**PRECLINICAL PHARMACOKINETIC REPORT**

**Developmental Biology and Solid Tumor Program**

**P-PKSR Study 33826-254371**

**STUDY TITLE:**

# OCULAR PHARMACOKINETICS OF TOPICAL PANOBINOSTAT IN MICE: PROJECT SUMMARY

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| **SHORT TITLE:** | **Ocular PK of Topical Panobinostat Project Summary** |
| **TEST ARTICLE:** | Panobinostat |
| **SECTION:** | Nonclinical Pharmacokinetics (Non-GLP) |
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| **SJCRH SRM2 O/R:** | 33826-254371 Preclinical Pharmacokinetic Shared Resource<br>31667-223828<br>27105-158873 |
| **REFERENCE STUDY NUMBERS:** | NA NA |
| **IN VIVO SCIENTIST(S)** | Pritchard, Eleanor |
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| **REPORT FORMAT:** | Project Summary |
| **REPORT STATUS:** | FINAL |
| **DATE:** | 2020-04-10 |

***NOTE: THIS REPORT IS A REFORMATTING ONLY OF REPORT POSTED UNDER SRM2 ORDER 33826***

## Quality Statement

This non-GLP study was conducted using sound scientific principles and established techniques in accordance with the relevant guidelines and standard operating procedures (SOPs) of the Preclinical Pharmacokinetic Shared Resource (P-PKSR) and St. Jude Children's Research Hospital (SJCRH), Memphis, TN, USA. This report accurately reflects the data obtained during the course of this study.

These results represent part of an early phase preclinical pharmacology program. This study has been conducted to provide preliminary insights into the pharmacokinetic (PK) properties of the compound(s) in the indicated preclinical model(s). This study and its results are not intended to provide a comprehensive PK evaluation of the compound(s). The applied bioanalytical method was validated/qualified to support this specific study and discovery-style sample analyses.

Substantial study-to-study and inter-animal variability in preclinical PK exists. Such variability depends upon the in vivo scientists' experience, variations in compound purity and formulation, animal strains, sex and age, and other situational fixed effects (i.e. husbandry conditions, chow constituents, presence or absence of disease, concomitant drugs). As such, the actual PK, plasma or tissue compound concentrations, or equivalent dose in other studies or preclinical models may vary significantly from that reported herein.

# Ocular PK of Topical Panobinostat Project Summary

## 1.0 BACKGROUND

A series of three murine in vivo ocular pharmacokinetic (PK) studies for panobinostat were designed and conducted by Eleanor Pritchard et al. over the time period from 03/13/2014 to 07/14/2014. The objectives of these studies were to estimate panobinostat concentrations in the mouse vitreous, retina, orthotopically placed retinoblastoma xenograft tumor (Y79 RB Luc+), and plasma after various routes of administration (topical, subconjunctival, direct intravitreal injection, intraperitoneal) and formulations. **Table 1.1** lists the study SRM2 Order/Request numbers with brief descriptions of the studies, as well as the number of experimental samples analyzed by the P-PKSR for each study. In total, 183 experimental unknown samples were prepared and analyzed by LC-MS/MS (William Caufield, Principle Bioanalytical Chemist, P-PKSR).

**Table 1.1: Panobinostat Ocular PK Studies Summarized in Report**

| SRM2 Order/Request (O/R) | Description | # Samples |
|---|---|---|
| 27105-158873 | In vivo date: 03/13/2014. Intravitreal injections of panobinostat in 0.9% NaCl. | 11 vitreous, 13 retina, 11 plasma (35 total) |
| 31667-223828 | In vivo date: 05/30/2014. Topical panobinostat in 5% HPBCD. Isoflurane vs awake. | 19 vitreous, 19 retina, 19 plasma (57 total) |
| 33826-254371 | In vivo date: 07/14/2014. Right eyes w/ Y79 RB (Luc+). SC, TOP, or IP. Control, untreated retina and vitreous also submitted. | 41 vitreous, 50 retina (91 total) |

## 2.0 METHODS

### 2.1 In Vivo Study Execution

See **Appendix 6.1**, provided by Eleanor Pritchard, summarizing the in vivo study designs and procedures.

### 2.2 Bioanalytical

A qualified method for mouse plasma, vitreous humor, and retina homogenate was implemented for bioanalysis of the submitted panobinostat samples. Briefly for plasma, a 25 µL aliquot of sample, calibrator or QC, was protein precipitated with 100 µL of 40 ng/mL panobinostat-d8 (IS) in ice cold methanol and a final volume of 100 µL was placed on the autosampler at 4°C. For vitreous and retina samples, the measured mass of the sample was diluted up to a known final volume with blank plasma, and a 25 µL aliquot was subjected to the same protein precipitation and handling as a pure plasma sample.

The extracted samples were analyzed using an HPLC equipped with a 5500 QTRAP™ mass spectrometer in positive ESI mode. MRM transitions of 350.180->158.180 and 357.912->147.193 were monitored for panobinostat and IS, respectively. The LC separation was performed using a Waters XBridge BEH C18 (2.5 µm, 75 mm x 2.1 mm) column with isocratic elution at a flow rate of 0.35 mL/min with a run time of approximately 5 minutes (including equilibration). The water-methanol-acetonitrile-100 mM ammonium formate mobile phase composition was held at 49:26:20:5 (v/v) for 1.6 minutes. The column was then rinsed for 3 minutes at 0:47.5:47.5:5 (v/v) water-methanol-acetonitrile-100 mM ammonium formate and then equilibrated at the initial conditions for 2 minutes. The analyte and deuterated IS eluted at 0.86 minutes under these conditions.

