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Exposure-Response Analysis of Vamorolone (VBP15) in Boys with Duchenne Muscular Dystrophy

Xiaonan Li, PhD¹, Laurie S. Conklin, MD^{2,3}, John van den Anker, MD, PhD^{2,3}, Eric P. Hoffman, PhD^{2,4}, Paula R. Clemens, MD⁵, William J. Jusko, PhD^{1,*}

¹Department of Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, NY, USA

²ReveraGen BioPharma, Rockville, MD, USA

³Children's National Hospital, Washington, DC, USA

⁴Binghamton University–SUNY, Binghamton, NY, USA

⁵University of Pittsburgh and Department of Veterans Affairs Medical Center, Pittsburgh, PA, USA

Abstract

Exposure-response (E-R) relationships of vamorolone, a novel dissociative steroidal anti-inflammatory drug, were investigated in clinical trials in boys with Duchenne Muscular Dystrophy (DMD). Variables were clinical outcome measures, Fridericia-corrected QT (QTcF) duration, and pharmacodynamic (PD) biomarkers. Exposure metrics were area under the plasma concentration time curve (*AUC*) and maximum plasma concentration (*C_{max}*), with a sigmoid *E_{max}* model applied. Significant improvements of clinical efficacy outcomes were observed after 24 weeks of daily dosing. The primary outcome, time to stand from supine (TTSTAND) velocity, exhibited the highest sensitivity to vamorolone, with the lowest *AUC* value providing 50% of maximum effect (*E50*=186 ng·hr/mL), followed by time to climb 4 stairs (TTCLIMB) (*E50*=478 ng·hr/mL), time to run/walk 10 meters (TTRW) (*E50*=1220 ng·hr/mL), and 6-minute walk test (6MWT) (*E50*=1770 ng·hr/mL). Week 2 changes of proinflammatory PD biomarkers showed exposure-dependent decreases. The *E50* was 260 ng·hr/mL for insulin-like growth factor binding protein 2 (IGFBP-2), 1200 ng·hr/mL for matrix metalloproteinase 12 (MMP12), 1260 ng·hr/mL for lymphotoxin α 1/ β 2 (LT α 1/ β 2), 1340 ng·hr/mL for CD23, 1420 ng·hr/mL for Interleukin-22 binding protein (IL22BP), and 1600 ng·hr/mL for Macrophage Derived Chemokine/C-C Motif Chemokine 22 (MDC/CCL22). No relationship was found between QTcF interval changes from baseline and *C_{max}* at

*Corresponding Author: William J. Jusko, PhD, Department of Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, NY, 14214. wj Jusko@buffalo.edu.

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Drs. Conklin, van den Anker, and Hoffman are part-time employees and own stock in ReveraGen BioPharma. Dr. Jusko receives contract funds from ReveraGen BioPharma. Dr. Clemens receives NIH grant funding directly and via subcontracts from ReveraGen for support of these studies.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

week 2 or 24. This analysis showed that improvements in clinical efficacy endpoints at week 24 and PD biomarkers at week 2 were achieved at typical vamorolone exposures of 2 mg/kg daily doses with the median AUC of 6 mg/kg doses (3651 ng·hr/mL) corresponding to approximately 95% of maximum effects for most response variables.

Keywords

Vamorolone; Duchenne muscular dystrophy (DMD); children; glucocorticoids; exposure-response; pharmacodynamics

Introduction

Vamorolone (VBP15), a first-in-class anti-inflammatory steroidal drug, is being developed as a replacement for traditional glucocorticoids (GCs) for the treatment of Duchenne muscular dystrophy (DMD). DMD is a progressive genetic muscle disease caused by the deficient or defective synthesis of dystrophin, a protein that supports myofiber integrity, force production and functionality in all muscles including skeletal, heart, and smooth muscle in gut and blood vessels.^{1, 2} The primary characteristics of DMD are progressive deterioration in skeletal muscle strength, which impairs motor and respiratory functions, and the later development of cardiomyopathy. DMD mainly affects boys, with symptom onset typically between 3 to 5 years of age. Developmental motor milestones are achieved in the first 6–10 years of life but are behind healthy controls. If untreated, DMD leads to loss of ambulation by approximately 8–12 years of age. Currently, there is no cure for DMD; the goal of treating DMD is to alleviate symptoms and slow down the disease progression.

Activation of the pro-inflammatory nuclear factor- κ B (NF- κ B) pathway is one of the earliest histological features of dystrophin deficiency in muscle in DMD and can be detected years before onset of clinical symptoms.^{3, 4} Primarily through targeting the NF- κ B pathway,⁵ chronic treatment with glucocorticoids (GCs) significantly delays the loss of muscle strength, respiratory function and prolongs ambulation, and thus is recommended as the standard of care for boys with DMD.⁶ However, the dose- and time-dependent side effects of GCs, such as muscle wasting, growth delay, weight gain, and bone fragility, greatly reduce patient adherence and limit the overall therapeutic outcomes.⁷ Therefore, developing alternative therapeutic drugs that are effective with a low level of adverse effects will enhance the management of patients with DMD.

