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REVIEW

# Recent Progress in the Development of Opaganib for the Treatment of Covid-19

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**Abstract:** The Covid-19 pandemic driven by the SARS-CoV-2 virus continues to exert extensive humanitarian and economic stress across the world. Although antivirals active against mild disease have been identified recently, new drugs to treat moderate and severe Covid-19 patients are needed. Sphingolipids regulate key pathologic processes, including viral proliferation and pathologic host inflammation. Opaganib (aka ABC294640) is a first-in-class clinical drug targeting sphingolipid metabolism for the treatment of cancer and inflammatory diseases. Recent work demonstrates that opaganib also has antiviral activity against several viruses including SARS-CoV-2. A recently completed multinational Phase 2/3 clinical trial of opaganib in patients hospitalized with Covid-19 demonstrated that opaganib can be safely administered to these patients, and more importantly, resulted in a 62% decrease in mortality in a large subpopulation of patients with moderately severe Covid-19. Furthermore, acceleration of the clearance of the virus was observed in opaganib-treated patients. Understanding the biochemical mechanism for the anti-SARS-CoV-2 activity of opaganib is essential for optimizing Covid-19 treatment protocols. Opaganib inhibits three key enzymes in sphingolipid metabolism: sphingosine kinase-2 (SK2); dihydroceramide desaturase (DES1); and glucosylceramide synthase (GCS). Herein, we describe a tripartite model by which opaganib suppresses infection and replication of SARS-CoV-2 by inhibiting SK2, DES1 and GCS. The potential impact of modulation of sphingolipid signaling on multi-organ dysfunction in Covid-19 patients is also discussed.

**Keywords:** opaganib, ABC294640, sphingolipid, sphingosine kinase, dihydroceramide desaturase, glucosylceramide synthase

## Covid-19 Overview

As of June 2022, Covid-19 has caused the deaths of more than 1,000,000 people in the United States and over 6,300,000 people worldwide (https://covid19.who.int). Beyond these mortality figures, the economic and social hardships caused by the pandemic reach virtually every country and person. Although outstanding work on the development, manufacturing and distribution of vaccines targeting SARS-CoV-2 is reducing the impact of Covid-19 in many countries, the ability of the virus to mutate into potentially unresponsive variants, as well as the lack of global access to vaccines and the certainty of breakthrough infections in many vaccinated individuals make it imperative that effective and easily administered drugs are available for the treatment of Covid-19 patients. Therapeutic antibodies against SARS-CoV-2 proteins have some efficacy in the very early stages of Covid-19, but their high cost and the need for intravenous administration reduce their broad application.[1] Additionally, recent viral variants are less responsive to the existing monoclonal antibody drugs. Similarly, the antiviral drug remdesivir requires intravenous administration and clinical trials have reported either modestly positive (25% reduction in mortality)[2] or negative (WHO Solidarity trial)[3] results in hospitalized Covid-19 patients. More recent clinical trial data show the efficacy of two new oral antivirals (the nucleoside analog Molnupiravir and the protease inhibitor Paxlovid) in SARS-CoV-2-infected individuals if the drugs are given very shortly after infection (3–5 days). However, Molnuporavir lacks efficacy in Covid-19 patients with moderate or severe disease[4] and Paxlovid is untested in this population. Consequently, it is essential that additional drugs be identified for use in hospitalized Covid-19 patients.

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## Development of Opaganib

Because sphingolipids regulate key pathologic processes, including tumor cell proliferation and pathologic inflammation (recently reviewed in[5–8]), we and others sought to identify inhibitors of sphingosine kinases (SK1 and SK2) that may have efficacy as anticancer and anti-inflammatory drugs. Opaganib (Figure 1) is an orally active, isozyme-selective inhibitor of SK2 that is competitive with respect to sphingosine.[9,10] Opaganib depletes sphingosine 1-phosphate (S1P) and elevates ceramides in tumor cells, thereby suppressing signaling through pERK and pAKT and promoting autophagy and/or apoptosis in tumor cells.[9–15] Opaganib also down-regulates the expression of c-Myc in a variety of cancer cell lines.[14,16–19] Because it acts as a sphingosine mimetic, opaganib also inhibits dihydroceramide desaturase (DES1), which accounts for the substantial increases in dihydroceramides in cells treated with the drug.[9,16] Additionally, opaganib reduces levels of hexosylceramides in cells, presumably by inhibition of glucosylceramide synthase (GCS). Therefore, opaganib has the unique ability to simultaneously target three key enzymes in the sphingolipid metabolism pathway (Figure 1).