All samples were quantified with plasma based, duplicate calibration curves, with within-run plasma QCs at 5 levels (n=6 at each level) – LLOQ, Low, Mid, High, and Dilution. The dynamic range of the assay spanned from a LLOQ of 5 ng/mL to 1000 ng/mL. Typically, a quadratic regression with $1/X^2$ weighing described the calibrator and QC data sufficiently for quantitation (R>0.99); however, in some instances a linear regression was applied with sufficient results (R>0.99). Pass/Fail criteria for a bioanalytical run consisted of the following:

- 75% of the calibrators in each curve are required to meet the 15% (20% at LLOQ) deviation, including both standards at the LLOQ and the ULOQ.
- 2/3rds of QC replicates must be within 15% (20% at the LLOQ level) of their nominal concentration with precision (%CV) of ≤ 15% (20% at the LLOQ level).
- The percentage of carryover relative to the mean area of the lowest calibrator (LLOQ) is ≤ 20%.

Stability of panobinostat in mouse plasma for 30 days at -80°C has been previously verified by Estella-Hermoso de Mendoza et al. [1], and internally by the P-PKSR. Long-term freezer stability (169 days at -80°C) for panobinostat biological samples was assessed by running stored plasma QCs at the high and low levels (n=7 and 6 respectively). Samples were deemed stable if the mean accuracies at both QC levels did not deviate more than 15% from the nominal concentrations.

### 2.3 Data Analysis

Bioanalytical data from each matrix run was outputted from instrument using Analyst 1.5.2 and MultiQuant 2.1.1 software in a standardized text format and converted to .xlsx files in Excel (**Appendices 6.2 through 6.6**). The files were then manually manipulated using Excel and R to generate the final bioanalytical results for the submitted experimental samples (**Appendix 6.7**).

For the purposes of graphical representation and summary statistics, all data that were below the lower limit of quantitation (BLOQ) were set to ½ the lower limit of quantitation (LLOQ), i.e. 2.5 ng/mL or 0.00715 µM. Summary statistics for concentrations by Study, Matrix, Group, and Time were generated using R. Likewise, scatter plots, boxplots and line graphs to summarize data were generated using R. Concentration data was converted to and presented in micromolar (µM) units (panobinostat MW = 349.43).

## 3.0 RESULTS

### 3.1 In Vivo Study Execution

See **Appendix 6.1**, provided by Eleanor Pritchard, summarizing the in vivo study designs, procedures, and events recorded during in vivo portions.

### 3.2 Bioanalytical

A number of bionalaytical runs were executed to quantitate panobinostat in the submitted samples. The samples for each matrix across studies were batched together and analyzed in separate matrix-specific runs. **Table 3.2.1** lists the run names, dates, and indicates whether the run resulted in usable bioanalytical data. There were technical run failures due to equipment issues and mid-run stoppages.

**Table 3.2.1: Summary of Attempted Bioanalytical Runs for Panobinostat Ocular PK Samples**

| Run Name | Run Date | Matrix | Comments | Usable Data? |
|---|---|---|---|---|
| 33826-254371 PLA 07-22-2014 | 07/22/2014 | Plasma | No technical issues | Yes |

| | | | | |
|---|---|---|---|---|
| 33826-254371 VH 07-24-2014 | 07/24/2014 | Vitreous | Inappropriate dilution discovered | No |
| 33826-254371 VH 07-24-2014_dil-reinj | 07/24/2014 | Vitreous | Technical failure: run stopped at 75% run completion | No |
| 33826-254371 VH 07-24-2014_dil-reinj-2 | 07/28/2014 | Vitreous | Technical failure: injector needle failed. | No |
| 33826-254371 VH 07-24-2014_dil-reinj-3 | 07/28/2014 | Vitreous | No technical issues, peak area ratios were ~30% lower than two previous runs | Yes |
| 33826-254371 VH 07-29-2014 | 07/29/2014 | Vitreous | No technical issues | Yes |
| 33826-254371 Retinae 08-01-2014 Quadratic | 08/01/2014 | Retina | No technical issues | Yes |
| 33826-254371 Retina 08-05-2014 | 08/05/2014 | Retina | No technical issues | Yes |

### 3.2.1 Detailed Descriptions of Bioanalytical Runs Resulting in Usable Data

The details regarding performance characteristics and comments or issues with each of the bioanalytical runs in **Table 3.2.1** above resulting in usable data are provided in **Table 3.2.1.1** below. Issues across the five valid runs involved choice of regression model (quadratic vs linear, weighting schemes), sample handling losses, split peaks with intravitreal injection samples, potential sample stability issues in matrix or extracts, and quantifiable panobinostat in putative untreated control vitreous and retina samples. An example LLOQ plasma chromatogram from Run 33826-254371 PLA 07-22-2014 is presented in **Figure 3.2.1.1** below. **Figure 3.2.1.2** depicts an example split peak from an experimental vitreous sample after an intravitreal injection of panobinostat. Similar split peaks were also noted in retina samples after intravitreal injection and in panobinostat dissolved in 0.9% NaCl (submitted intravitreal formulation sample). Red indicates the internal standard response (panobinostat-d8, 40 ng/mL), while blue indicates panobinostat.

**Table 3.2.1.1: Detailed Summary of Successful Bioanalytical Runs Resulting in Usable Data**

| Run Name/Details | Performance | Comments/Issues |
|---|---|---|
| 33826-254371 PLA 07-22-2014<br><br>Matrix: Plasma<br><br>All studies | ▪ Linear, $1/X^2$ weighting<br>▪ R>0.99<br>▪ Intra P&A: < 8.6% CV and 109-113% above LLOQ, 119% at LLOQ<br>▪ Run PASSED<br>▪ **Appendix 6.2** | ▪ The maximal number of CALs that could be dropped for a valid run was reached (25%)<br>▪ 2/6 QC-LLOQs were out of specification, biasing high.<br>▪ Plasma samples were <u>NOT STABLE</u> when stored for 169 days at -80°C<br>o QC-L mean accuracy 40.1%<br>o QC-H mean accuracy 28.6% |

