COMBATTING FUTURE BIOLOGICAL THREATS – HOST-DIRECTED INTERVENTIONS TO EMERGING THREATS FOR RAPID RESPONSE

The New World Alphavirus Inhibitor Bardoxolone Methyl Affects Host Kinases And Impacts Nonstructural Protein Production

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New world alphaviruses, including Venezuelan equine encephalitis virus (VEEV) and eastern equine encephalitis virus (EEEV) are arboviruses that pose a threat to human and animal health. There are currently no FDA approved therapeutics for the treatment or prevention of disease caused by these alphaviruses in humans. Bardoxolone methyl (BARM) is a therapeutic which was developed to treat kidney disease and functions by impacting the ubiquitin-mediated signaling pathway. We have previously shown that BARM has robust antiviral activity against VEEV TC-83, fully virulent VEEV TrD, and EEEV FL-93 in vitro. Our data suggested that BARM exerted alphavirus inhibition in a cell type independent manner in multiple relevant cell types of the central nervous system (CNS). Time of addition studies have revealed that BARM is able to exert potent inhibition of EEEV even when treatment is delivered several hours after infection in the in vitro model. We have expanded our work to investigate the mechanism by which BARM impacts alphavirus replication by assessing its effects on viral protein production and impact on host kinases. We have found that BARM impacts the production of VEEV nonstructural proteins in a dose dependent fashion as determined by western blot studies. Additional work will be done to assess if the localization of the nonstructural proteins are also affected by microscopy methods. Ongoing studies are focused on confirming the effects of BARM on host kinases and determining if these kinases impact viral replication by siRNA knockdown. Through a kinome screen, our early studies suggest that BARM downregulates various tyrosine and serine/threonine kinases in human vascular endothelial cells. We have collected data from proteomic inquiry of signal transduction events in the context of BARM treatment to synergize with the kinome screen and data analysis is in progress. We anticipate to show that the effects of BARM on host kinases may be impacting the production of the viral nonstructural proteins and pinpoint which kinases are important for the establishment of a productive infection by alphaviruses in CNS cells. With the broadly relevant role of host-based, ubiquitin-driven signaling in the establishment of productive infections by multiple vector-transmitted, RNA-genome containing viruses, we anticipate that BARM will offer broad-spectrum therapeutic solutions to address the challenge posed by several emerging viral pathogens that put the warfighter at risk of exposure.