	Report *R # XTN025365*	#	R#XTN025365
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Efficacy *in vivo* VZV study

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List of Abbreviations

IV	intravenous
IP	intraperitoneal
Subq	subcutaneous
mg/kg	milligram per kilogram
ng/mL	nanogram per milliliter
DNA	deoxyribonucleic acid
D5W	5% dextrose in water (278 mmol/L dextrose)
LPX	lipoplex
GLP	Good Laboratory Practice
g	gram
min	minute
hr	hour
LPX	lipoplex
LC	lethal concentration
LD	lethal dose
LC50	concentration causing death in 50%
ppm	parts per million parts
MTD	maximum tolerated dose
OSHA	Occupational Safety and Health Administration
CFR	Code of Federal Regulations

1. STUDY OBJECTIVE

The objective of this non-GLP study is to evaluate preclinically the *in vivo* therapeutic efficacy of topical application of compound of interest (modified Hexagen Wound Dressing, original formula is 95% petrolatum and the other 5% is liquid) in animal model of VZV as described in the article Chronic Uveitis in Guinea Pigs Infected with Varicella-Zoster Virus Expressing Escherichia coli b-Galactosidase, *Journal of Infectious Diseases*.

2. STUDY ANIMALS AND IACUC REVIEW

Altogen Labs received IACUC study LC03397 protocol approval on August 20, 2018 (Altogen Labs IACUC protocol number 4-05782). Twenty (20) Dunkin Hartley Guinea Pigs were purchased from the Harlan Laboratories (Envigo) and went through mandatory 1-week acclimatization per IACUC guidelines. All animal procedures and maintenance were conducted in accordance with the institutional guidelines. Mice were housed at Altogen Labs animal facility and carcasses frozen and disposed at the end of the study per institutional guidelines.

3. STUDY DESIGN

The administration of the test articles and the animal numbers in each study group are shown in the following experimental design table.

Table 1. Study Design

Group	N	Treatment	Dosing Route	VZV Infection	Schedule
1	10	No-treatment Control	-	Yes	-
2	10	Test Compound	*Topical*	Yes	Daily

*Note: N = animal number

Total of 20 animals. Study type: non-GLP.

Thirty (30) day study.

4. MATERIALS AND EQUIPMENT

Animals

Species: Dunkin Hartley Guinea Pig, Female

Strain: HsdDhl:DH

Age: 8-10 weeks old

Total number: 20

Animal supplier: Harlan Laboratories (Envigo)

VZV administration: Intravitreal. Animal model of VZV as described in the article Chronic Uveitis in Guinea Pigs Infected with Varicella-Zoster Virus Expressing Escherichia coli b-Galactosidase

VZV strain: VR-1433 (ATCC)

Test article administration: Topical/Ocular

Endpoint: Perform eye isolation and dissection for quadrant analysis, followed by tissue processing and DNA isolation for TaqMan-based qPCR to quantitate VZV expression level

The animals were housed in individual ventilated cages (up to 2 guinea pigs mice per cage) under the following conditions:

- Temperature: 22-25ºC
- Humidity: 40-60%
- Light cycle: 12 hours light and 12 hours darkness
- Diet: Standard guinea pig diet, dry granule food
- Water: sterile water, autoclaved before using
- Cage identification label: per Altogen Labs IACUC
- Animal identification: per Altogen Labs IACUC
- Acclimatization (adapt housing): 7 days mandatory acclimatization period

Test and reference articles

Test article was delivered from Global Health Solutions. All articles received in good condition. Test article is modified formulation of Hexagen Wound Dressing (original formulation is 95% petrolatum and the other 5% is liquid).

5. STUDY DEVIATIONS

Changes to the Study Plan

Guinea pigs eye dissection into separate tissues was technically challenging due to a small size of the eye and couldn't guarantee accurate tissue dissection, therefore eye enucleation was performed for each animal.

6. EXPERIMENTAL METHODS

VZV administration

VZV strain VR-1433 was obtained from ATCC and administered via intravitreal injection. The right eyes of twenty (20) guinea pigs were intravitreally injected with 10 µL of VR-1433.

Test article administration

Test article was administered as topical application daily. All work performed in a Biological Safety Cabinet to prepare for topical application.

Tissue dissection

Guinea pigs eye dissection into separate tissues was technically challenging due to a small size of the eye and couldn't guarantee accurate tissue dissection, therefore eye enucleation was performed for each animal. After euthanasia, eyes were enucleated, and washed twice in 0.01M TRIS-HCl (pH 7.5), 0.1M sodium chloride, and 0.05M EDTA buffer.