| | | |
|---|---|---|
| 33826-254371<br>VH 07-24-2014_dil-reinj-3<br><br>Matrix: Vitreous<br><br>27105-158873 & 31667-223828 | ▪ Linear, $1/X^2$ weighting<br>▪ R>0.99<br>▪ Intra P&A: <7.9 % CV and 102- 110% above LLOQ, 114% at LLOQ<br>▪ Run PASSED<br>▪ **Appendix 6.3** | ▪ Prepared, extracted samples remained in autosampler at deg for 4 days – possible stability issues, as indicated by 30% loss of signal on average.<br>▪ All intravitreal injection samples had split peaks (**Figure 3.2.1.2**). Only 1 peak at the known elution time of panobinostat was integrated, so actual concentrations may be higher. |
| 33826-254371<br>VH 07-29-2014<br><br>Matrix: Vitreous<br><br>33826-254371 | ▪ Linear, $1/X^2$ weighting<br>▪ R>0.99<br>▪ Intra P&A: < 3.2% CV and 107- 109% above LLOQ, 109% at LLOQ<br>▪ Run PASSED<br>▪ **Appendix 6.4** | ▪ Quantifiable panobinostat in 5/12 control vitreous samples, averaging 47.6 ng/mL (range: 20.5 - 84.3 ng/mL)<br>▪ Blank samples after highest CAL suggested no quantifiable carryover |
| 33826-254371<br>Retinae 08-01-2014 Quadratic<br><br>Matrix: Retina<br><br>27105-158873 & 31667-223828 | ▪ Quadratic, $1/X^2$ weighting<br>▪ R>0.99<br>▪ Intra P&A: < 3.1% CV and 97- 112% above LLOQ, 105% at LLOQ<br>▪ Run PASSED<br>▪ **Appendix 6.5** | ▪ Two samples were misweighed (2 hr #4 IVT RH 03-13-14 & 0.5 hr #1 iso RH 05-30-14) and were assigned average weights for retinas for their collection group prior to dilution and homogenization.<br>▪ All intravitreal injection samples had split peaks. Only 1 peak at the known elution time of panobinostat was integrated, so actual concentrations may be higher. |
| 33826-254371<br>Retina 08-05-2014<br><br>Matrix: Retina<br><br>33826-254371 | ▪ Quadratic, $1/X^2$ weighting<br>▪ R>0.99<br>▪ Intra P&A: < 5.4% CV and 101- 126% above LLOQ, 104% at LLOQ<br>▪ Run FAILED<br>▪ **Appendix 6.6** | ▪ >2/3rd of the individual QC-L (15 ng/mL) results were out of specification (> 15% bias) resulting in a run failure by acceptance criteria.<br>▪ >85% of experimental results were estimated to be above second calibrator (30 ng/mL) – bias at low end of curve can be considered negligible in this instance.<br>▪ Quantifiable panobinostat in 7/14 control retina samples, averaging 26.4 ng/mL (range: 9.84 – 80.1 ng/mL)<br>▪ Blank samples after highest CAL suggested no quantifiable carryover<br>▪ Experimental samples were diluted with 20 uL of blank plasma. No attempt to scale the amount of plasma diluent according to the retina/tumor weight was made. The higher amount of tumor tissue (~10 mg) compared with standard retina samples (3-5 mg) may result in a matrix effect, biasing concentration estimates in such samples in an unknown direction. |

**Figure 3.2.1.1: Example LLOQ plasma chromatogram from Run 33826-254371 PLA 07-22-2014 (5 ng/mL or 0.0143 µM)**

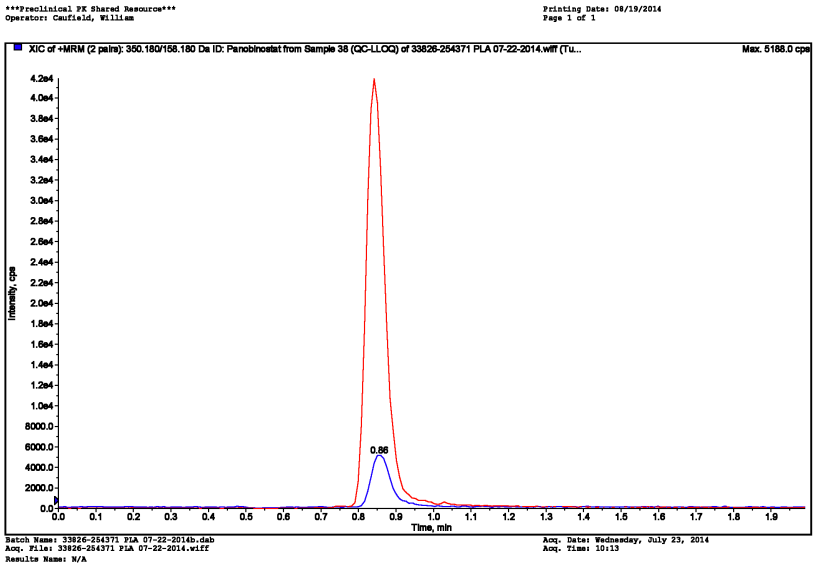
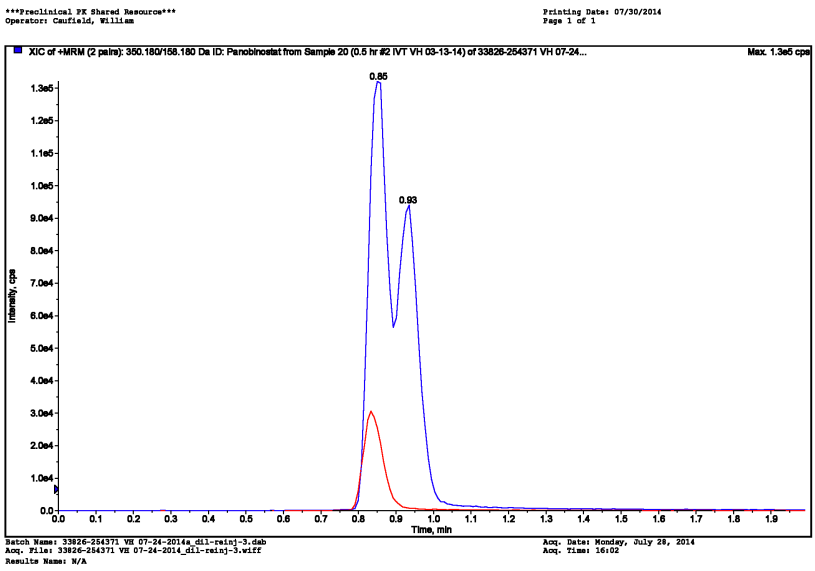



**Figure 3.2.1.2: Example Split Panobinostat Peak from an Experimental Vitreous Sample after Intravitreal Injection**



## 3.3 Data Analysis

### 3.3.1 Quantifiable Panobinostat in Control Vitreous and Retina Samples

For unknown reasons, panobinostat was quantifiable in a majority of control vitreous and retina samples from untreated mice in the O/R 33826-254371 submission. Analytical run blanks ruled out potential carryover from other samples as the cause. Potential sources could be contamination of harvesting equipment during in vivo execution or bioanalytical processing contamination.