Vamorolone shows multi-functional properties including anti-inflammatory, membrane stabilization, and mineralocorticoid receptor antagonist activities.^{8–10} In preclinical studies,^{9, 11–14} vamorolone was a potent inhibitor of the pro-inflammatory NF- κ B pathway, which is the mechanism of anti-inflammatory efficacy that is shared by vamorolone and GCs. This anti-inflammatory mechanism of action has been monitored in human trials and studies through reductions of blood biomarkers (CD23, IGFBP2, IL22BP, MMP12, MDC/CCL22, and Lymphotoxin α 1/ β 2).^{15, 16}

A first-in-human phase I study in 86 healthy male adults showed that the pharmacokinetics (PK) of vamorolone was linear within a broad dose range (0.1–20 mg/kg/day), and

vamorolone was safe and well-tolerated at all doses as indicated by biomarker measurements. Serum biomarkers reflecting typical side effects associated with traditional GCs such as increased fasting insulin and glucose levels (insulin resistance) and decreased osteocalcin (bone formation) showed no significant change in any vamorolone dose group compared to placebo.¹⁷ First-in-patient phase IIa clinical trials included a 2-week open-label multiple ascending dose (0.25, 0.75, 2, and 6 mg/kg/day) study in 48 DMD boys (4-<7 years old, steroid naive) (VBP15-002)¹⁶ and a subsequent 24-week extension phase (VBP15-003) at the same doses.¹⁸ The phase IIa studies demonstrated that vamorolone was safe at all doses tested, with decreased steroid-associated safety concerns predicted by biomarker studies. Both dose-responsive improvements in clinical outcomes (e.g., time to stand from supine velocity and 6-minute walk test, etc.), and decreases in pro-inflammatory corticosteroid-responsive biomarkers, were observed.^{16, 18}

The FDA has a guidance regarding the importance of exploring exposure-response relationships in drug development (<https://www.fda.gov/media/71277/download>). While PK provides key data to link drug dosing to blood concentrations, in their guidance the FDA notes, “Far less attention has been paid to establishing the relationship between blood concentrations and pharmacodynamic (PD) responses and possible differences among population subsets in these concentration-response (often called PK-PD) relationships.” Defining the quantitative relationships between first-in-class vamorolone drug exposure and efficacy and safety outcomes enables dose selection (therapeutic window). Integrating blood biomarkers into vamorolone exposure-response models can provide outcome measures for efficacy that are more objective and rapidly responding to the drug (e.g. 2-weeks treatment) compared to traditional clinical outcomes at 6-months treatment. Finally, the definition of exposure-response relationships of vamorolone in young DMD subjects (4 to <7 years) could contribute to extrapolations of dose selection in broader age ranges of DMD (younger and older patients), and in trials of other chronic inflammatory states where GCs are standard of care.

We asked the question of whether vamorolone exposure in first-in-patient trials in DMD boys predicted change in pro-inflammatory biomarkers after 2 weeks of treatment, later functional outcomes at 6 months, and a safety assessment required of all drugs (QTc interval prolongation; a biomarker associated with risk of life-threatening arrhythmia). We have previously reported the response of all variables to vamorolone dose groups, but here extend this to drug exposure (PK/PD) modeling. This analysis assessed a wide range of drug concentrations (including supratherapeutic in phase I), and collection of PK and QTc data from both healthy adults and children with DMD.

Methods

Data sources

The current E-R analysis focuses on data from the Phase I trial in healthy adults (VBP15-001) and two consecutive open-label phase IIa clinical trials in steroid-naïve DMD boys ages 4 to <7 years old (from the 4th birthday to one day before the 7th birthday). VBP15-002 was a first-in-patient, 2-week multiple ascending dose (0.25, 0.75, 2.0, and 6.0 mg/kg/day) study of vamorolone, with two- week follow-up off drug. There were 12 patients assigned to each

of the four dose groups. Participants in VBP15–002 were subsequently enrolled in VBP15–003, a 24-week extension phase at the same doses. Further details for all studies have been published.^{16–18} A summary of these studies is shown in Table 1.

Pharmacokinetic (PK) data collection

The single- and multiple-dose PK of oral vamorolone in both healthy male volunteers and DMD boys (Table 1) were assessed previously based on noncompartmental analysis and population PK modeling.¹⁹ Overall, vamorolone exhibited moderate variability in PK, with maximum plasma concentration (C_{max}) usually occurring at 2–4 hours and a half-life of approximately 2–3 hours for all the doses and days tested. The PK of vamorolone is linear within the dose range studied in both healthy men (0.1~20.0 mg/kg/day) and DMD boys (0.25~6.0 mg/kg/day), with no evidence of accumulation after multiple daily doses. Similar PK were observed in healthy men and boys with DMD with apparent clearance averaging 2.0 L/h/kg in men and 1.9 L/h/kg in boys.¹⁹ Given the linear and stationary PK properties of vamorolone in DMD boys, the observed area under the curve (AUC) and the C_{max} values on day 14 were used, if available. Otherwise, corresponding values on day 1 were used as the PK exposure for the present E-R analysis (Table 2).

Response variables

The response variables assessed in this E-R analysis included clinical efficacy endpoints (week 24), exploratory PD biomarkers (week 2), and clinical safety endpoints (weeks 2 and 24) (Table 3). A table of patient characteristics was published in Conklin et al., 2018,¹⁶ and is also publicly accessible via clinicaltrials.gov (NCT02760264; NCT02760277).

The 24-week dose-ranging study (VBP15–003) evaluated the effect of 4 doses of vamorolone over a 24-fold dose range (0.25, 0.75, 2.0, and 6.0 mg/kg/day; n=12/dose group; n=48 DMD boys total) on motor function tests that are validated measures of clinical efficacy. It is important to note that the PK values were after only 2 weeks of dosing, whereas the clinical assessments were after 6 months of dosing.

Serum biomarkers were measured from 39 subjects using the SOMAscan™ assay, which measured 1,310 serum proteins, using modified nucleic acid probes that are specific and sensitive for each of the proteins (aptamers). The number of subjects evaluated at each dose level was 7 at 6.0 mg/kg, 10 at 2.0 mg/kg, 11 at 0.75 mg/kg and 11 at 0.25 mg/kg. Numbers of subject per dose level varied because Somalogic™ ended performance of contractual assays and therefore only samples accessed by December 1, 2017 were available for testing. Two serum samples were tested for each subject: Baseline (pre-treatment), and after two weeks of daily treatment with vamorolone at the indicated dose level. Data are reported in Relative Fluorescence Units (RFUs). A data filter was then applied so that only data from the pre-specified 7 efficacy and 6 safety biomarkers were analyzed. These data have been published.¹⁶ The 6 efficacy biomarkers selected for this analysis, which were previously known to respond to GCs and vamorolone, were IGFBP2, MMP12, LTα1/β2, CD23, IL22BP, and MDC/CCL22. The assessed exploratory PD biomarkers are pro-inflammatory mediators related to NF- κ B pathways that were previously shown to be responsive to GC treatment in children with DMD, inflammatory bowel disease, juvenile dermatomyositis and

ANCA-associated vasculitis.^{15, 20} We hypothesized that defining exposure-response relationships of these serum proteins at 2 weeks treatment with vamorolone would be an objective means of evaluating drug mechanism of action, and potentially predicting later clinical improvements at 6 months treatment.