Opaganib has antitumor activity in a wide range of mouse models,[9,13,14,16,17,20–26] as well as anti-inflammatory activity in several rodent models.[27–31] A Phase 1 clinical trial with opaganib administered to patients with advanced solid tumors was conducted to assess the drug's safety and tolerability when given orally on a twice-daily continuous schedule to fasted patients.[32] This trial demonstrated that opaganib is well tolerated, with 2 patients receiving more than 40 weeks of drug treatment, including a patient with refractory cholangiocarcinoma who experienced a prolonged Partial Response. Overall, 64% of patients who completed 2 cycles of opaganib treatment had Stable Disease or better, suggesting that the drug has activity in many cancer patients. Although the Maximum Tolerated Dose was not formally defined in this study, the recommended phase 2 dose was established as 500 mg twice-daily. A subsequent food–effect study of opaganib given to healthy volunteers indicated that adverse events were milder in fed subjects than in fasted subjects. Therefore, in a following trial in patients with refractory multiple myeloma, opaganib was escalated to 750 mg twice-daily. In this trial, 58% of the evaluable patients achieved Stable Disease or better, and one developed a Very Good Partial Response that was associated with marked decreases in plasma levels of TNFα, EGF and VEGF. Opaganib is currently in Phase 2 clinical testing in patients having cholangiocarcinoma (NCT03377179) or prostate cancer (NCT04207255). To date, more than 400 people have been treated with opaganib in oncology and Covid-19 clinical trials (summarized in Table 1), demonstrating the safety and efficacy of the drug.

## Anti-Viral Activity of Opaganib

Several studies have demonstrated that SKs and S1P enhance the replication of influenza, measles and hepatitis B viruses (reviewed in[33]). We have previously shown that SK2 maintains the latency of Kaposi's sarcoma-associated herpesvirus (KSHV)-infected endothelial cells,[13] and opaganib therefore suppresses KSHV-induced tumor growth in vivo.[13,34,35] Reid et al demonstrated that opaganib inhibits the replication of chikungunya virus (CHIKV), which contains a +single-stranded RNA genome as does SARS-CoV-2.[36] In recent studies by Xia et al, opaganib suppressed the replication of influenza A virus in A549 cells in vitro with an $EC_{50}$<2 μM,[37] which is well below the $C_{max}$ for opaganib in cancer patients.[32] Furthermore, two oral doses of opaganib markedly reduced the viral load in the lungs of mice exposed to influenza A virus, and this substantially improved the survival of infected mice.[37] Combined activity against the influenza virus and SARS-CoV-2 could be very important for individuals with Covid-19 during the flu season. To evaluate the in vitro effects of opaganib on SARS-CoV-2 replication, opaganib was studied in a 3D tissue model of human bronchial epithelial cells (EpiAirway™) which

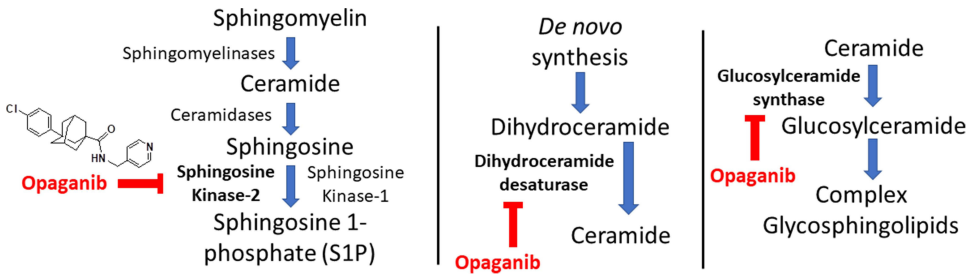


**Figure 1** Multitargeting of sphingolipid metabolism by opaganib. Opaganib (aka ABC294640; Yeliva®; 3-(4-chlorophenyl)-N-(pyridin-4-ylmethyl)-1-adamantanecarboxamide, hydrochloride salt) inhibits SK2 decreasing S1P synthesis, DES1 elevating dihydroceramides, and GCS reducing hexosylceramides.

