 \text{ mg/mL}} = 1.35 \text{ mL}$$

The subject will receive a dose volume of 1.35 mL per dose throughout the 14-day Treatment Period. Dose will be administered using a 3 mL volumetric syringe rounded to the nearest 0.1 mL, or 1.4 mL daily.

Example 4: Dose volume calculation for a subject enrolled in Dose Level Group 4 (6.0 mg/kg) with a body weight of 23 kg:

$$\frac{\text{Subject Weight (23 kg)} \times \text{Dose Level (6.0 mg/kg)}}{40 \text{ mg/mL}} = 3.45 \text{ mL}$$

The subject will receive a dose volume of 3.45 mL per dose throughout the 14-day Treatment Period. Dose will be administered using a 5 mL volumetric syringe rounded to the nearest 0.2 mL, or 3.4 mL daily.

Each subject enrolled in the study will be dispensed 100 mL bottle(s) of vamorolone 4% suspension at the Day 1 Visit, for use throughout the Treatment Period, Days 1-14. Each bulk dispensed bottle should be used for one subject only. Each bottle dispensed to the subject and ready for administration to subjects will be labeled with subject study number, dispense date, protocol number, dose level and volume to dispense per dose. Any unused or partially used drug product should be returned at the Week 2 Visit and retained at the study site for investigational drug accountability monitoring.