The clinical trial database from VBP15–002 and VBP15–003 was queried for the following data: QTcF interval (ms) from the VBP15–002 Screening and Baseline, VBP15–002 Week 2 visit, and VBP15–003 Week 24 visit. If a patient had a re-screening measure of the QTcF interval, this value was used instead of the Screening measure. From the Week 2 visit in the VBP15–002 trial and Week 24 visit in the VBP15–003 trial, the following data were recorded: time of medication dose, time of electrocardiogram, QTcF interval. The QTcF interval changes from Baseline (QTcF) at both week 2 and week 24 were calculated.

Definition of sensitive clinical outcomes for E-R analysis

Motor outcomes assessed in the VBP15–002 and VBP15–003 clinical trials were time to stand from supine (TTSTAND), time to run/walk 10 meters (TTRW), time to climb 4 stairs (TTCLIMB), 6-minute walk test in meters walked (6MWT), and North Star Ambulatory Assessment (17 test score) (NSAA). In DMD subjects at the relatively young age studied (4 to <7 years at enrollment), TTSTAND, TTRW, TTCLIMB, and 6MWT are generally stable (do not decline or improve). Vamorolone treatment showed dose-responsive improvements of motor outcomes for all these measures except NSAA.¹⁸ The inability to see a drug effect with NSAA is due to improvement in untreated DMD subjects in this age range owing to a neurodevelopmental component that increases scores in DMD subjects independent of drug effect. For this reason, NSAA was not considered for E-R relationships.

The TTSTAND, TTRW, and TTCLIMB results are measured in seconds, but data are often subjected to a non-linear data transformation into velocity (1/results for TTSTAND and TTCLIMB; 10/results for TTRW). This transformation has the advantage of retaining data if a subject becomes unable to perform the test (velocity=0), and also compresses the high degree of variance introduced by particularly severely affected subjects (severe outliers).

To determine if seconds or velocity was best utilized to define E-R relationships, we carried out a sample size calculation (power analysis) using the data from the VBP15–002/VBP15–003 clinical trials. For these sample size calculations, we combined the 0.25 and 0.75 mg/kg/day vamorolone-treatment groups as a ‘Pseudo-Placebo’ Group, and the 2.0 and 6.0 mg/kg/day vamorolone-treatment groups as the ‘Treatment’ Group. Our rationale for combining the 0.25 and 0.75 mg/kg/day vamorolone-treatment groups as a pseudo-placebo for a 24-week treatment period included:

- The 0.25 and 0.75 mg/kg/day groups showed no statistically significant difference from steroid-naïve natural history comparators over a 24-week period.¹⁸
- The use of low-dose vamorolone-treated groups as a pseudo-placebo would lead to conservative sample size calculations in the event there may be a limited drug effect.

- The use of low-dose vamorolone-treated groups as pseudo-placebo should include some element of ‘placebo effect’, as these subjects were in a trial, given active drug.

Mixed effect Model Repeat Measurement (MMRM) models were applied to baseline, 12-week, and 24-week data of motor outcomes adjusted for Baseline and age. The MMRM change from Baseline was determined for Pseudo-Placebo Group ($0.25 + 0.75$ mg/kg/day) and the Treatment Group ($2.0 + 6.0$ mg/kg/day) (Table S1). Comparison of the Treatment Group ($2.0 + 6.0$ mg/kg/day) vs. the Pseudo-Placebo Group ($0.25 + 0.75$ mg/kg/day) showed improvement in all outcomes, the most significant findings were for TTSTAND velocity ($P > |t| = 0.0035$).

Sample size calculations for the VBP15–004 study were performed using the same MMRM model that was used for the VBP15–002/VBP15–003 24-week analysis.¹⁸ For each efficacy endpoint (TTSTAND seconds and velocity, TTRW seconds and velocity, TTCLIMB seconds and velocity, and 6MWT meters walked), the Treatment Group ($2.0 + 6.0$ mg/kg/day) was compared to the Pseudo-Placebo Group ($0.25 + 0.75$ mg/kg/day), and estimates of the mean change from baseline at Week 24 using LS means were made for each of the Groups. For the estimates of standard deviation we used simple descriptive statistics. We then calculated the sample size using two-sided t-tests assuming unequal variance, with power =90% and alpha =0.05. An example of the analysis for the TTSTAND velocity is as follows:

- Two-Sample T-Tests Allowing Unequal Variance
- Numeric Results for Two-Sample T-Test Allowing Unequal Variance
- Alternative Hypothesis: $H1: \delta = \mu_1 - \mu_2 \neq 0$
- Target Power = 0.90, Actual Power = 0.90813, $N1 = 30$, $N2 = 30$, $N = 60$, $\mu_1 = 0.0$, $\mu_2 = 0.0$, $\delta = -0.1$, $\sigma_1 = 0.1$, $\sigma_2 = 0.1$, and Alpha = 0.050.

where: Target Power is the desired power value (or values) entered in the procedure. Power is the probability of rejecting a false null hypothesis. Actual Power is the power obtained in this scenario. Because $N1$ and $N2$ (numbers of items sampled from each population, $N =$ total) are discrete, this value is often (slightly) larger than the target power.

Symbols are: μ_1 and μ_2 the assumed population means, $\delta = \mu_1 - \mu_2$ is the difference between population means at which power and sample size calculations are made, σ_1 and σ_2 are the assumed population standard deviations for groups 1 and 2, and Alpha is the probability of rejecting a true null hypothesis.