**Table 1** Clinical Studies of Opaganib

| Study Code and Title | Country | Subjects Treated with Opaganib | Primary Objective |
|---|---|---|---|
| ABC-101: A Phase I, Open-Label, Dose-Escalation, Safety, Pharmacokinetic and Pharmacodynamic Study of ABC294640 in Patients with Advanced Solid Tumors [NCT01488513][32] | USA | 21 | To assess safety and determine the maximum tolerated dose (MTD) and the dose limiting toxicities (DLT) of opaganib in patients with solid organ tumors. |
| ABC-103: A Phase Ib/II Safety and Efficacy Study of ABC294640 in Patients with Refractory or Relapsed Multiple Myeloma Who Have Previously Been Treated with Proteasome Inhibitors and Immunomodulatory Drugs [NCT02757326] | USA | 13 | Assess overall treatment response rate and overall survival in patients with relapsed or refractory multiple myeloma treated with single-agent opaganib. |
| ABC-107: A Phase II Study of the Addition of Opaganib to Androgen Antagonists in Patients with Prostate Cancer Progression on Enzalutamide or Abiraterone [NCT04207255] | USA | 61 | To measure the proportion of patients with disease control during opaganib (plus abiraterone or enzalutamide) therapy, using a composite metric based on PSA, bone scan, and RECIST measurements per Prostate Cancer Working Group 3 (PCWG3) criteria. For purposes of this study, disease control is defined as stable disease or better after four cycles (16 weeks) of treatment. |
| ABC-108: A Phase I/IIA Study of ABC294640 Alone and in Combination with Hydroxychloroquine Sulfate in the Treatment of Patients with Advanced, Unresectable Intra-hepatic, Perihilar and Extra-Hepatic Cholangiocarcinoma [NCT03377179] | USA | 65 | Part 1: determine the Response Rate (RR) of cholangiocarcinoma defined as objective responses, ie complete and partial responses plus stable disease (SD) of at least 4 months to treatment with opaganib.<br>Part 2: determine Durable Disease Control Rate (DCCR) of cholangiocarcinoma, defined as a DCR of at least a period of ≥4 4 months of treatment with opaganib and HCQ. |
| ABC-109: A Phase 1, Single-Dose, Open-Label, Randomized, Three-Period Crossover Study to Evaluate the Effect of Food and Nasogastric Administration on the Pharmacokinetics of ABC294640 in Healthy Subjects [RedHill Biopharma unpublished data] | USA | 23 healthy subjects | Assessment of the effect of a standardized meal on the absorption and bioavailability of opaganib. |
| ABC-110: Opaganib, a Sphingosine Kinase-2 (SK2) Inhibitor in COVID-19 Pneumonia: a Randomized, Double-blind, Placebo-Controlled Phase 2a Study, in Adult Subjects Hospitalized With SARS-CoV-2 Positive Pneumonia [NCT04414618][39] | USA | 22 | Phase 2a Proof of concept study. To assess the safety and tolerability of opaganib administered orally at 500 mg Q 12 hours, for up to 14 days, in patients with COVID-19 pneumonia and to assess to evaluate the total oxygen requirement (area under the curve) using daily supplemental oxygen flow (L/min) over 14 days (Day 1 to Day 14) as primary efficacy. |
| ABC-201: Opaganib, a Sphingosine Kinase-2 (SK2) Inhibitor in COVID-19 Pneumonia: a Randomized, Double-blind, Placebo-Controlled Phase 2/3 Study, in Adult Subjects Hospitalized With Severe SARS-CoV-2 Positive Pneumonia [NCT04467840][40] | Italy, Poland, Russia, Brazil, Mexico, Israel, Colombia | 230 | A phase 2/3 multi-center randomized, double-blind, parallel arm, placebo- controlled study in Adult Subjects Hospitalized with Severe SARS-CoV-2 Positive Pneumonia to determine the potential of opaganib to improve and/or stabilize the clinical status of the patient. |