DNA isolationTissue dissection

DNA was isolated using Qiagen DNA Isolation Kit QIAamp DNA Mini Kit (51304) following manufacturer recommended protocol.

qPCR analysis

Primers and TaqMan probes (IDT):

GAPDH was amplified as an internal reference to adjust for well-to-well variances in the

amount of starting template. The MSI2 values were corrected to the GAPDH values (internal reference).

"% Remaining Expression Levels" were calculated as a percentage of VZV expression relative to the negative control (no-treatment group). A value of 100% would represent an untreated sample. Values less than 100% exhibit the percent reduction of the target (VZV).

1. Cavia Porcellus housekeeping gene (GAPDH):

a.GAPDH FWD: 5'-CACCCAGAAGACTGTGGATG-3'

b.GAPDH REV: 5'-GATGCGGGGATGATGTTCT-3'

c.GAPDH Probe: 5'-FAM-CCTCTGGGAAGCTGTGGC-3'

2.VZV primers and TaqMan probe:

a.VZV FWD: 5'-CGGCATGGCCCGTCTAT-3'

b.VZV REV: 5'-TCGCGTGCTGCGGC-3'

c.VZV Probe: 5'-FAM-ATTCAGCAATGGAAACACACGACGCC-3'

TaqMan Fast Advanced Master Mix (ThermoFisher, #4444556) was used for qPCR analysis (384-well format / standard run mode protocol per manufacturers recommended protocol). Concentration of DNA samples were measured prior to analysis and all templates were normalized to 10 ug/ml concentration.

Observation and data collection

Aanimals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any adverse health effects of VZV infection and test article treatment on normal behavior such as mobility, visual estimation of food and water consumption, body weight gain/loss, eye/hair matting and any other abnormal effects. Death and observed clinical signs were recorded per Altogen Labs IACUC

Handling of animals with body weight loss (BWL) during the study and the humane end points of the animals. Body weight loss during the study.

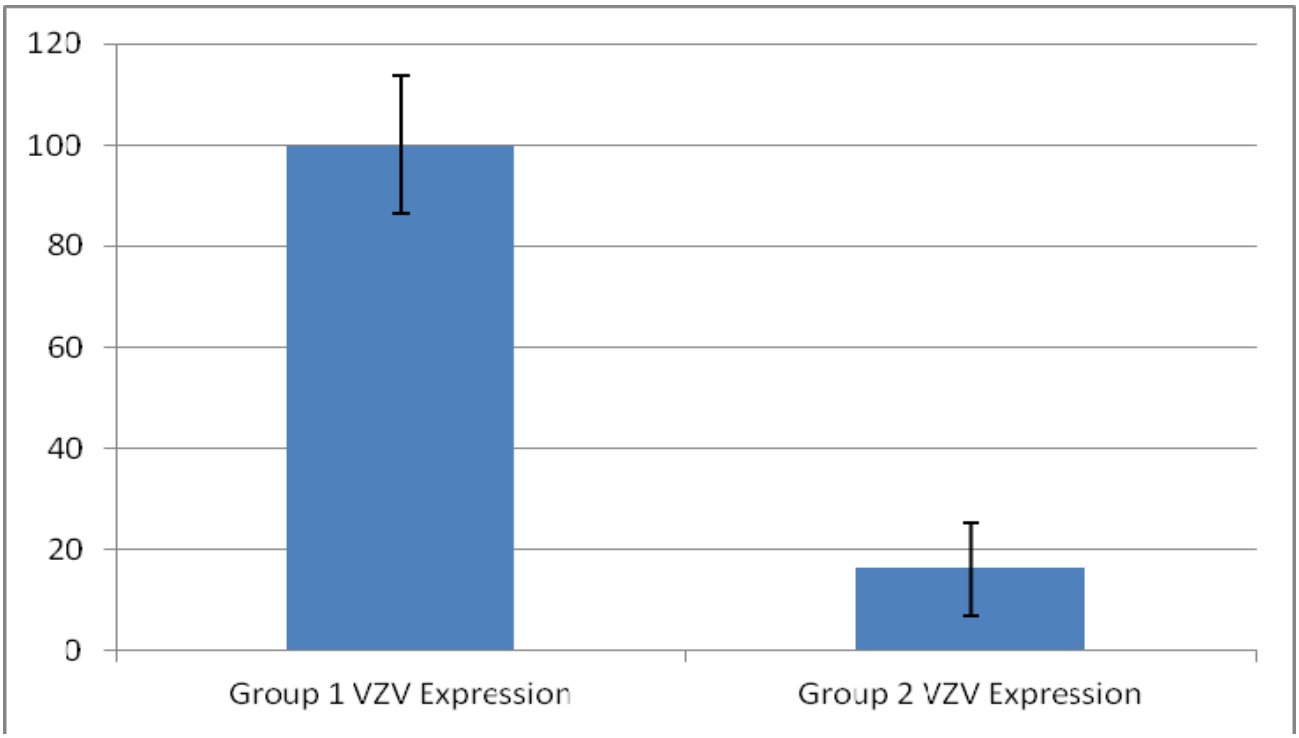
Sacrifice the mouse when one measurement of body weight loss (BWL)> 20%. Animal research associated with this study was conducted under Altogen Labs IACUC regulatory space. Altogen Labs IACUC guidelines were followed in case of BWL and any other conditions.