Control samples from the right eyes containing the Y79 RB tumors had higher quantifiable panobinostat concentrations in both vitreous and retina, averaging 0.125 µM (43.5 ng/mL) and 0.07 µM (24.4 ng/mL) in vitreous and retina, respectively. There was no apparent within-mouse correlation between the right and left eyes for quantifiable panobinostat for either control retina or vitreous. There was a small within-mouse, within-eye correlation for quantifiable panobinostat between matched control vitreous and retina pairs (Spearman R= -0.255). **Figure 3.3.1.1** and **Figure 3.3.1.2** depict visual scatter plots of quantifiable panobinostat concentrations in control vitreous and retina samples, respectively. The solid horizontal bar in the figures indicates the LLOQ of 0.0143 µM (5 ng/mL). **Table 3.3.1.1** reports the control results for mouse and eye by matrix.

**Figure 3.3.1.1: Quantifiable Panobinostat Concentrations in Control Vitreous Samples**

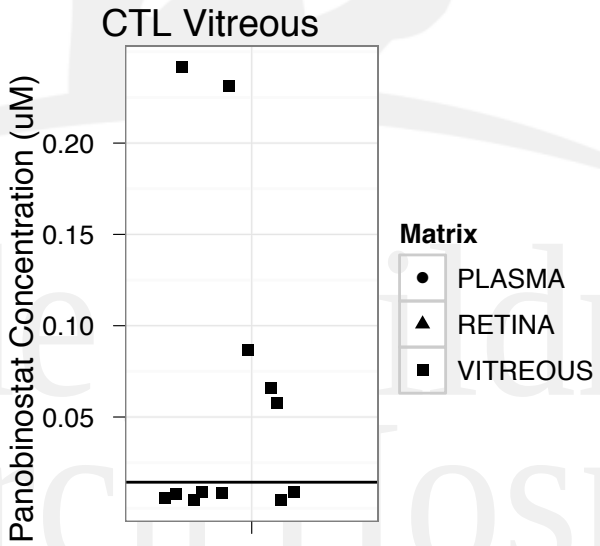


**Figure 3.3.1.2: Quantifiable Panobinostat Concentrations in Control Retina Samples**

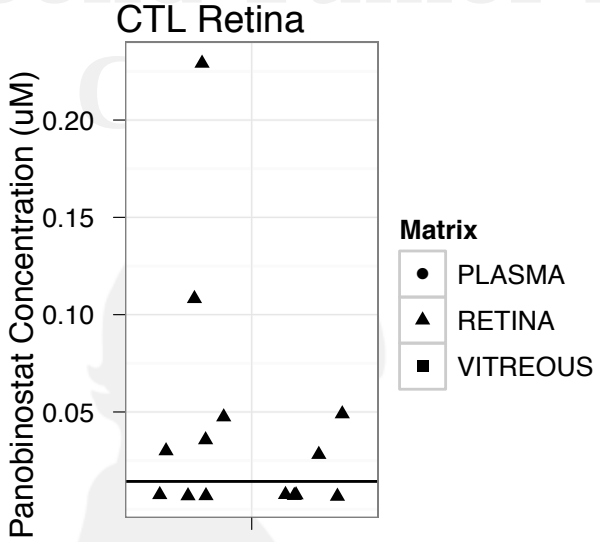



**Table 3.3.1.1: Quantifiable Panobinostat in Control Matrices – Mouse and Eye by Matrix**

| Mouse | Eye | Retina Conc. (µM) | Vitreous Conc. (µM) |
|---|---|---|---|
| 1 | Left | - | - |
| 2 | Left | 0.108 | - |
| 3 | Left | - | 0.0664 |
| 4 | Left | - | - |
| 5 | Left | - | - |
| 6 | Left | - | 0.241 |
| 7 | Left | - | - |
| 1 | Right | 0.0479 | - |
| 2 | Right | 0.229 | - |
| 3 | Right | 0.0494 | 0.0586 |
| 4 | Right | - | - |
| 5 | Right | 0.0295 | 0.0842 |
| 6 | Right | 0.0355 | 0.231 |
| 7 | Right | 0.0282 | - |

- : Result was below the lower limit of quantitation (BLOQ), < 0.0143 µM

### 3.3.2 Results - O/R 27105-158873

Mice were dosed with 5 µL of 40 µM panobinostat in sterile 0.9% NaCl via intravitreal injection, with vitreous, retina, and plasma samples taken at 0.5, 1, and 2 hours post-dose.