morphologically and functionally resembles the human airway.[38] Opaganib treatment of cells infected with SARS-CoV-2 resulted in a dose-dependent inhibition of virus production at pharmacologically relevant concentrations ($IC_{50}$~0.5 μM) 72 hours after infection without compromising cell viability. SARS-CoV-2 replication was completely blocked by <3 μM opaganib. This first study used the alpha (Washington) strain of SARS-CoV-2, and subsequent similar studies demonstrated that opaganib also inhibits the replication of the beta, gamma, delta and omicron SARS-CoV-2 variants. Because opaganib targets host proteins instead of viral proteins, mutation of the SARS-CoV-2 virus is not expected to generate opaganib-resistant variants as commonly occurs with virus-directed antivirals. Furthermore, opaganib is relatively easy to synthesize and manufacture into capsules and has exceptional chemical stability, making it a very suitable drug for global use. These preclinical studies demonstrating the anti-inflammatory and antiviral efficacies of opaganib in multiple models (summarized in Table 2) support the potential for opaganib to help mitigate the current Covid-19 pandemic, as well as potentially successfully treat other viral diseases.

## Safety and Efficacy of Opaganib in Covid-19 Patients

Testing for opaganib efficacy in Covid-19 patients was originally rationalized based on its ability to suppress pathologic inflammation in multiple preclinical models, its preclinical antiviral activity, and its safety in oncology clinical trials. However, the direct demonstration of the ability of opaganib to inhibit SARS-CoV-2 replication in vitro[38] provided compelling support for testing opaganib for the treatment and/or prevention of Covid-19. A Phase 2a proof-of-concept study of opaganib administered to hospitalized patients with severe Covid-19 pneumonia requiring supplemental oxygen was conducted in the USA in 2020. In addition to the Standard of Care treatment, patients received opaganib (n = 23) or placebo (n = 19) for up to 14 days and were followed for 28 days after their last dose. There were no material differences in adverse events between the opaganib and placebo treatment groups, indicating that opaganib can be safely administered to these patients.[39] Although the small study size precluded definitive demonstration of safety and/or efficacy, patients who received oral opaganib required less supplemental oxygen and achieved earlier hospital discharge than the placebo control patients.[39] This positive outcome supported the conduct of a Phase 2/3 multinational randomized, double-blind, parallel arm, placebo-controlled study to evaluate the ability of opaganib to improve the clinical status of hospitalized patients with severe Covid-19.[40] The study enrolled 475 adult patients who were randomized to either 500 mg oral opaganib every 12 hours or matching placebo, in addition to Standard of Care for 14 days. Patients were followed for 42 days from their first dose of opaganib. As with the Phase 2a study, adverse events were similar in both treatment groups, further demonstrating the safety of opaganib for Covid-19 patients. More specifically, in the setting of a 14-day course of treatment, opaganib had a favorable safety profile with mostly low-grade nausea, insomnia and anxiety being clearly treatment related adverse events, all occurring in less than 10% of patients. The pre-specified analyses for the primary clinical outcome (the proportion of patients breathing room air without oxygen support by Day 14) did not demonstrate a statistically significant treatment benefit in the entire patient population. However, the subpopulation of patients (54%) requiring at or below the median oxygen supplementation (median fraction inspired oxygen ($FiO_2$) at baseline was 60%) demonstrated a clear clinical benefit with opaganib treatment.[40] Most importantly, opaganib reduced the incidences of intubation/mechanical ventilation and death by Day 42 by 62% each. Additionally, a more rapid clearance of the virus was observed in patients treated with opaganib (median = 10 days) compared to control patients receiving standard of care (median >14 days) for the entire treated population.[41] Overall, the data suggest therapeutic benefit from the treatment of moderately severe Covid-19 patients with opaganib. Although untested to date, it is likely that the clinical benefit of opaganib would be even greater in less-severe Covid-19 patients including non-hospitalized individuals at risk for hospitalization or long-term sequelae.

