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

Ubiquitin E3 Ligases As Therapeutic Targets Towards Achieving Broad-spectrum Inhibition Of Vector-transmitted, Neurotropic Viruses.

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Vector-transmitted viral pathogens that pose a significant threat to the deployed warfighter include alphaviruses and flaviviruses that display neurotropism. Examples among neurotropic alphaviruses and flaviviruses include Venezuelan equine encephalitis virus (VEEV), Eastern Equine Encephalitis Virus (EEEV), and West Nile virus (WNV). Other alphaviruses such as chikungunya virus (CHIKV) and flaviviruses such as Dengue virus (DENV) are also known to exhibit pathology involving the central nervous system (CNS), although less prominent than bona fide neurotropic viruses. These viruses, by virtue of their abilities to establish a strong productive infection in the CNS and the potential to elicit robust inflammatory responses, also contribute to tissue damage resulting in neurological disease. There are currently no FDA-approved therapeutic intervention strategies that can address the CNS manifestations of these viral infections, including the ensuing tissue damage.

Host-based therapeutic strategies offer the advantage of the potential for broad-spectrum outcomes and a high barrier to the evolution of resistance. Post-translational modification of viral proteins by the engagement of the host enzymatic machinery, especially ubiquitination, is well established as being a central requirement for multiple enveloped viruses. While some viral proteins can mimic host ubiquitin ligases, for the vast majority, the host enzymatic machinery is essential for achieving appropriate modification of viral targets. We hypothesize that host-derived ubiquitin E3 ligases will play essential roles in various aspects of the viral life cycle of neurotropic viruses, involving ubiquitination of nonstructural and structural proteins. We have started to address this hypothesis by optimizing a methodology to achieve the targeted depletion of 35 human ubiquitin E3-ligases by a siRNA approach and assessing the impact of the depletion on productive infection using VEEV as a prototype pathogen.

A total of 35 E3 ubiquitin ligases were employed in our optimized transfection and viral infectivity assessment assays in Vero cells. At 50nm concentration of each siRNA, the impact on viral load was quantified by luciferase assay following infection by nanoluciferase-tagged VEEV TC-83. Concomitantly, cell survival following E3 ligase depletion was also assessed by cell titer glo assay. Selected siRNA candidates were subjected to independent validation in Vero and HEK293T cell lines to ascertain dose dependency and address potential mechanisms of action.

Our initial screen of 35 E3 ligase targets showed little to no cytotoxicity in Vero cells under our optimized transfection conditions. Based on viral inhibition, 6 candidates were identified as exerting an impact on VEEV TC-83. Ongoing studies are focused on additional mechanism of action assessments including impact on specific aspects of viral life cycle, and ubiquitination of viral proteins.

Lead candidates based on the level of viral inhibition against TC-83 will be explored further in other RNA viruses including virulent alphaviruses (VEEV TrD, EEEV FL-93) and flaviviruses to address broad-spectrum inhibitory potential. Given the wide-ranging requirement of ubiquitination of viral proteins for multiple enveloped RNA viruses, this approach has the potential to deliver broadly relevant therapeutic targets that will apply to emerging and reemerging viral pathogens that pose challenges to the civilian population and the warfighter.

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