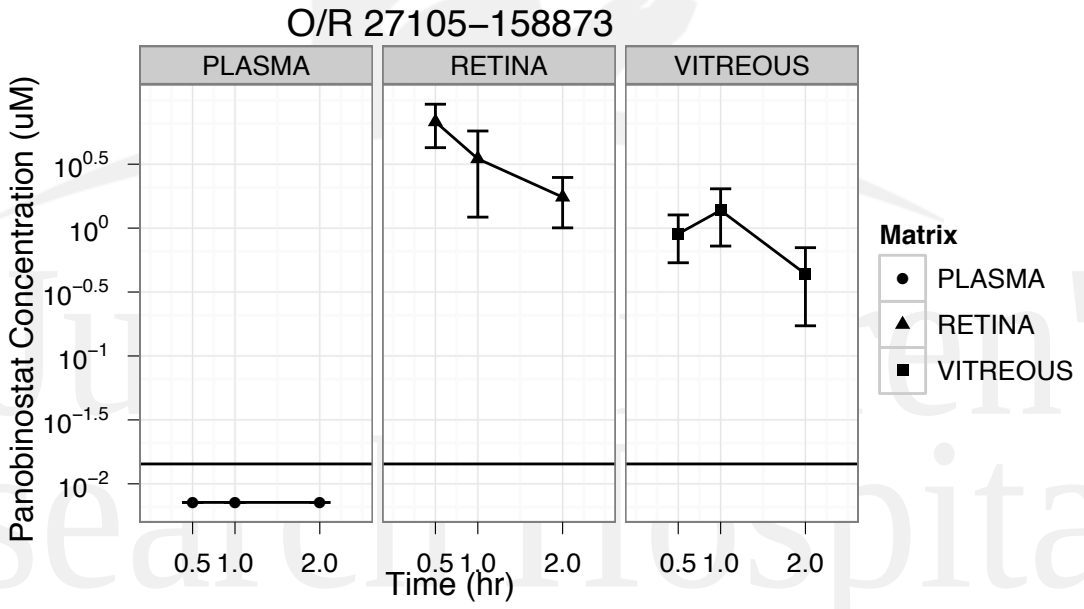
Retinal concentrations of panobinostat were higher than those in the vitreous, and no quantifiable panobionstat was noted in the plasma. During the 2-hour sampling period, retinal concentrations ranged from 0.838 to 9.34 µM, while vitreous concentrations ranged from 0.159 to 1.16 µM. The mean retina $C_{max}$ of 6.79 µM was noted at 0.5 hours post dose, while the mean vitreous $C_{max}$ of 1.38 µM was observed at 1 hour. Note: actual retina and vitreous concentrations may be higher

by ~1.5-fold secondary to peak splitting. **Table 3.3.2.1** presents the summary statistics for the panobinostat concentrations, with all BLOQ data set to ½ the BLOQ (0.00715 µM). Mean (SD) concentration-time (Ct) profiles by each matrix are presented in **Figure 3.3.2.1**. Note that the horizontal line in the figure indicates the LLOQ for the assay (0.0143 µM).

**Table 3.3.2.1: Summary Ct Statistics by Matrix for O/R 27105-158873**

| Matrix | Side | Time (hr) | N | Mean (µM) | SD (µM) | Median (µM) | Min (µM) | Max (µM) |
|---|---|---|---|---|---|---|---|---|
| PLASMA | NR | 0.5 | 3 | 0.00715 | 0 | 0.00715 | 0.00715 | 0.00715 |
| PLASMA | NR | 1 | 3 | 0.00715 | 0 | 0.00715 | 0.00715 | 0.00715 |
| PLASMA | NR | 2 | 5 | 0.00715 | 0 | 0.00715 | 0.00715 | 0.00715 |
| RETINA | NR | 0.5 | 3 | 6.79 | 2.53 | 6.74 | 4.28 | 9.34 |
| RETINA | NR | 1 | 5 | 3.48 | 2.27 | 2.7 | 1.09 | 7.08 |
| RETINA | NR | 2 | 5 | 1.75 | 0.743 | 1.81 | 0.838 | 2.56 |
| VITREOUS | NR | 0.5 | 2 | 0.902 | 0.366 | 0.902 | 0.643 | 1.16 |
| VITREOUS | NR | 1 | 4 | 1.38 | 0.653 | 1.19 | 0.824 | 2.3 |
| VITREOUS | NR | 2 | 5 | 0.438 | 0.266 | 0.377 | 0.159 | 0.869 |

**Figure 3.3.2.1: Mean (SD) Panobinostat Ct Profiles by Matrix**




### 3.3.3 Results – O/R 31667-223828

Two groups of mice (isoflurane anesthetized vs. awake) were dosed with 5 µL of 50 mM panobinostat in 5% HPBCD in both eyes, with plasma, vitreous, and retina samples taken at 0.5 and 1 hour post-dose.

The overall variability in concentrations between mice and groups was high, with a median [range] CV of 70% [27.5% - 129%]. The isoflurane anesthetized mice tended to have higher

panobinostat concentrations in all matrices, with slightly less variability, particularly for the vitreous. Plasma concentrations were quantifiable, indicating likelihood of systemic absorption from the topical route. Retinal panobinostat concentrations were lower than those observed from intravitreal injections in O/R 27105-158873. Vitreous panobinostat concentrations tended to peak faster and higher at the 0.5 hour time point for topical administration, but declined rapidly compared with intravitreal injections.