This same model for sample size calculations was used to carry out analyses for each outcome measure (both seconds and velocity). Data are summarized in Table S2.

This analysis showed that the clinical outcomes most sensitive for detecting a vamorolone effect were TTSTAND velocity, TTRW seconds, TTCLIMB seconds, and 6MWT meters walked. These outcomes were then carried forward into the E-R relationship analyses.

E-R analysis

The E-R analysis population is defined as the subset of patients from the full population for which both exposure data and the assessed response variables were available. The E-R analysis was performed using nonlinear mixed effects modeling program NONMEM (Version 7.4; ICON Development Solutions, Dublin, Ireland) with the first-order conditional estimation (“FOCE”) and the INTERACTION option. Data manipulation and plotting were done using R (version 3.4.3) and GraphPad Prism (version 5.01). Preliminary model assessments were performed for each of the response variables, where linear, E_{max} and sigmoid E_{max} model were compared, different residual error models (additive, proportional, and additive plus proportional error models) were tested, between-subject variability (BSV) was considered on each of the model parameters. The evaluation of model performance was based on goodness-of-fit (GOF) plots, precision of parameter estimates and the objective function value. Finally, the relationships between vamorolone exposure and all the response variables in Table 3 except QTcF interval were characterized using the sigmoid E_{max} model with baseline values (Eq. 1):

$$E = E_0 \cdot \left(1 \pm \frac{E_{max} \cdot Exposure^\gamma}{E_{50}^\gamma + Exposure^\gamma} \right) \quad (1)$$

where E indicates the absolute value of each response variable at the assessed time point; E_0 represents the baseline value; $Exposure$ indicates vamorolone AUC on day 14; E_{max} is the maximum effect of vamorolone, E_{50} is the AUC value of vamorolone achieving 50% maximal effect, and γ is the Hill coefficient. For individual i , the model parameters are:

$$E_{0,i} = \widetilde{E_0} \cdot e^{\eta_i} \quad (2)$$

$$E_{50,i} = \widetilde{E_{50}} \quad (3)$$

$$E_{max,i} = \widetilde{E_{max}} \quad (4)$$

$$\gamma_i = \widetilde{\gamma} \quad (5)$$

where bars indicate typical values of parameters; between-subject random effects were modeled using exponential function for baseline response (E_0) only (Eq. 2) and η_i is the random between-subject effect on E_0 for the i^{th} individual which was assumed to be normally distributed with mean 0 and variance $\omega^2 E_0$. Residual variability was described using an additive error model for the clinical efficacy endpoints except 6MWT (Eq. 6) and a proportional error model for the exploratory PD biomarkers and 6MWT (Eq. 7).

$$E_{ij} = \widehat{E}_{ij} + \varepsilon_{1ij} \quad (6)$$

$$E_{ij} = \widehat{E}_{ij} \cdot (1 + \varepsilon_{2ij}) \quad (7)$$

where E_{ij} and $\hat{E}_{ij}$ are the j^{th} observed and predicted responses for the i^{th} individual; and ε_{1ij} and ε_{2ij} are the additive and proportional random residual effects. Both ε were normally distributed ($\varepsilon_{1ij} \sim N(0, \sigma_{\text{add}}^2)$, $\varepsilon_{2ij} \sim N(0, \sigma_{\text{prop}}^2)$).

Results

E-R analysis of clinical efficacy endpoints

The relationships between week 24 change of clinical efficacy endpoints from baseline and vamorolone AUC on day 14 were reasonably characterized by the E_{max} model as illustrated in Figure 1. The parameter estimates of the E-R analysis are listed in Table 4. The GOF plots of the final population model for clinical endpoints are shown in Supplemental Figures S5–S8.

The primary efficacy endpoint was week 24 change of TTSTAND velocity from baseline. The typical baseline value of TTSTAND velocity in DMD boys was 0.2 rises/s, with the between-subject variability (BSV) of 32.6%. The estimated maximum effect of vamorolone on TTSTAND velocity was an increase from baseline of 0.04 rises/s, accounting for 19.4% of the baseline value. Vamorolone showed potent effects on TTSTAND velocity change from baseline, with E_{50} =186 ng·hr/mL, which was close to the median AUC value of the lowest dose group (0.25 mg/kg). Median AUC values of the 2 and 6 mg/kg doses (1113 and 3651 ng·hr/mL) correspond to average 85.7% and 95.2% of the maximum effect of vamorolone on TTSTAND velocity change at week 24.

The typical baseline value of 6MWT is 330 meters distance walked, with the BSV of 16.6%. The estimated maximum effect of vamorolone on 6MWT at week 24 was a change from baseline of 63.4 meters, accounting for 19.2% of the baseline value. The AUC value of vamorolone producing 50% maximum effect (E_{50}) on 6MWT was 1770 ng·hr/mL. The median AUC value associated with the 6 mg/kg dose produced an average of 76.4% maximum effect on meters change at week 24 (48.4 meters).

The TTRW change from baseline showed exposure-related decreases with a maximum decrease of 0.68 seconds (11.3% of the baseline). The typical baseline value of TTRW was 6 s, with the BSV of 20.1%. The E_{50} value was estimated to be 1220 ng·hr/mL, which was close to the median AUC value at 2 mg/kg dose group. On average, the median AUC value of the highest dose group would achieve 99.6% of the maximum effect on TTRW change at week 24.

The TTCLIMB change from baseline to week 24 showed exposure-related changes, with a maximum decrease of 0.74 s (E_{max} =0.164) from baseline (E_0 =4.5 s, BSV=40.7%). The estimated E_{50} value was 478 ng·hr/mL, close to the typical AUC value observed at the 0.75 mg/kg dose (534 ng·hr/mL). Greater decreases of TTCLIMB at week 24 would be expected at 2 and 6 mg/kg groups, accounting for approximately 98.6% and 100%, of the maximum change from the baseline.