## Mechanism for the Antiviral Efficacy of Opaganib

Increasing attention is being paid to the involvement of sphingolipids in viral infection and replication in general (reviewed in[33,42,43]) and SARS viruses in particular.[44–48] Entry of SARS viruses into target cells is primarily mediated by binding to angiotensin-converting enzyme 2 (ACE2), followed by proteolytic cleavage by TMPRSS2 and internalization via endocytosis mediated by lipid rafts, which are cholesterol- and sphingolipid-rich membrane domains.[49] McGowan et al[44] provided an excellent review of the roles of sphingolipid metabolism in viral replication, activation of the host

**Table 2** Nonclinical Studies of Opaganib Relating to Covid-19

| Pathology | Model | Key Findings | Reference |
|---|---|---|---|
| **Inflammation (in vivo studies only)** | | | |
| Ulcerative colitis | Dextran sulfate sodium | Opaganib reduced colon damage and markers of inflammation including neutrophil infiltration and cytokine induction | [29] |
| Crohn's Disease | Trinitrobenzene sulfonic acid | Opaganib reduced colon damage and markers of inflammation including neutrophil infiltration and cytokine induction | [30] |
| Colitis-driven colon cancer | Azoxymethane + dextran sulfate sodium | Opaganib decreased the incidence and multiplicity of colon tumors | [24] |
| Vascular permeability | Intradermal VEGF or Streptozotocin | Opaganib reduced vascular leakage in skin and retinas | [31] |
| Rheumatoid arthritis | Subcutaneous collagen or Intradermal adjuvant | Opaganib reduced disease severity and degradation of bone and cartilage | [28] |
| Osteoarthritis | Monosodium iodoacetate | Opaganib attenuated knee joint histological damage and pain | [27] |
| Liver transplantation failure | Cold storage of donor liver | Opaganib in organ storage solution improved survival following surgery and decreased damage and markers of inflammation in the transplanted liver | [94] |
| Hepatic ischemia-reperfusion injury | Liver ischemia-reperfusion surgery | Opaganib reduced hepatic necrosis and markers of inflammation including iNOS, TNFα and neutrophil infiltration, resulting in protection against death | [96] |
| Bacterial pneumonia | *Pseudomonas aeruginosa* | Opaganib reduced lung damage and markers of inflammation including cell infiltration and cytokine induction | [118] |
| Lupus nephritis | MRL/lpr transgenic mice | Opaganib attenuated glomerular pathology but not vascular or interstitial pathology | [127] |
| Psoriasis | Imiquimod | Opaganib reduced psoriatic markers including erythema, scaling and epidermal thickness, as well as the sizes of the inguinal lymph nodes and spleen | [128] |
| Renal fibrosis | Unilateral ureteral obstruction | Opaganib reduced renal fibrosis | [62] and RedHill Biopharma unpublished data |
| Acute Kidney Injury | Ischemia-reperfusion or lipopolysaccharide injection | Opaganib reduced kidney damage and inflammation in both models | Apogee Biotechnology manuscript submitted |
| Atherosclerosis | ApoE knockout mice | Opaganib reduced the number of aortic plaques and increased survival duration | Apogee Biotechnology unpublished data |
| Gastrointestinal acute radiation syndrome | Ionizing radiation | Opaganib reduced colon damage and improved survival following exposure to X-rays | Apogee Biotechnology unpublished data |
| Pulmonary fibrosis | Ionizing radiation | Opaganib reduced lung fibrosis and increased survival duration following exposure to X-rays | Apogee Biotechnology unpublished data |

(*Continued*)

**Table 2** (Continued).

| Pathology | Model | Key Findings | Reference |
|---|---|---|---|
| **Viral (in vivo and in vitro studies)** | | | |
| Kaposi Sarcoma Herpes Virus associated lymphoma | Primary effusion lymphoma (PEL) cells/ xenografts | Opaganib induced tumor regression and promoted viral lytic gene expression in PEL cells | [13,34,35] |
| Arthralgic febrile illness | Chikungunya virus (CHIKV) in cells and mice | Opaganib inhibited CHIKV replication in HepG2 cells | [36] |
| Influenza | Influenza A virus (IAV) in cells and mice | Opaganib improved viability of mice following IAV infection and attenuated viral replication in vitro | [37] |
| Covid-19 | SARS-CoV-2 in EpiAirway model | Opaganib inhibited the replication of multiple SARS-CoV-2 variants (alpha (Washington), beta (South African), gamma (Brazilian), delta (Indian) and omicron) | RedHill Biopharma unpublished data |