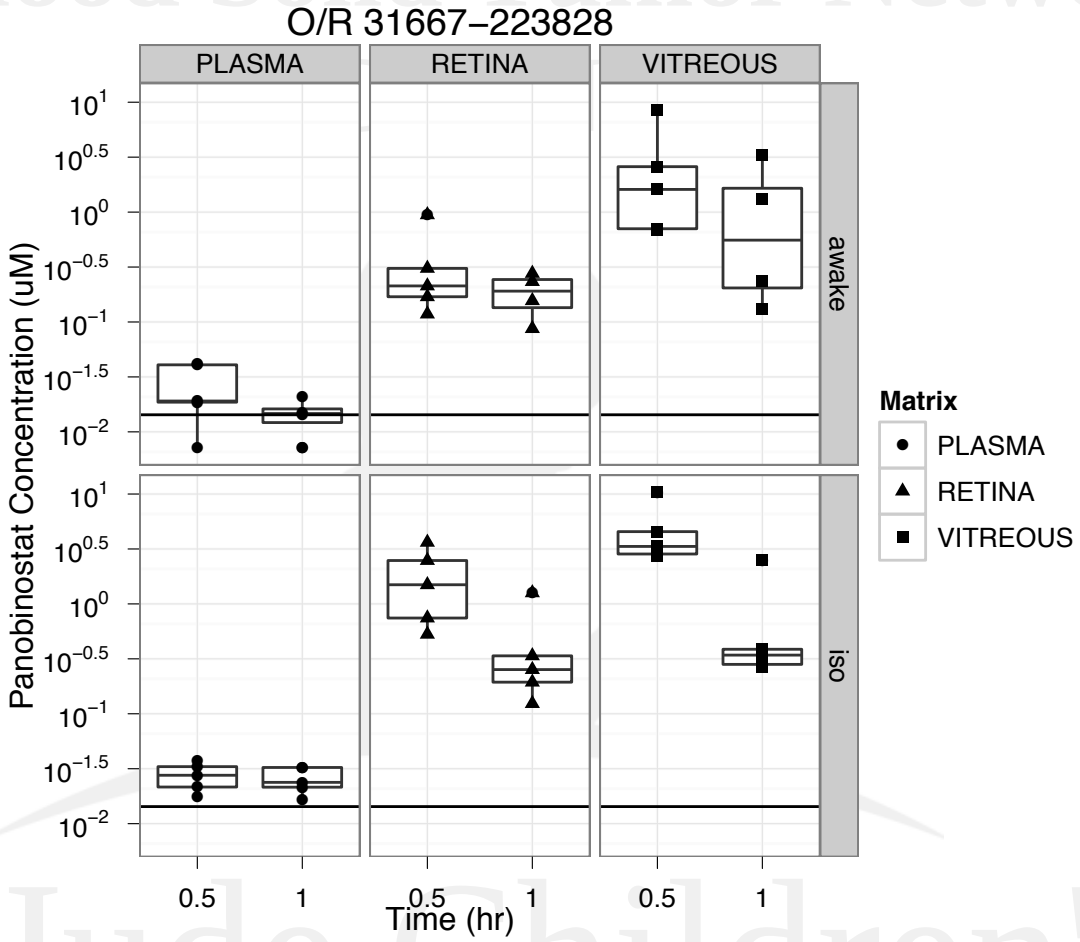
**Table 3.3.3.1** presents the summary statistics for the panobinostat concentrations, with all BLOQ data set to ½ the BLOQ (0.00715 µM). Scatter plots and Median-Quartile boxplots by each group, matrix, and time point are presented in **Figure 3.3.3.1**. Note that the horizontal line in the figure indicates the LLOQ for the assay (0.0143 µM).

**Table 3.3.3.1: Summary Ct Statistics by Group and Matrix for O/R 31667-223828**

| **Group: Isoflurane** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Matrix** | **Side** | **Time (hr)** | **N** | **Mean (µM)** | **SD (µM)** | **Median (µM)** | **Min (µM)** | **Max (µM)** |
| PLASMA | NR | 0.5 | 5 | 0.0275 | 0.00818 | 0.0276 | 0.0177 | 0.0377 |
| PLASMA | NR | 1 | 5 | 0.0254 | 0.00698 | 0.0238 | 0.0167 | 0.0325 |
| RETINA | NR | 0.5 | 5 | 1.78 | 1.29 | 1.49 | 0.529 | 3.63 |
| RETINA | NR | 1 | 5 | 0.434 | 0.469 | 0.253 | 0.124 | 1.26 |
| VITREOUS | NR | 0.5 | 5 | 4.77 | 3.23 | 3.33 | 2.73 | 10.4 |
| VITREOUS | NR | 1 | 5 | 0.755 | 0.977 | 0.342 | 0.265 | 2.5 |

| **Group: Awake** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Matrix** | **Side** | **Time (hr)** | **N** | **Mean (µM)** | **SD (µM)** | **Median (µM)** | **Min (µM)** | **Max (µM)** |
| PLASMA | NR | 0.5 | 5 | 0.0255 | 0.0152 | 0.0191 | 0.00715 | 0.0418 |
| PLASMA | NR | 1 | 4 | 0.0143 | 0.0056 | 0.0147 | 0.00715 | 0.0208 |
| RETINA | NR | 0.5 | 5 | 0.351 | 0.34 | 0.213 | 0.118 | 0.945 |
| RETINA | NR | 1 | 4 | 0.188 | 0.0839 | 0.195 | 0.087 | 0.277 |
| VITREOUS | NR | 0.5 | 5 | 2.81 | 3.25 | 1.61 | 0.685 | 8.45 |
| VITREOUS | NR | 1 | 4 | 1.24 | 1.46 | 0.773 | 0.132 | 3.28 |

**Figure 3.3.3.1: Median (Quartile) Panobinostat Ct Profiles by Group and Matirx**




### 3.3.4 Results – O/R 33826-254371

Mice bearing Y79 RB luciferase tumor in their right vitreous only were divided into three separate groups and dosed in both eyes with 1) 5 µL of 4 mM panobionstat in "ocular formulation" subconjunctivally, 2) 5 µL of 50 mM panobinostat in 5% HPBCD topically, or 3) 8 mg/kg panobinostat by IP injection. Vitreous and retinas were harvested at 1 and 2 hours post-dose. Untreated mice were also harvested as controls (see **Section 3.3.1**).

There was very high variability in panobinostat concentrations in the vitreous and retina (median [range] CV: 121% [36.5% - 268%]), with the topical (TOP) route resulting in more variability vs. intraperitoneal (IP) or subconjunctival (SC) administrations. Despite the variability, the topical route trended toward higher vitreous and retina concentrations. Generally, no differences in concentrations between the 1 and 2 hour time points could be noted, except for the Topical Left Vitreous samples, which tended to be lower at 2 hours. No differences between retina and vitreous concentrations for the same administration route were evident. The right side bearing the vitreal Y79 RB Luc+ tumors tended to have higher retina and vitreous concentrations relative to the non-tumor bearing left side, regardless of the route of administration. Finally, the panobinostat vitreous concentrations after IP administration of 8 mg/kg were in line with those anticipated given