E-R analysis of exploratory PD biomarkers

Following 2 weeks of daily vamorolone dosing, 6 out of the 7 pre-specified exploratory efficacy PD biomarkers tested in the VBP15–002 trial showed dose-dependent decreases from baseline with increasing doses.¹⁶ The relationships between week 2 change of these PD biomarkers and vamorolone exposure (day-14 *AUC*) were further explored and were adequately described by the sigmoidal E_{max} model (Figure 2 and Table 5). The GOF plots of the final population model for exploratory PD biomarkers are shown in Supplemental Figures S9–S14.

The maximum effect of vamorolone on CD23 was a decrease of 2620 ($E_{max}=0.295$) from the baseline ($E_0=8880$, $BSV=20.6\%$). The estimated E_{50} (1340 ng·hr/mL) was within the exposure range of the 2 mg/kg dose group (578–2202 ng·hr/mL). Minimal changes of CD23 were observed at *AUC* values less than the median *AUC* at 0.75 mg/kg (534 ng·hr/mL), which corresponds to only 1% of the maximum response. The average effect of vamorolone on CD23 increased from 28.3% of the maximum effect to 99.3% when doses increased from 2 to 6 mg/kg.

The typical baseline of MDC/CCL22 was 2420 ($BSV=19.2\%$), and the average maximum change (820.4) by vamorolone accounted for 33.9% of the baseline value. MDC/CCL22 demonstrated relatively lower sensitivity to vamorolone treatment as compared to other PD biomarkers ($E_{50}=1600$ ng·hr/mL). Similar to CD23, no significant drug effect was observable for MDC/CCL22 when exposure was lower than the median *AUC* at 0.75 mg/kg. A sharp decrease from baseline was observed from 114.9 (14% maximal response) to 807.2 (98.4% maximal response) when drug exposure increased from the median *AUC* of 2 to that of 6 mg/kg.

The typical baseline value of IL22BP was estimated to be 6790 ($BSV=19.1\%$). The maximum effect of vamorolone on IL22BP was a change of 2655 from baseline ($E_{max}=0.391$). Week 2 change of IL22BP with increased vamorolone exposure also showed a steep E-R relationship, as reflected by the minimal IL22BP changes at the two lower doses and a marked change from 24.6% maximal effect at 2 mg/kg to 98.7% maximal effect when doses increased to 6 mg/kg.

The maximum decrease of LT $\alpha 1/\beta 2$ from baseline by vamorolone was estimated to be 169.6 (32.8% of the baseline value), with the half maximal change achieved at the *AUC* value of 1260 ng·hr/mL. Similarly, significant changes of LT $\alpha 1/\beta 2$ from baseline were observed starting from the dose of 2 mg/kg, with an average of 99.4% maximal change achieved at 6 mg/kg.

The typical baseline RFU of IGFBP-2 in DMD boys was 891, with BSV of 28.5%. The estimated maximum effect of vamorolone on IGFBP-2 change from baseline was 331.5, accounting for 37.2% of the baseline value. The E_{50} value (260 ng·hr/mL) on IGFBP-2 change was well below the median *AUC* of the 0.75 mg/kg dose group (534.5 ng·hr/mL). On average, approximately 67.3%, 81% and 93.4% of the maximum inhibition on week 2 change of IGFBP-2 from baseline can be achieved at corresponding 0.75, 2 and 6 mg/kg doses of vamorolone.

Vamorolone decreased MMP12 with the highest capacity ($E_{max}=0.512$). The average maximum change from baseline ($E_0=3450$) was about 1766 within the dose/exposure range assessed. The E_{50} value was estimated to be 1200 ng·hr/mL, which was close to the median AUC value at 2 mg/kg dose group. The average change of MMP12 by vamorolone was minimal at 0.25 and 0.75 mg/kg doses (less than 1.7% of the maximum change). The drug effect became stronger as its dose increased to 2 and 6 mg/kg, corresponding to an average of 40.7% and 99.6% maximum inhibition on MMP12 change at week 2.

E-R analysis of clinical safety endpoint-QTcF

The relationship between individual exposures of vamorolone (C_{max} values on day 14) and individual QTcF interval changes from baseline (QTcF) at both week 2 and week 24 in DMD boys was evaluated graphically to detect relevant trends. As shown in the scatter plots in Figure 3, there is no obvious positive relationship between vamorolone exposure and QTcF interval change from baseline at either week 2 or week 24. The QTcF interval change in each C_{max} quantile was also plotted in the boxplots in Figure 3; there was also no statistically significant difference between the QTcF interval values in each C_{max} quantile as compared to those in the first C_{max} quantile (7.19~64.45 ng/mL). No DMD boys had a QTcF >30 ms at week 2. The QTcF values >30 ms were observed in 4 out of 45 patients at week 24. All the QTcF values were <60 ms (Table S3).

The relationship between placebo-adjusted QTcF (QTcF) and vamorolone C_{max} in healthy male adults from the previous phase I study¹⁷ was also examined. The results are shown in the Supplemental Materials. Overall, the increase of QTcF after vamorolone was minor; most QTcF values at any time point were < 10 ms. There was no trend of increasing incidence of QTcF>10 ms with dose (Figure S3). The time-averaged QTcF values stratified by dose also demonstrated no trend of increasing QTcF values with dose (Figure S4).

Given the linear and stationary PK of vamorolone, as well as the lack of proportionality of QTcF with dose and higher exposures but smaller QTcF during the FED study (vamorolone given with food produced much higher AUC values), the QTcF changes in relation to individual plasma drug concentrations were not further assessed.

Discussion

We used model-based E-R analyses on efficacy and safety biomarkers/endpoints in a clinical trial of vamorolone in DMD to facilitate the benefit-risk evaluation of different dose levels and also explore biomarker exposure/dose changes at 2-weeks treatment to determine if they aligned with clinical outcomes at 6-months of treatment. For the safety signal of cardiac long QT interval (QTcF), Phase I data of vamorolone treatment of adult volunteers was also studied. The relationships between AUC and week 24 changes of clinical efficacy outcomes and week 2 changes of exploratory PD biomarkers from baseline were quantitatively characterized by the sigmoid E_{max} model. Significant relationships were also found between C_{max} and all of the efficacy biomarkers studied (Figures S1 and S2).

