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

Antiviral Activity Of Opaganib, A First-in-class Sphingolipid Modulator

Rekha Panchal USAMRIID **Eric Nguyen** USAMRIID **Raina Kumar** USAMRIID **LTC Jeffrey Kugelman** USAMRIID **Janet Skerry** USAMRIID **Xiaoli Chi** USAMRIID **Ashley Mcaleese** USAMRIID **Aura Garrison** USAMRIID **Mark Levitt** RedHill Biopharma Ltd **Gilead Raday** RedHill Biopharma Ltd **Patricia Anderson** RedHill Biopharma Ltd **Reza Fati** RedHill Biopharma Ltd **LTC Robert Haupt** USAMRIID

Sphingolipids are known to regulate the infection, replication and pathogenesis of many viruses, including Ebolavirus (EBOV). Opaganib is a clinical-stage small molecule inhibitor of sphingosine kinase-2 (SPHK2) and dihydroceramide desaturase (DES1) with anticancer, anti-inflammatory and antiviral activity. Opaganib has undergone clinical testing in cancer patients and was evaluated in Global Phase 2/3 studies in patients with severe Covid-19. It is hypothesized that selective inhibition of SPHK2 by Opaganib disrupts the activation of PI3K/AKT pathway which is known to reduce EBOV infection. To further test the antiviral activity of Opaganib, dose-response studies were performed using primary human lung fibroblasts (MRC5) that were pre-treated with the compound and then infected with EBOV, Sudan virus (SUDV) or Marburg virus (MARV). Viral infection was detected by immunofluorescence staining using viral-specific antibodies and quantified using an Opera Phenix confocal imaging system and the Harmony image analysis software. Genedata software was used to determine the antiviral potency (EC50) and cellular toxicity (CC50) which is based on reduced cell number. Opaganib had average EC50 values of 6-10.4 μM and CC50 ≥60 μM against SUDV and EBOV but was not as potent against MARV. Opaganib was then tested in a mouse model of EBOV infection. Balb/c mice were infected via intraperitoneal injection with 100 pfu of mouse-adapted EBOV Zaire and then treated via the oral route with Opaganib at 50, 100 or 150 mg/kg, twice daily for a total of eight days. Virus exposure was 100% lethal to vehicle-control mice, whereas 30% survival was observed at 150 mg/kg Opaganib ($p<0.03$). Next, the in vitro combined effects of Opaganib and Remdesivir, a direct acting RNA dependent RNA polymerase inhibitor. MRC5 cells were pretreated with varying concentrations of Opaganib and/or Remdesivir and then infected with EBOV. After 48 hours, the antiviral activity was quantitated using an image-based immunofluorescence assay. Opaganib and Remdesivir show an average synergy score of around 25 across four synergy models at concentration of Opaganib at 0.074 μM and 0.66 μM of Remdesivir. A synergy score greater than 10 was indicative of synergistic activity. Further exploration of the in vitro and in vivo antiviral activity of Opaganib alone and in combination is ongoing. Overall, the results suggest host-directed therapeutics that target key signaling pathways could improve treatment outcome for infections caused by deadly pathogens such as filoviruses and can prevent or reduce development of microbial resistance. Opaganib is believed to be the first host-directed investigational drug to show in vivo activity against EBOV.

This project was supported by Defense Threat Reduction Agency (DTRA) under project Number CB10642. The opinions, interpretations, conclusions, and recommendations presented are those of the author and are not necessarily endorsed by the U.S. Army or Department of Defense