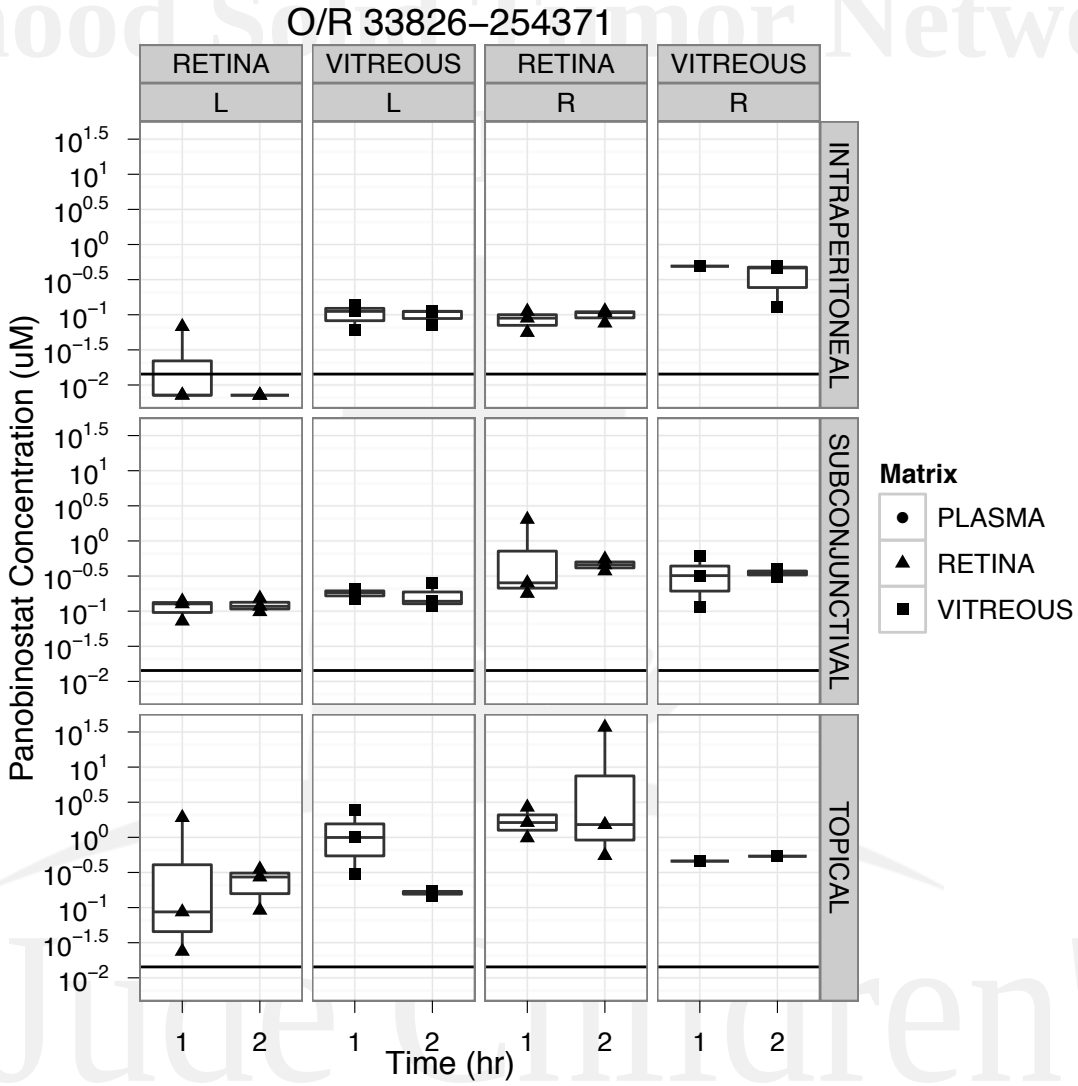
the previous systemic "screening" PK study in rhabdomyosarcoma bearing mice (O/R 19516-89512). In this previous study, retina and vitreous concentrations were similar; however, in the currently reported IP study, the vitreous concentrations were higher than retina.

**Table 3.3.4.1** presents the summary statistics for the panobinostat concentrations, with all BLOQ data set to ½ the BLOQ (0.00715 µM). Scatter plots and Median-Quartile boxplots by each route, matrix, side, and time point are presented in **Figure 3.3.4.1**. Note that the horizontal line in the figure indicates the LLOQ for the assay (0.0143 µM).

**Table 3.3.4.1: Summary Ct Statistics by Route, Matrix, and Side for O/R 33826-254371**

| Route | Matrix | Side | Time (hr) | N | Mean (µM) | SD (µM) | Median (µM) | Min (µM) | Max (µM) |
|---|---|---|---|---|---|---|---|---|---|
| IP | RETINA | L | 1 | 3 | 0.0274 | 0.035 | 0.00715 | 0.00715 | 0.0678 |
| IP | RETINA | L | 2 | 3 | 0.00715 | 0 | 0.00715 | 0.00715 | 0.00715 |
| IP | RETINA | R | 1 | 3 | 0.0858 | 0.0283 | 0.0894 | 0.0558 | 0.112 |
| IP | RETINA | R | 2 | 3 | 0.0989 | 0.02 | 0.107 | 0.076 | 0.113 |
| IP | VITREOUS | L | 1 | 3 | 0.103 | 0.0386 | 0.112 | 0.0607 | 0.136 |
| IP | VITREOUS | L | 2 | 3 | 0.0981 | 0.0241 | 0.111 | 0.0703 | 0.113 |
| IP | VITREOUS | R | 1 | 1 | 0.491 | NA | 0.491 | 0.491 | 0.491 |
| IP | VITREOUS | R | 2 | 3 | 0.36 | 0.201 | 0.464 | 0.128 | 0.489 |
| SC | RETINA | L | 1 | 3 | 0.113 | 0.0356 | 0.127 | 0.0722 | 0.139 |
| SC | RETINA | L | 2 | 3 | 0.123 | 0.0276 | 0.117 | 0.0983 | 0.153 |
| SC | RETINA | R | 1 | 3 | 0.819 | 1.04 | 0.253 | 0.179 | 2.02 |
| SC | RETINA | R | 2 | 3 | 0.461 | 0.0885 | 0.457 | 0.374 | 0.551 |
| SC | VITREOUS | L | 1 | 3 | 0.179 | 0.029 | 0.184 | 0.148 | 0.206 |
| SC | VITREOUS | L | 2 | 3 | 0.17 | 0.0737 | 0.138 | 0.118 | 0.254 |
| SC | VITREOUS | R | 1 | 3 | 0.346 | 0.244 | 0.321 | 0.116 | 0.602 |
| SC | VITREOUS | R | 2 | 2 | 0.353 | 0.0637 | 0.353 | 0.308 | 0.398 |
| TOP | RETINA | L | 1 | 3 | 0.674 | 1.07 | 0.0868 | 0.0238 | 1.91 |
| TOP | RETINA | L | 2 | 3 | 0.239 | 0.134 | 0.272 | 0.0915 | 0.353 |
| TOP | RETINA | R | 1 | 3 | 1.76 | 0.859 | 1.63 | 0.982 | 2.68 |
| TOP | RETINA | R | 2 | 3 | 13 | 20.7 | 1.52 | 0.55 | 36.9 |
| TOP | VITREOUS | L | 1 | 3 | 1.24 | 1.08 | 0.996 | 0.297 | 2.42 |
| TOP | VITREOUS | L | 2 | 3 | 0.162 | 0.0149 | 0.166 | 0.145 | 0.174 |
| TOP | VITREOUS | R | 1 | 1 | 0.459 | NA | 0.459 | 0.459 | 0.459 |
| TOP | VITREOUS | R | 2 | 1 | 0.539 | NA | 0.539 | 0.539 | 0.539 |

