

substantial and unmet need for therapies to treat coronavirus infections, and to treat the sequelae resulting from coronavirus infection.

[0005] Rho-associated coiled-coil kinase (ROCK) is a serine/threonine kinase from the AGC (PKA, PKG, and PKC) kinase family and comprises two isoforms, ROCK1 and  
5 ROCK2. The two isoforms are expressed and regulated differently in specific tissues. For example, ROCK1 is ubiquitously expressed at a relatively high level, while ROCK2 is preferentially expressed in certain tissues including heart, brain and skeletal muscle. ROCK is a target of the small GTPase Rho and is involved in diverse cellular activities achieved by phosphorylating downstream effector proteins (MLC, LIMK, ERM, MARCKS, CRMP-2,  
10 etc.). Studies have shown that various diseases (*e.g.*, pulmonary fibrosis, cardiac-cerebral vascular disease, neurological disease and cancer etc.) are related to the pathways mediated by ROCK. As such, ROCK has been considered as an important target in the development of novel drugs.

[0006] The present disclosure relates to the previous unrecognized and surprising potent  
15 anti-viral effects of ROCK2 inhibitors and their use for the treatment of viral infections, including coronavirus infections such as a SARS-CoV-1 infection, a SARS-CoV-2 infection, or a MERS-CoV infection. Additionally, ROCK2 inhibition treats many of the secondary conditions that may be result from the viral infection, including inflammation, fibrosis, and cytokine storm. As a result of treating such secondary conditions, ROCK2 inhibition may  
20 also be useful for treating or preventing long COVID.

## SUMMARY

[0007] In one example, the disclosure provides antiviral compositions comprising a Rho-associated coiled-coil kinase (ROCK2) inhibitor, such as a ROCK2 inhibitor disclosed  
25 herein. In one example, the disclosure provides methods of treating a subject by administering to the subject a therapeutically effective amount of a ROCK2 inhibitor, such as a ROCK2 inhibitor disclosed herein, or a composition comprising the ROCK2 inhibitor. Such methods and compositions described herein may be useful for the treatment of an individual afflicted with or suspected of being afflicted with a viral infection. In one aspect,  
30 the disclosure provides use of a ROCK2 inhibitor in the manufacture of a medicament for treating a viral infection in a subject in need thereof. In one aspect, the disclosure provides use of a ROCK2 inhibitor in the manufacture of a medicament for preventing a viral infection in a subject at risk for the viral infection. In examples, the viral infection is a coronavirus infection. In examples, the viral infection is a SARS-CoV-1 infection, a SARS-CoV-2 infection, or a MERS-CoV infection. In some examples, the viral infection is caused by  
35 SARS-CoV-2. In some examples, the viral infection is caused by a Delta or an Omicron variant of SARS-CoV-2. In one aspect, the disclosure provides use of a ROCK2 inhibitor in the manufacture of a medicament for treating Kawasaki disease, PMIS, or PIMS-TS associated with a viral infection in a subject in need thereof. In examples, the methods and compositions described herein are useful for the treatment and prevention of sequelae  
40 resulting from the

viral infection, including sequelae resulting from coronavirus infection. In one aspect, the disclosure provides use of a ROCK2 inhibitor in the manufacture of a medicament for the treatment or prevention of sequelae resulting from a viral infection in a subject in need thereof. In examples, the methods and compositions described herein are useful for the treatment and prevention of sequelae resulting from a SARS-CoV-1 infection, a SARS-CoV-2 infection, or a MERS-CoV infection. In some examples, the sequelae result from a viral infection is caused by SARS-CoV-2. In some examples, the sequelae result from a viral infection is caused by a Delta or an Omicron variant of SARS-CoV-2. In examples, the sequelae include one or more of the group consisting of fatigue, dyspnea (difficulty breathing), cough, arthralgia (joint pain), myalgia, headache, chest pain, fever, palpitations, myocardial inflammation, ventricular dysfunction, stroke, pulmonary function abnormalities, pulmonary fibrosis, renal dysfunction rash, alopecia, olfactory and/or gustatory dysfunction, sleep dysregulation, cognitive impairment altered, memory impairment, depression, anxiety, changes in mood and combinations thereof. In examples, the sequelae include fibrosis. In some examples, the fibrosis is pulmonary fibrosis. As a result of treating one or more sequelae of resulting from a SARS-CoV-2 infection, the ROCK2 selective inhibitor may be useful for treating or preventing long COVID.