immune response and maintaining vascular integrity. The authors also discussed how manipulating the SK-S1P pathway may be beneficial to Covid-19 patients and provided a brief overview of the potential for repurposing sphingolipid-directed drugs for the treatment of Covid-19, including opaganib and two other drugs in clinical trials at that time. Since its publication, clinical testing of opaganib has markedly progressed and the results from the recently completed multinational Phase 2/3 clinical trial with hospitalized severe Covid-19 patients have been reported.[40] Because of the positive efficacy of opaganib against Covid-19, definition of the biochemical mechanism(s) for this activity requires further investigation. We herein focus on potential antiviral mechanisms that are directly relevant to the biochemical actions of opaganib (Figure 2).

## Inhibition of Spike Protein-ACE2 Binding

Blocking the ability of the SAR-CoV-2 spike protein to interact with ACE2 is clearly expected to inhibit infection and viral replication, and this is a likely mechanism for several antibody-based therapeutics, including convalescent plasma. Edwards et al recently demonstrated that binding of the SARS-CoV-2 spike protein to ACE2 is potently inhibited by sphingosine.[50] By inhibiting sphingosine phosphorylation to S1P by SK2, opaganib at least transiently elevates sphingosine levels that may be sufficient to disrupt SARS-CoV-2 binding to target cells. Alternatively, because opaganib is competitive with sphingosine for

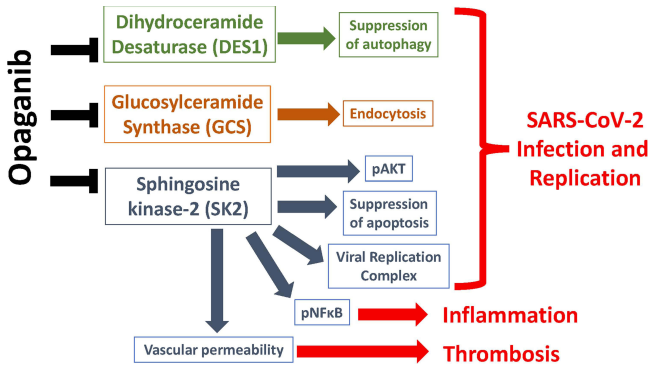


**Figure 2** Model for the therapeutic activity of opaganib against Covid-19. Sphingolipids regulate the ability of SARS-CoV-2 to replicate and thereby cause Covid-19. Firstly, pro-autophagic dihydroceramide levels are normally maintained at low levels by DES1. Inhibition of DES1 by opaganib elevates dihydroceramides and promotes autophagy which suppresses viral replication. Secondly, hexosylceramides are necessary for the endocytosis of virus bound to ACE2. Inhibition of GCS by opaganib reduces hexosylceramides thereby impairing the ability of the virus to enter target cells. Thirdly, SK2 regulates several signaling pathways, as well as the viral replication complex, that are required for viral replication. Inhibition of SK2 by opaganib therefore has multifaceted suppressive effects on viral infection and replication. Furthermore, opaganib suppression of inflammation and thrombosis mediated by SK2 may protect against multi-organ dysfunction in Covid-19 patients.

inhibition of SK2,[9,10] ie acts as a "sphingosine mimetic", it may directly disrupt binding of the spike protein to ACE2 thereby suppressing the internalization and replication of SARS-CoV-2.

