CHARACTERIZATION OF RVU-27065 A NOVEL SMALL-MOLECULE STING AGONIST SUITABLE FOR SYSTEMIC ADMINISTRATION

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Background STimulator of INterferon Genes (STING) is a key signaling protein involved in activation of the immune system in response to self-DNA. In recent years, STING signaling has been demonstrated to play a major role in activating the anti-tumor immune response and therefore is considered an attractive drug target in immuno-oncology. The first wave of STING agonists, cyclic-dinucleotide analogues of the internal ligand cGAMP, were developed for local, intratumoral administration. Herein we present the most recent profiling results of our frontrunner molecule RVU-27065, a potent and selective systemic STING agonist with a favorable drug profile.

Methods Binding to recombinant STING protein was examined using Fluorescence Thermal Shift and Fluorescence Polarisation. Primary activity screen was performed in THP-1 Dual reporter cells. Selectivity was confirmed in THP-1 reporter cells with knocked out STING or expressing STING variants. T cell viability and proliferation was assessed by flow cytometry using activated human T cells. PBMCs were isolated by density gradient from whole blood of healthy donors. Downstream STING pathway activation in cells treated with RVU-27065 was confirmed using Western blot analysis. BALB/c mice were inoculated with EMT6 tumor cells and the compound was administered intravenously followed by regular monitoring of tumor growth. Cured animals were rechallenged by repeated inoculation of EMT6 cells.

Results RVU-27065 binds and strongly thermostabilizes recombinant STING proteins of all tested species. Binding to the protein results in activation of downstream signalling pathway, confirmed by western blot analysis. The agonist is characterized by selectivity and excellent potency in THP-1 dual reporter cells as well as in human PBMCs and dendritic cells. Short term incubation of RVU-27065 has no impact on T cell viability, activation or proliferation. Furthermore, STING activation with RVU-27065 leads to repolarization of immunosuppressive M2 macrophages into pro-inflammatory M1-like phenotype. In vivo efficacy of RVU-27065 was confirmed, leading to significant tumor growth inhibition and complete tumor regressions in an EMT6 mouse breast cancer syngeneic tumor model.

Conclusions RVU-27065 is a novel representative of a 3rd generation of Ryvu STING agonists – small-molecule, non-macrocyclic molecules built around a unique chemotype. The compound is characterized by high in vitro potency which translates to efficacy in vivo in preclinical animal models. Drug-like properties, excellent selectivity and a good safety profile make RVU-27065 an attractive candidate for further development for standalone as well as targeted delivery, which holds high potential for improved immunotherapy in cancer patients.

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