[0008] In one example, the disclosure provides a method of treating a viral infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a ROCK2 inhibitor, such as a ROCK2 selective inhibitor, of the disclosure. In some examples, the ROCK2 inhibitor is a ribonucleic acid (RNA). In some examples, the ROCK2 inhibitor is an antisense RNA against ROCK2 transcription. Optionally, the RNA is a small interfering RNA (siRNA) or a micro RNA (miRNA). In some examples, the ROCK2 inhibitor is a compound having the structure of Formula I to IV, as defined herein, or a pharmaceutically acceptable salt, ester, stereoisomer, polymorph, solvate, N-oxide, or isotopically labeled compound thereof, and particularly (2-(3-(4-((1H-indazol-5-yl)amino)quinazolin-2-yl)phenoxy)-N-isopropylacetamide). In some examples the ROCK2 inhibitor has the structure of Compound 1, as defined herein, or a pharmaceutically acceptable salt, ester, stereoisomer, polymorph, solvate, N-oxide, or isotopically labeled compound thereof. In some examples, the ROCK2 inhibitor is belumosudil. In some examples, the subject is a human subject. In some examples, the subject is a veterinary subject.

[0009] In some examples, the method comprises administering to the subject a therapeutically effective amount of at least one other therapeutic agent. The at least one other therapeutic agent may be another antiviral agent, a corticosteroid, an anti-inflammatory signal

transduction modulator, a  $\beta$ 2-adrenoreceptor agonist bronchodilator, an anticholinergic, a mucolytic agent, hypertonic saline, or a combination thereof.

[0010] In one example, the disclosure provides a method of treating a viral infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a ROCK2 inhibitor, such as a ROCK2 selective inhibitor, of the disclosure, and a therapeutically effective amount of at least one other antiviral agent. In some examples, the ROCK2 inhibitor is a compound having the structure of Formula I to IV, as defined herein, or a pharmaceutically acceptable salt, ester, stereoisomer, polymorph, solvate, N-oxide, or isotopically labeled compound thereof, and particularly (2-(3-(4-((1H-indazol-5-yl)amino)quinazolin-2-yl)phenoxy)-N-isopropylacetamide). In some examples the ROCK2 inhibitor has the structure of Compound 1, as defined herein, or a pharmaceutically acceptable salt, ester, stereoisomer, polymorph, solvate, N-oxide, or isotopically labeled compound thereof. In some examples, the ROCK2 inhibitor is belumosudil. The at least one other antiviral agent may be a nucleoside or nucleotide analog, or a pharmaceutically acceptable salt or prodrug thereof. In some examples, the subject is a human subject. In some examples, the subject is a veterinary subject.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0011] **FIGURE 1** provides the viral titers of SARS-CoV-2 (CPN mL<sup>-1</sup>) in immortalized human airway epithelial cells (Calu-3) after 24 h and 48 h that were infected with the virus and then treated with Compound 2 (10  $\mu$ L of 10  $\mu$ M solution) at 0, 2, 4, and 8 h post-infection in triplicate.

[0012] **FIGURE 2** shows the viral titers of SARS-CoV-2 (CPN mL<sup>-1</sup>) in Calu-3 cells after 24 h that were infected with the virus and then treated with Compound 2 (10  $\mu$ L of 10  $\mu$ M solution) or a control solution (DMSO at PBS) at 0, 2, 4, and 8 h post-infection in triplicate.

[0013] **FIGURE 3** shows the viral titers of SARS-CoV-2 (CPN mL<sup>-1</sup>) in Calu-3 cells after 48 h that were infected with the virus and then treated with Compound 2 (10  $\mu$ L of 10  $\mu$ M solution) or a control solution (DMSO at PBS) at 0, 2, 4, and 8 h post-infection in triplicate.

[0014] **FIGURE 4** Cellular Toxicity: LDH Assay. Calu-3 cells were treated with the listed concentrations of Compound 2 for 2 h at 37 °C followed by challenge with SARS-CoV-2 for 6 h. After washing, the cells were maintained for 48 h and the LDH assay performed.

[0015] **FIGURE 5** Cellular Toxicity: LDH Assay. Calu-3 cells were treated with negative control (DMSO), positive control (Saponin; SAP), or the listed concentrations of Compound