7. STATISTICS

Summary statistics, the mean and the standard error of the mean (SEM), are provided for the tumor volume of each group at each time point. Statistical analysis of difference in tumor volume among the groups and the analysis of drug interaction were conducted on the data obtained after the final dose.

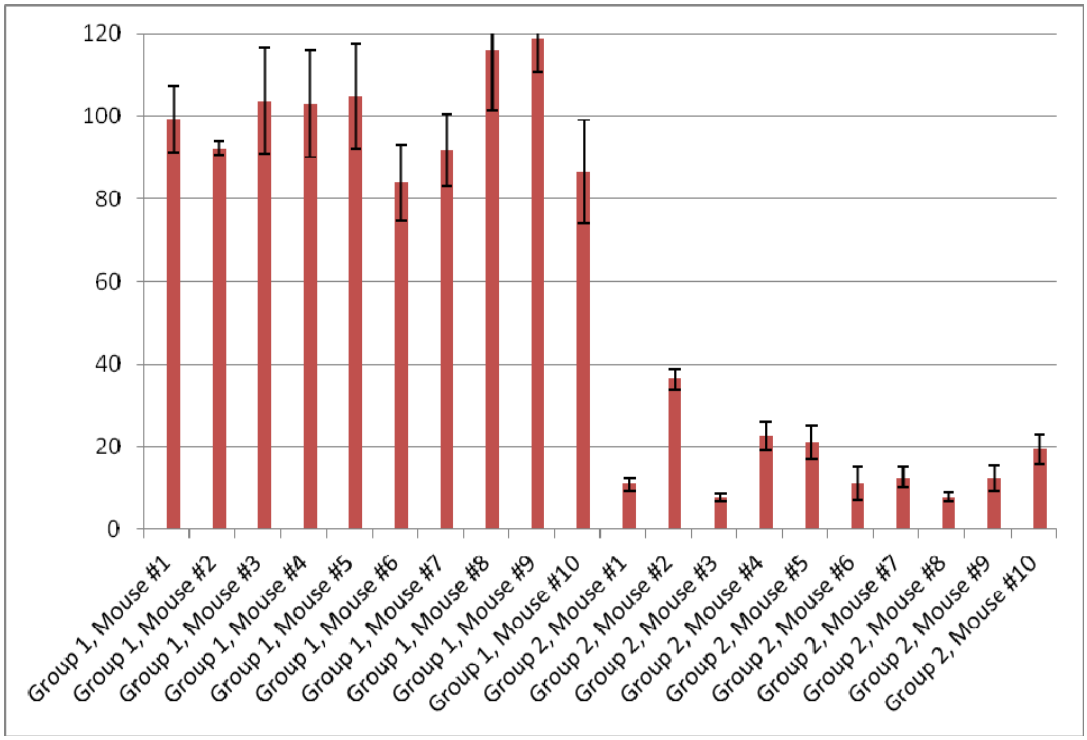
8. RESULTS

Figure 1. Percent decrease of VZV expression of Group 1 vs Group 2 (average value of Group 1 is denoted as 100%). Test article ID and administration schedule indicated in Table 1 above.



*Please see attached Excel file (data.xls) for raw data record.

Figure 2. Percent decrease of VZV expression of individual animals (average value of Group 1 is denoted as 100%). Test article ID and administration schedule indicated in Table 1 above.



*Please see attached Excel file (data.xls) for raw data record.

Survival Time

There was no animal death observed within the study.

Clinical Observations

All animals that received intravitreal injection of VZV strain developed redness of the eye, no other symptoms were observed. There were no other clinical signs or behavioral phenotypes observed within the study (daily cage intensive observation for adverse effect were performed). No animals were observed to have >20% BWL within the study period.

9. ATTACHMENT

Row data attached in Excel file data format (data.xls)