## Inhibition of Akt Signaling

A strong body of evidence demonstrates that viruses commonly commandeer host machinery required for viral replication, including activation of the PI3K/AKT/mTOR signaling pathway (reviewed in[51]). For example, Mizutani et al demonstrated that infection of Vero E6 cells by coronavirus activates the AKT signaling pathway,[52] and that pAKT is essential for establishing persistent viral infection of these cells.[53] Recent work confirms Akt activation by SARS-CoV-2,[54] and further shows that inhibition of AKT by GSK690693 blocks viral-induction of cytokine and chemokine expression by lung epithelial cells.[55] Additionally, the pan-AKT inhibitor capivasertib was shown to inhibit the entry of SAR-CoV-2 into Vero cells.[56] Similarly, the AKT inhibitor MK-2206 suppressed viral replication, most likely by inducing autophagy.[54,57] Together, these results led to the proposal that inhibitors of the PI3K/AKT/mTOR signaling pathway may be effective for Covid-19 therapy;[51,58] however, none of the clinical trials assessing such drugs have reported positive data to date.[51] Beyond direct inhibition of viral replication, AKT inhibitors may attenuate excessive inflammation, cytokine storm, fibrosis, and thrombosis in Covid-19 patients.[58] We and others have demonstrated that opaganib efficiently inhibits AKT phosphorylation in multiple cell types.[9,10,12,14,16,17,20,22–25,59–63] The biochemical mechanism for reduction of pAKT by opaganib has not been fully defined, but likely involves the stimulation of protein phosphatase 2A (PP2A)-mediated pAKT dephosphorylation due to reduction of S1P levels (which suppress PP2A activity[64]) and/or inactivation of Inhibitor 2 of PP2A (SET) due to elevation of ceramide levels.[65,66] It is also possible that opaganib directly binds to and inhibits SET as has been suggested for fingolimod (FTY720).[67] Finally, AKT contains an N-terminal pleckstrin homology (PH) domain which enables its membrane translocation and subsequent activation by upstream kinases. Because SK2 also contains a PH domain that directs its localization to membranes,[68] opaganib may alter potential SK2-AKT co-localization necessary to allow viral replication. Through one or more of these mechanisms, inhibition of AKT phosphorylation may underlie the ability of opaganib to suppress infection by SARS-CoV-2.

## Induction of Autophagy

The primary roles of autophagy in normal cells are to recycle intracellular materials and to eliminate intracellular pathogens. Viruses, including coronaviruses, suppress autophagy to promote their own replication, and consequently activation of autophagy can effectively limit viral replication.[69–72] SARS-CoV-2 blocks autophagy, leading to the suggestion that autophagy-inducing agents may overcome infection by reactivating intracellular viral destruction.[73–79] Supporting this concept, SARS-CoV-2 was shown to promote Beclin-1 degradation,[80] and several autophagy-modifying compounds suppress SAR-CoV-2 replication in vitro (reviewed in[75]). It is established that: dihydroceramides induce autophagy;[81–87] that opaganib elevates dihydroceramides by inhibition of DES1;[16,88,89] and that opaganib promotes autophagy.[11,15,34] Therefore, inhibition of DES1 may be involved in the ability of opaganib to suppress infection by SARS-CoV-2 through the induction of protective autophagy in the host cells.

## Induction of Apoptosis

One mechanism that limits viral spread is the induction of apoptosis of infected host cells, and consequently viruses frequently suppress apoptosis to facilitate their replication. Coronavirus infection modulates both the extrinsic and intrinsic apoptosis pathways via the Death Domain (DD) superfamily of proteins and the Bcl-2 family of proteins, respectively.[90] For example, Zhong et al demonstrated that the avian coronavirus infectious bronchitis virus (IBV) induces the expression of Mcl-1 (which inhibits apoptosis) and Bak (which promotes apoptosis), and that genetic ablation of Mcl-1 accelerates IBV-induced apoptosis.[91] Furthermore, viruses, including SARS-CoV-2, activate signaling through NFκB which suppresses apoptosis via both pathways. Sphingosine kinase-2 plays important roles in regulating apoptosis and NFκB pathways, and the effects of opaganib on these processes have been examined. For example, SK2 contains a BH3 domain, and therefore overexpression of SK2 induces apoptosis.[92] Opaganib promotes apoptosis alone and in combination with the DD ligand TRAIL in cancer cells[7,93] and suppresses NFκB activation both in vitro and in vivo.[12,94–96] Mechanistically, opaganib promotes apoptosis by suppressing the expression of Mcl-1[17,97,98] and survivin.[99] Furthermore, we have previously shown that in conjunction with suppressing its kinase activity, opaganib increases SK2 expression, and this overexpression of the BH3 domain could provide a magnified pro-

death stimulus.[10] Therefore, similar to studies with KSHV-infected cells,[13,35] opaganib may suppress Covid-19 through the induction of protective apoptosis in SARS-CoV-2-infected host cells.