An analysis of clinical outcomes after 24-weeks treatment with vamorolone showed that four measures were most sensitive to detecting a drug-related improvement: TTSTAND velocity, 6MWT meters walked, TTRW seconds, and TTCLIMB seconds (Tables S1 and S2). A fifth clinical outcome measure, NSAA (17-test combined score) showed neurodevelopmental improvement in all DMD boys and was insensitive to drug effect, and was not studied further. According to the modeling results (Table 4), the primary efficacy outcome, TTSTAND velocity change, showed the highest sensitivity to vamorolone treatment among all the assessed clinical efficacy endpoints, as reflected by the lowest E_{50} value (186 ng-hr/mL) that was close to the median AUC of the lowest dose group (Table 4). The maximum increase of TTSTAND velocity after 24 weeks of daily vamorolone treatment was estimated to be 0.04 rises/s. On average, the 0.75, 2 and 6 mg/kg doses would increase the TTSTAND velocity by corresponding 0.03, 0.034 and 0.038 rises/s, suggesting significant vamorolone-related improvements as compared to the untreated historical control group taken from the Cooperative International Neuromuscular Group Duchenne Natural History Study (CINRG DNHS) (mean week 24 change = 0.01 rises/s). TTSTAND is an important predictor of changes in 6MWT and, more generally, the disease progression of DMD.²¹ A pre-defined threshold value of 5 seconds differentiates between patients who are likely to show disease progression (TTSTAND>5 seconds) versus those who are likely to show stability or improvement (TTSTAND<5 seconds).²¹ The estimated typical baseline of TTSTAND was 5.15 seconds, which decreased at week 24 to an average value of 4.6 and 4.27 seconds, with the doses of 2 and 6 mg/kg (data not shown), indicating that the improvement in TTSTAND by vamorolone is clinically meaningful.

The week 24 changes of TTRW and TTCLIMB both exhibited exposure-dependent decreases. TTRW showed little change compared to baseline at the doses of 0.25 and 0.75 mg/kg, while a greater decrease in time to complete the task was observed starting from the 2 mg/kg dose (Figure 1). The maximum decrease in TTRW at week 24 was estimated to be 0.678 s and half of the maximum response was achieved at 1220 ng-hr/mL. Average improvement in TTRW by 2 and 6 mg/kg doses were decreases from baseline of 0.26 seconds and 0.677 seconds, respectively (Table 4). Compared to the effect on TTRW, vamorolone was more effective for TTCLIMB with a lower E_{50} (478 ng-hr/mL) that was smaller than the typical median AUC of 0.75 mg/kg (Table 2). As a result, more than 98.56% (0.727 seconds) of the maximum decrease (0.738 seconds) from baseline (4.5 seconds) can be expected for TTCLIMB by giving vamorolone at doses higher than 2 mg/kg.

The 6MWT has been shown to be a feasible, safe, and reliable endpoint in ambulant boys with DMD.²² As a secondary clinical outcome in this study, 6MWT exhibited the lowest sensitivity to vamorolone treatment. The estimated maximum change of 6MWT at week 24 was 63.4 meters. However, given the relatively large E_{50} value (1770 ng·hr/mL), only 32% (20.3 meters) and 76.4% (48.4 meters) of the maximum response can be achieved at 2 and 6 mg/kg doses. In contrast, week 24 changes of all the other clinical efficacy endpoints approached their maximum value with vamorolone exposure at the highest dose tested. Nevertheless, 6MWT still showed a clinically meaningful and significant improvement (>40 meters) with 6 mg/kg daily doses of vamorolone.²²

We also report exposure-response relationships for six pharmacodynamic biomarkers of efficacy measured after 2-weeks of drug treatment. These biomarkers had been previously defined as GC-responsive in four disorders, and were pre-specified as exploratory PD biomarkers in the vamorolone trials as acute read-outs of anti-inflammatory steroid-like effect.^{15, 16} In the exposure-response modeling, vamorolone showed the highest potency for changes in IGFBP-2, as indicated by the lowest E_{50} value (260 ng·hr/mL) among all the tested PD biomarkers. With this, the typical vamorolone exposure at a dose as low as 0.75 mg/kg would be sufficient to achieve more than half (67.3%) of the maximum effect on IGFBP-2 inhibition, and the highest two doses would exert averages of 81.1% and 93.4% maximal effects for 2 mg/kg and 6 mg/kg respectively. In contrast to IGFBP-2, all the other biomarkers exhibited comparable sensitivities to vamorolone, with E_{50} values ranging from 1200 to 1600 ng·hr/mL. The order of E_{50} from highest to lowest was MDC/CCL22>IL22BP>CD23>LT α 1/ β 2>MMP12, which were all within the AUC range of the 2 mg/kg dose (578~2202 ng·hr/mL). According to the model, approximately 50% of the maximum response can be achieved for these biomarkers at the dose of 2 mg/kg and more than 98% maximal effect can be expected at the highest dose after 2 weeks of vamorolone treatment. In addition, the predicted E-R curves of all the biomarkers were steeper than that of IGFBP-2 as their estimated γ values were around 5 while that value for IGFBP-2 was 1, suggesting a much narrower exposure window of vamorolone for these biomarkers, from no effect to almost maximum effect.

In the present study, significant ER relationships between vamorolone exposure and week 2 changes of all the PD biomarkers were quantitatively demonstrated (Figure 2, Table 5), which strongly supports the anti-inflammatory mechanisms of vamorolone in DMD boys and establishes a predictive PK/PD relationship. This model could be used to support dose-selection and assess the effect of vamorolone in early phase trials of vamorolone in expanded age ranges and other indications. Additional trials testing vamorolone in a broader age range of DMD (2 to 18 years), and in two additional indications (pediatric ulcerative colitis and Becker muscular dystrophy) are in the design phase.

