BROAD-SPECTRUM THERAPEUTICS FOR VIRAL DISEASES: A MEDICAL COUNTERMEASURE PLATFORM FOR EMERGING THREATS

Inhibitors Of The Ubiquitin-proteasome Pathway As Broad-spectrum Countermeasures Against Acutely Infectious Viruses

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Acutely infectious viruses including alphaviruses (Venezuelan equine encephalitis virus [VEEV] and Eastern Equine Encephalitis Virus [EEEV], bunyaviruses (Rift Valley fever virus [RVFV]), and flaviviruses (Yellow Fever Virus [YFV]) are vector transmitted, resulting in increased disease burden in the civilian and military population. Several of these pathogens are highly infectious and transmissible as aerosols, thus posing an increased threat to the warfighter. Studies from our laboratory and from several others have demonstrated the critical role of the host ubiquitination pathway in the establishment of a productive infection for acutely infectious viruses. For example, we have demonstrated that proteasome inhibitor Bortezomib is a broad-spectrum inhibitor of alphaviruses and bunyaviruses. Published studies have demonstrated that many viral proteins including the capsid protein of VEEV, the NS1, 3 and 5 proteins of Dengue Virus, VP35 protein of Ebola virus are ubiquitinated in infected cells. As part of our efforts to characterize the host: viral protein interactome of alphaviruses using VEEV and EEEV as prototypes, we have identified several proteins involved in the ubiquitin proteasome pathway as interacting with all the viral nonstructural proteins. These observations and evidence in the scientific literature prompted us to hypothesize that ubiquitin proteasome inhibitors will constitute an excellent broad-spectrum strategy to address emerging/re-emerging viral challenges. As several viral proteins are known to be ubiquitinated in multiple sites, inhibiting such a broadly critical post translational modification event is highly unlikely to result in resistant viruses.

We examined multiple inhibitors of the ubiquitin proteasome pathway using alphaviruses as prototype pathogens. Of several inhibitors that were queried for antiviral activities against the TC-83 strain of VEEV in three different cell lines (Vero, HMC3, and SVG-p12), NSC697923, Bardoxolone methyl (BARM), Omavelexelone (OMA) demonstrated robust inhibition of TC-83 at 1μM concentration. In vitro dosing studies conducted thus far have quantified viral loads following combined pre/post-treatment of cells, pre-treatment only, and post-treatment only conditions. The results identify combined pre/post-treatment as the most effective strategy in robustly reducing viral load between 2-4 logs and that inhibition is exerted in many events of the viral life cycle. Ongoing studies include, quantification of genomic copy numbers and infectious titers in the context of the virulent strains of VEEV, EEEV and Chikungunya virus, as well as quantification of inflammatory cytokine expression for the selected inhibitors. Additionally, we are evaluating the significance of the inhibitors in the context of a three-dimensional organ-on-chip (OOC) model of the human blood brain barrier (BBB) to address if the inhibition of virus and inflammatory events can preserve barrier integrity following VEEV infection, as well as in case of when the pathogen gains access to the brain side without needing to pass through the BBB (aerosol). Inhibitors that showcase the ability to preserve barrier integrity will be queried in the context of other acutely infectious viral pathogens that are known to cause encephalitic outcomes (RVFV, YFV).

We envision ubiquitin proteasome inhibitors as being broadly effective countermeasure strategies that can be applicable to several emerging/re-emerging viral pathogens that pose challenges to the military and civilian population.

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