CAMO (COMPARING ANIMAL MODELS TO ORGANOIDS) - TESTING MEDICAL COUNTERMEASURES WITH MICROPHYSIOLOGICAL SYSTEMS AND COMPARING TO TRADITIONAL ANIMAL MODELS AND CLINICAL TRAILS

Comparison Of Therapeutic Effectiveness Of Alphavirus NsP2 Protease Inhibitors In In Vitro And In Vivo Models Of Infection By Venezuelan Equine Encephalitis Virus

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The application of microphysiological systems (MPS) in the preclinical countermeasures development pipeline will greatly accelerate therapeutics discovery and development against several pathogens of high relevance to the DTRA mission. Additionally, many pathogens that pose challenges to the warfighter in the form of aerosols do not have animal models that faithfully recapitulate clinically relevant features of human disease. Alphaviruses, including Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV), and Western Equine Encephalitis Virus (WEEV) are highly infectious as aerosols, posing a major challenge to the warfighter. There is a concerted effort by several investigators to address this challenge by developing both pathogen- and host-directed countermeasures to treat exposed individuals. An important challenge in the countermeasures development process is that traditional small animal models (murine models) do not recapitulate human CNS disease manifestations, while larger animal models (nonhuman primates) pose other challenges including robust sample numbers.

We have expanded our early preclinical countermeasures efficacy testing process to include conventional 2-dimensional in vitro models involving CNS-derived cells, a 3-dimensional organ on a chip (OOC) model representative of the human blood brain barrier (BBB) prior to evaluating candidate efficacy in the murine model. We have thus far assessed both pathogen-targeted and host-targeted small molecule candidates in these simple and complex in vitro models and in mouse models. Our studies with novel small molecule inhibitors that target the alphaviruses nsP2 protease were carried out in all of the above-mentioned experimental platforms. The in vitro studies in simple 2D cell culture with astrocytes, pericytes and endothelial cells demonstrated robust inhibition of virulent VEEV titer, thus facilitating our down selection process. When the prioritized candidates were evaluated in the 3-dimensional BBB OOC, we observed that the inhibitors were capable of preserving endothelial barrier integrity and differentially modulating viral and inflammatory loads in the brain and vascular compartments. The metrics around viral and inflammatory load changes in the different compartments of the NVU also provided insight into the potential of the compound to cross the BBB, thus laying the foundation for next generation compound development strategy. In vivo studies have shown these inhibitors to be non-toxic in mice and provide protection from infection in a VEEV TC-83 lethal challenge mouse model. Notably, our comparisons of endothelial gene expression patterns in the NVU and mouse brain samples showed interesting similarities in responses, while also identifying differences between our 2-dimensional, OOC and in vivo models. Our ongoing efforts are also focused on expanding these observations for other host-targeted, FDA-approved small molecules to ascertain how host-targeted inhibitors compare with pathogen-targeted direct acting antivirals in these different experimental systems.