INTRODUCTION

• Chikungunya virus (CHIKV) is often characterized in affected individuals by inflammation produced by pro-inflammatory cytokines in the plasma, the severity of which is directly correlated to the severity of the disease
• The cleavage of furin, an important mammalian cell protein convertase, is crucial for viruses to enter and exit host cells
• Inhibitors of furin have been shown to inhibit alphavirus propagation
• Boston Pharmaceuticals has identified a furin inhibitor, BOS-981, as a potential treatment option for CHIKV
• Past studies have evaluated the tolerability of furin inhibitors in the BALB/c mouse model
• Furin inhibitors, similar to that of BOS-981, have been successful at decreasing the expression of pro-inflammatory cytokines, as well as total number of macrophages, in the BALB/c mouse model.

OBJECTIVES

1. To determine the tolerability of BOS-981, a furin inhibitor, at previously evaluated doses in C57BL/6 mice
2. To determine the effect of a furin inhibitor in a mouse model of Chikungunya disease

MATERIALS AND METHODS

Toxicity Study [completed]

Animals: C57BL/6 mice, all female, 3 mice/group.
Virus: N/A
Vehicle: D-Mannitol and Citric Acid
Osmotic pumps: 1 Alzet Model 2001 osmotic pump per mouse was filled with the designated treatment regimen, primed in saline and incubated at 37°C until implantation.
Implantation:
Animals were anesthetized using Ketamine Xylazine, at which time their lumbar region was shaved, and disinfected using 10% betadine. An incision was made in this lumbar region, the pump was removed from its saline bath and inserted, subcutaneously, into the mouse. The incision site was then wound-clipped.
Parameters:
Individual weights and toxicity signs, recorded daily.

Antiviral Study [projected Dec. 2021]

Animals: C57BL/6 mice, 25 male and 34 female, 10 animals/infected group, 3 animals/uninfected group, 59 mice total.
Virus: La Reunion Island isolate CHIKV (LR 2006-OPYI)
Vehicle: D-Mannitol and Citric Acid
Osmotic pumps: 1 Alzet Model 2001 osmotic pump per mouse will be filled with the designated treatment regimen, primed in saline and incubated at 37°C until implantation.
Implantation:
Animals will be anesthetized using Ketamine Xylazine, at which time their lumbar region will be shaved, and disinfected using 10% betadine. When an incision is made in this lumbar region, the pump will be removed from its saline bath and inserted, subcutaneously, into the mouse. The incision site will then be wound-clipped.
Parameters:
Footpad measurements, recorded daily. Serum collection from G2, only, at 24- and 72-hours post-implantation. Virus titration in hind limb to be completed on day 6. Necropsy to collect and record spleen weights is to be completed on day 6.

RESULTS

Toxicity Study [completed]
All mice in the toxicity study survived for its entirety, except a single mouse that was euthanized one day before the study’s termination as a result of a protruding osmotic pump. No toxicity signs were recorded for the full length of the study and as is visible in Figure 2, individual weights generally increased throughout the duration of the study.

Antiviral Study [anticipated results]
In groups treated with the BOS-981 compound, we expect to see less severe footpad swelling, and spleen weights lighter than those recorded in our placebo groups. The placebo groups will most likely show a downward trend in overall weight, more severe footpad swelling, and higher spleen weights. Lastly, the treatment groups will have a lower virus titer than that of any placebo group.

CONCLUSION

The furin inhibitor, BOS-981, was not toxic to the C57BL/6 mouse model in the chosen concentrations employed for the toxicity study. Not only was the compound found to be non-toxic, but it was also well tolerated, evident in the overall healthy appearance of the mice and gradual increase in individual weights throughout the study. This is unlike the dwindling weights which are usually observed in sick or dying mice. Provided once the antiviral study has been completed that there is less footpad swelling, smaller recorded spleen weights, and a lower virus titer for the treatment groups in comparison to the placebo groups, we can conclude that BOS-981’s properties as a furin inhibitor characterize it as an effective treatment of the inflammatory symptoms associated with CHIKV.

REFERENCES