**Figure 3.3.3.1: Median (Quartile) Panobinostat Ct Profiles by Route, Side, and Matirx**




## 4.0 CONCLUSIONS

- Reported results herein should be considered qualitative due to bioanalytical issues and study designs. Results provide rough rank-order of formulation and route performance, and concentrations should not be considered of adequate precision. Thus concentration results should be de-emphasized for use in inferences, extrapolations, or modeling exercises.
- Submitted, control retina and vitreous matrices from untreated mice contained significant amounts of panobinostat in roughly half of the samples, from the right tumor bearing retinas in particular. Analytical carryover ruled out as cause; likely contamination from harvesting equipment or bioanalytical processing.
- Panobinostat is NOT STABLE in mouse plasma stored for 169 days (~6 months) at -80°C, resulting in a loss of ~ 66%, impacting reported bioanalytical results.
  - Loss of stability occurs between 30 and 169 days

- Plasma findings on stability likely to apply to retina, vitreous, and other mouse tissues
- Submitted samples were stored for between 9 and 145 days at -80°C

- Panobinostat concentrations in retina and vitreous after intravitreal injection in 0.9% NaCl may be higher than reported (~1.5-fold) due to matrix effect / peak splitting.
  - Likely an effect from relatively large volume of 0.9% NaCl in vitreous and retina after intravitreal injection; loss of vitreous volume due to needle puncture may exacerbate.
  - Submitted intravitreal formulation in 0.9% NaCl also had same split peak phenomenon.
  - Panobinostat has only been reported as formulated in 5% dextrose (D5W) for preclinical IP, IV, and clinical IV administration. Stability could be compromised in 0.9% NaCl.

- After intravitreal injection, retina concentrations of panobinostat were slightly higher than vitreous, and peaked more rapidly. Plasma concentrations were all BLOQ.

- After topical administration, variability in panobinostat concentrations was high-to-extreme in all matrices, and included quantifiable panobinostat in plasma (systemic absorption). While median retina and vitreous concentrations were modestly higher than other routes, variability raises concerns about exposure consistency during therapeutic application. Isoflurane anesthesia, as a chemical restraint to permit better eye surface retention, reduced the variability, but not to a practically significant extent.

- Subconjunctival administration resulted in low-to-moderate variability in panobinostat concentrations, and also included quantifiable panobinostat in plasma indicating systemic absorption. Retina and vitreous concentrations from subconjunctival were on par with those from intraperitoneal injection of the murine equivalent dosage of 8 mg/kg (similar plasma AUC to humans at recommended Phase 2 dose).

- Regardless of route, panobinostat concentrations in the retina and vitreous of the right, Y79 RB Luc+ cell bearing eyes tended to be higher than the non-tumored left eyes. This could be an artifact of implantation or a bioanalytical matrix effect, as control tumor bearing retina and vitreous also tended to have higher levels of panobinostat.

- Rank-ordering of routes and formulations by pertinent favorable characteristics:
  - Variability (low to high): IP < IVT < SC < TOP
  - Concentration (high to low): TOP > IVT > SC = IP
  - Plasma Conc. (low to high): IVT < TOP (awake) < TOP (iso) < IP
  - Abbreviations: IP, intraperitoneal; IVT, intravitreal; SC, subconjunctival; TOP, topical

## 5.0 REFERENCES

[1] A. Estella-Hermoso de Mendoza, I. Imbuluzqueta, M. A. Campanero, D. Gonzalez, A. Vilas-Zornoza, X. Agirre, H. Lana, G. Abizanda, F. Prosper, and M. J. Blanco-Prieto, “Development and validation of ultra high performance liquid chromatography–mass spectrometry method for LBH589 in mouse plasma and tissues,” *J. Chromatogr. B*, vol. 879, no. 30, pp. 3490–3496, Nov. 2011.

## 6.0 APPENDICES

**Appendix 6.1** Summary of panobinostat samples dropped off 07-22-2014.docx
**Appendix 6.2** 33826-254371 PLA 07-22-2014.xlsx
**Appendix 6.3** 33826-254371 VH 07-24-2014_dil-reinj-3.xlsx
**Appendix 6.4** 33826-254371 VH 07-29-2014.xlsx
**Appendix 6.5** 33826-254371 Retinae 08-01-2014 Quadratic.xlsx

**Appendix 6.6** 33826-254371 Retina 08-05-2014.xlsx
**Appendix 6.7** Panobinostat Ocular Final