Bromodomain and extraterminal (BET) protein inhibitor, apabetalone, reduces ACE2 expression and attenuates SARS-CoV-2 infection in vitro

D. Gilham[1], A.L. Smith[2], L. Fu[1], D.Y. Moore[2], A. Muralidharan[3], S.P.M. Reid[3], S.C. Stotz[1], J.O. Johansson[4], M. Sweeney[4], N.C.W. Wong[1], D. El-Gamal[2], E. Kulikowski[1]

[1]Resverlogix Corp., Calgary, Canada; [2]University of Nebraska Medical Center, Eppley Institute for Research in Cancer and Allied Diseases, Omaha, United States of America; [3]University of Nebraska Medical Center, Department of Pathology and Microbiology, Omaha, United States of America; [4]Resverlogix Inc., San Francisco, United States of America

Funding Acknowledgement: Type of funding sources: Public Institution(s). Main funding source(s): COVID-19 Rapid Response Grant from the College of Medicine at the University of Nebraska Medical Center (to DE and SPMR), and by University of Nebraska Medical Center start-up funds (to DE).

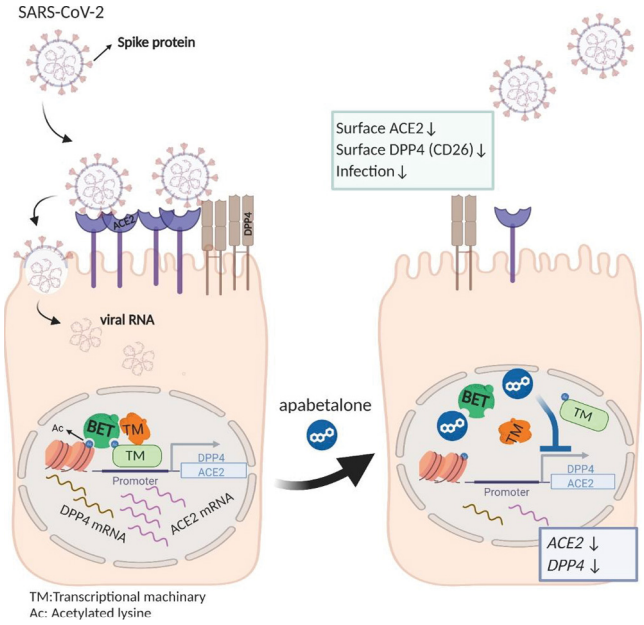
Background/Introduction: SARS-CoV-2 causes life threatening COVID-19 complications including acute coronary syndrome, venous thromboembolism, hyperinflammation and damage in multiple tissues. The SARS-CoV-2 "spike protein" binds cell surface receptors including angiotensin-converting enzyme 2 (ACE2) for entry into host cells to initiate infection. Host cell dipeptidyl peptidase-4 (DPP4 / CD26) is implicated as a cofactor in uptake. Recent evidence indicates expression of factors involved in SARS-CoV-2 uptake into host cells is regulated by BET proteins, epigenetic readers modulating gene expression. Apabetalone, the most clinically advanced BET inhibitor (BETi), is in phase 3 trials for cardiovascular disease (CVD) (a,b). In cultured human cardiomyocytes, apabetalone suppressed infection with SARS-CoV-2 and prevented dysfunction of cardiac organoids induced by the cytokine-storm that arises in patients with severe symptoms (c). However, anti-viral properties of apabetalone in other cell types are not known.

Purpose: To examine effects of apabetalone on SARS-CoV-2 infection in cell culture via downregulated expression of cell surface receptors involved in viral entry. Cell systems used mimic initial sites of infection in the lung as well as cell types contributing to complications in late stages of infection.

Methods: Gene expression was measured by real-time PCR, protein levels by immunoblot or flow cytometry, and binding of recombinant SARS-CoV-2 spike protein by flow cytometry. Infection with SARS-CoV-2 was determined in a BSL3 facility. Infectivity was quantified by determining levels of viral spike protein amongst total cells via imaging on an Operetta CLS.

Results: In Calu-3, a human bronchial epithelial cell line, apabetalone dose-dependently downregulated ACE2 gene expression (up to 98%), reduced ACE2 protein levels (up to 84%) and diminished binding of SARS-CoV-2 spike protein (up to 77%, $p<0.001$ for all parameters). Further, apabetalone abolished infection of Calu-3 cells with live SARS-CoV-2, which was comparable to other antiviral agents. Apabetalone-driven ACE2 downregulation was also observed in extrapulmonary cell types including HepG2, Huh-7 or primary hepatocytes (up to 90%, $p<0.001$ for all cell types), and Vero E6, a monkey kidney epithelial cell line (up to 38%, $p<0.05$). DPP4/CD26, a potential cofactor for SARS-CoV-2 uptake, was also downregulated by apabetalone in Calu-3 cells (mRNA ~65% and protein ~40%, $p<0.001$), which may be synergistic with ACE2 reductions to impede SARS-CoV-2 infection.

Conclusions: Apabetalone, an investigational drug for CVD, reduced cell surface receptors (ACE2 and DPP4) involved in SARS-CoV-2 uptake into host cells and dramatically attenuated SARS-CoV-2 infection/propagation in vitro. Our results suggest apabetalone can mitigate SARS-CoV-2 replication in multiple organs, which together with an established safety profile supports clinical evaluation of apabetalone to treat



Graphical Abstract