Exposure-response studies can aid in the definition of a therapeutic window, and we included one potential safety signal in the studies here. All drugs require studies of QT prolongation as an important safety signal. For vamorolone, the QTcF interval change at weeks 2 and 24 showed no trend with the increase of exposure (Figure 3), indicating that vamorolone was well tolerated and had minimal proarrhythmic potential in DMD boys within the exposure range tested. Most changes of QTcF interval in DMD boys were modest and well below the 30 or 60 ms change from baseline in QTc interval threshold of concern by the FDA (Table S3).²³ The FDA Guidance²³ states, “Drugs that prolong the mean QT/QTc interval by >20 ms have a substantially increased likelihood of being proarrhythmic, and might have clinical arrhythmic events captured during drug development.” VBP15 does not produce prolongations of mean QT/QTc intervals that exceed 20 ms.

Despite the quantitative demonstration of E-R relationships in DMD boys, this work is not without limitations. DMD is a progressive degenerative disease, which shows age-dependent disease progression. As demonstrated recently,²⁴ disease progression of DMD in younger

subjects <7 years is in parallel with growth and development, which may result in a temporary net gain in strength or abilities, albeit to a lesser extent than in healthy children of the same age. This has also been confirmed by Lennie et al²⁵ who applied an indirect response model to describe DMD disease progression as measured by the 6MWT over a wide pediatric age range and compared it to those of healthy subjects. Their analysis showed that DMD trajectory reached a maximum value at 8.9 years before decline and fell below 1 meter at 18.0 years. It would be ideal to use a disease progression model for DMD together with the placebo effect model to characterize the treatment effect over time. However, this would require extensive time-course data over long study periods in all groups (e.g., disease control group, placebo group and treatment groups of various doses), which is not applicable in the current analysis due to the short study period and the narrow age range studied (4~7 years). According to an analysis on the natural history data of DMD in 5–12.9 years-old, no significant changes in function over 1 year were observed in boys <7 years.²⁶ Therefore, it is reasonable to assume a stable disease condition for the age range of 4~7 years during 24 weeks, as was done in our analysis. The method used for conducting E-R analysis depends highly on the key questions that are being addressed, and relative simple models are often recommended as the starting point.²⁷ The basic modeling approach applied here seems sufficient to address the primary objective of this analysis, e.g. whether the treatment effect of vamorolone relates to exposures/doses at an early stage of the clinical trial and what the predicted effect of exposure/dose changes would be.

Overall, changes of clinical efficacy endpoints at week 24 and changes of pro-inflammatory PD biomarkers at week 2 exhibited vamorolone exposure-dependent improvements within the 24-fold dose range tested. The inhibitory effect on PD biomarkers after 2 weeks of daily treatment provided an early sign of its clinical efficacy at week 24. The E-R relationships were quantitatively characterized by a sigmoid E_{max} model incorporating the variability (BSV) in baseline responses, better accounting for the high inter-subject variability of the baseline. With oral daily dosing of vamorolone, significant improvement for most of the response variables generally can be achieved at exposures higher than 1113 ng·hr/mL, the median *AUC* value of the 2 mg/kg dose. This is in line with the previous dose-response assessments where statistically significant efficacy was demonstrated at the 2 and 6 mg/kg dose levels.¹⁸

In DMD, loss of the ability to stand from the floor, climb stairs, or walk are important milestones for the patient. Improvements in such functions are directly related to improved quality of life.²¹ In the present analysis, the estimated maximum improvements in these functions at week 24 were between 10~20% from the baseline, which is comparable to the reported minimal clinically important difference values (MCIDs) that indicates clinically relevant changes with treatments in DMD (8~8.9% for 6MWT and 18.9~33.9% for timed function tests).²⁸ The PK-PD relationships defined here provide evidence supporting our dose selection strategy and the use of PD biomarkers in proof-of-concept trials of vamorolone in other diseases and in studies in expanded ages of DMD boys.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Exposure-response relationship between AUC on day 14 and percent changes of clinical efficacy endpoints from baseline at week 24. The dotted line represents the zero change line. Open circles are observed percent change from baseline and solid lines are population model fittings. Horizontal solid lines on the bottom panels are the range of minimum to maximum AUC values for 0.25 (red), 0.75 (purple), 2 (orange) and 6 mg/kg (green) doses of vamorolone.

Figure 2.

Exposure-response relationship between *AUC* on day 14 and percent changes of exploratory PD biomarkers from baseline at week 2 demonstrated by percent change in Relative Fluorescence Unites (RFU). Graph metrics are the same as in Figure 1.

Figure 3.

The relationship between C_{max} on day 14 and QTcF interval change from baseline (QTcF) at weeks 2 and 24. Dots with different colors reflect dose groups: 0.25 (red); 0.75 (purple); 2 (orange); 6 mg/kg (green).

Table 1.

Summary of Studies Analyzed.

Study	Description	Dosing/Administration	Study population	Major results
Phase 1 VBP15-001	PK, acute tolerability and safety, MIST. Non-randomized, sequential dose cohorts.	Oral administration SAD: 0.1–20.0 mg/kg MAD: 1.0–20.0 mg/kg/day	Adult volunteers N=86	PK and safety through highest dose tested (20 mg/kg/day) Lack of change in safety biomarkers as expected with glucocorticoids
Phase 2a VBP15-002	PK, acute tolerability and safety, MIST. Non-randomized, sequential dose cohorts	Daily oral administration of vamorolone (0.25, 0.75, 2.0, 6.0 mg/kg/day). 2-week treatment period, 2-week washout	DMD ages 4 to <7 years, steroid naïve. N=48	Pediatric PK and MIST similar to adults. Improvements in exploratory pro-inflammatory efficacy biomarkers and lack of effect on safety biomarkers expected with glucocorticoids
Phase 2a extension VBP15-003	Follow-up of VBP15-002; long-term safety and tolerability, clinical efficacy, non-randomized, open-label, sequential dose cohorts	Daily oral administration of vamorolone (0.25, 0.75, 2.0, 6.0 mg/kg/day). 24- week treatment period.	DMD ages 4-<7 years, steroid-naïve. N=48	Primary outcome measure: time to stand from supine. 2.0 mg/kg vs CINRG DNHS p= 0.02

Abbreviations: SAD=single ascending dose; MAD=multiple ascending dose; PK=pharmacokinetics; DMD=Duchenne muscular dystrophy; MIST=metabolites in safety testing; CINRG DNHS=Cooperative International Neuromuscular Research Group Duchenne Natural History Study

Table 2.

Summary of vamorolone Steady-State Exposures (AUC and C_{max}) on day 14 in DMD boys after receiving daily oral doses of vamorolone.

Dose (mg/kg/day)	Mean (CV%) Median [Minimum, Maximum]	
	AUC (ng·hr/mL)	C_{max} (ng/mL)
0.25	163.8 (39.2)	32.2 (49.7)
	163.8	34.5
	[58, 272]	[7.2, 56.6]
0.75	543.7 (29.7)	124.7 (35.6)
	534.5	127.5
	[288, 830]	[55.7, 200]
2	1138 (42.9)	252.5 (39.7)
	1113	255.5
	[578, 2202]	[121, 440]
6	3487 (28.8)	893.1 (36.2)
	3651	919
	[2068, 5319]	[475, 1600]

Table 3.

Clinical response variables included in the E-R analysis

Category	Response variable ^a	Drug effect ^b
Clinical efficacy endpoints	Time to stand from supine (TTSTAND) velocity	↑
	6-minute walk test (6MWT)	↑
	Time to climb 4 stairs (TTCLIMB)	↓
	Time to run/walk 10 meters (TTRW)	↓
Exploratory PD biomarkers	CD23	
	Macrophage Derived Chemokine/ C-C Motif Chemokine 22 (MDC/CCL22)	
	Interleukin-22 binding protein (IL22BP)	
	Lymphotoxin $\alpha 1/\beta 2$ (LT $\alpha 1/\beta 2$)	↓
	Insulin-like growth factor binding protein 2 (IGFBP-2)	
	Matrix metalloproteinase 12 (MMP12)	
Clinical safety endpoints	Fridericia-corrected QT (QTcF) interval	NA

^aUnits for response variables rises/s for TTSTAND velocity, meters for 6MWT, and seconds for both TTRW and TTCLIMB. The response variables assessed were week 24 data of clinical efficacy endpoints, week 2 data of exploratory PD biomarkers, and both week 2 and week 24 data of clinical safety endpoints.

^bDirection of change indicating drug effect: ↑ increase, ↓ decrease on increasing vamorolone exposure.

NA: not applicable.

Table 4.

Parameter estimates of the population E-R analysis of clinical efficacy endpoints.

Parameter (Unit)	Estimates (RSE%)			
	TTSTAND velocity	6MWT	TTRW	TTCLIMB
$E_{\max}$	0.194 (28.4)	0.192 (48.9)	0.113 (22.9)	0.164 (62.8)
E_{50} (ng-hr/mL)	186 (91)	1770 (95.5)	1220 (46)	478 (28)
E_0^a	0.2 (5.4)	330 (2.8)	6 (3.2)	4.5 (6.9)
γ	1 (Fixed)	1.62 (53.2)	5 (Fixed)	5 (Fixed)
Between-subject variability	CV% (RSE%)			
$\omega^2 E_0$	32.6 (15.7)	16.6 (12.8)	20.1 (10.2)	40.7 (12.9)
Residual variability	Estimates (SD, RSE%)			
$\sigma_{\text{add}}^2^b$	0.0013 (0.03, 21.4)	--	0.32 (0.57, 25.8)	1.1 (1.05, 31.8)
	CV% (RSE%)			
σ_{prop}^2	--	0.005 (21.4)	--	--

Abbreviations: $E_{\max}$, maximum stimulatory/inhibitory effect of vamorolone; E_{50} , AUC value of vamorolone resulting in 50% maximum effect; E_0 , baseline of the response variable; SD, standard deviation; RSE, relative standard error; CV, coefficient of variation, σ_{add}^2 = variance of additive random residual effects, σ_{prop}^2 = variance of proportional random residual effects.

^aUnits for parameter E_0 are: risecs/s for TTSTAND velocity, meters for 6MWT, and seconds for both TTRW and TTCLIMB.

^bThe units for the SD of σ_{add}^2 are the same as those for E_0 .

Table 5.

Parameter estimates of the population E-R analysis on exploratory PD biomarkers

Parameter (Unit)	Estimates (RSE%)					
	CD23	MDC/CCL22	IL22BP	LTα1/β2	IGFBP-2	MMP12
$E_{\max}$	0.295 (22.3)	0.339 (18.7)	0.391 (11.3)	0.328 (18.8)	0.372 (44.4)	0.512 (10.4)
E_{50} (ng-hr/mL)	1340 (62.4)	1600 (18.3)	1420 (13.9)	1260 (34.9)	260 (183.8)	1200 (11.2)
E_0 (RFU)	8880 (3.8)	2420 (3.4)	6790 (3.5)	517 (2.9)	891 (5.6)	3450 (5.3)
γ	5 (Fixed)	5 (Fixed)	4.59 (39)	4.8 (74)	1 (Fixed)	5 (Fixed)
Between-subject variability						
	CV% (RSE%)					
$\omega^2 E_0$	20.6 (12.1)	19.2 (12.0)	19.1 (16.8)	11.2 (25.0)	28.5 (13.6)	26.4 (13.3)
Residual variability						
	CV% (RSE%)					
σ^2_{prop}	11.9 (11.1)	14.5 (11.1)	11.7 (10)	16.9 (10)	17.8 (11.6)	16.9 (9.5)

Abbreviations: σ^2_{prop} = variance of proportional random residual effects.