## Inhibition of Endocytosis

Entry of SARS-CoV into cells occurs via ACE2-mediated, pH-dependent endocytosis that does not involve clathrin or caveolae, but does require sphingolipid-containing membrane lipid rafts.[49] More specifically, glycosphingolipids are abundant components of the extracellular surface of the plasma membrane that are essential for endocytosis,[100–103] and so play major roles in the penetration of target cells by viruses. For example, exposure of cells to influenza virus elevates levels of sphingomyelin and glucosylceramide,[104,105] and inhibition of GCS suppresses influenza virus infection[106] and maturation.[107] More specifically, GCS activity is required for the entry of influenza virus[108] and thrombocytopenia syndrome virus[109] via endocytosis. This concept was extended by Vitner et al who demonstrated that SARS-CoV-2 infection of Vero E6 cells significantly elevated glycosphingolipid and sphinganine levels, and that similar increases occurred in mice infected with the virus.[110] Importantly, two GCS inhibitors strongly suppressed an early step in the replication of influenza virus and SARS-CoV-2 in Vero E6 cells,[111] demonstrating that GCS is a potential new target for anti-Covid-19 drugs. Therefore, the ability of opaganib to inhibit GCS may be involved in the ability of opaganib to suppress replication of SARS-CoV-2.

## Association with the Viral Replication-Transcription Complex (RTC)

SARS-CoV-2 RNA synthesis is conducted using RTC, which is anchored by the transmembrane viral proteins Nsp3, Nsp4 and Nsp6. The importance of sphingolipids in determining the structure and function of membrane domains such as lipid rafts is well established, and consequently, alteration of sphingolipids by opaganib may disrupt the ability of SARS-CoV-2 to efficiently establish functional RTCs. Furthermore, Reid et al demonstrated that SK2 is a host factor that co-localizes with the RTC of CHIKV by interaction with Nsp3.[36] This appears to be essential for optimal function of the RTC because inhibiting SK2 by opaganib or genetic knockout inhibited CHIKV transcription.[36] Conversely, over-expression of SK2 in response to opaganib may further suppress viral replication by competing with G3BP binding to the RTC.[112] Nsp3 plays a parallel membrane anchoring role for SARS-CoV-2 replication,[113] and therefore, disruption of the viral RTC may be involved in the ability of opaganib to suppress replication by SARS-CoV-2.

# Potential for Opaganib to Attenuate Multi-Organ Dysfunction in Covid-19 Patients

Sphingolipid metabolism is critically involved in the pathogenesis of lung damage, including pulmonary failure in Covid-19 patients (reviewed in[114]). Therefore, beyond the focused antiviral effects discussed above, the ability of opaganib to suppress pathologic inflammation is expected to benefit Covid-19 patients by limiting multi-organ damage due to excessive cytokine production and activity. For example, several studies have examined the role of SK2 in a murine *Pseudomonas aeruginosa* (*PA*)-induced pneumonia lung inflammation model. Genetic deletion of SK2, but not SK1, suppressed NADPH oxidase 4 induction,[115] and decreased levels of inflammatory cytokines, proteins and cell counts in bronchoalveolar lavage, as well as neutrophil infiltration into the alveolar space, in mice exposed to intratracheal *PA*.[116] This was associated with reduced expression of NFκB-regulated inflammation-associated genes in the lung tissue.[117] Most importantly, the administration of opaganib to mice nearly completely ameliorated *PA*-induced lung injury,[118] specifically by decreasing inflammatory cell infiltration on histologic examination, markedly reducing infection-induced increases in TNFα, IL-6, and $H_2O_2$ in bronchoalveolar lavage fluids, and improving survival of infected mice. Additionally, opaganib inhibits IL-6 secretion from human bronchial epithelial cells in vitro (RedHill Biopharma unpublished data). Consequently, opaganib may suppress Acute Respiratory Distress Syndrome and subsequent pulmonary fibrosis in Covid-19 patients.

Sphingolipids also have critical roles in acute kidney injury (AKI) and renal fibrosis (reviewed in[119,120]). For example, Park et al demonstrated that genetic knockout of SK2 decreased AKI following ischemia-reperfusion, whereas knockout of SK1 increased injury.[121] Additionally, Bajwa et al showed that the SK2 inhibitor SLP120701 reduced folic

acid-induced renal fibrosis in mice,[122] and Zhu et al demonstrated that opaganib inhibits extracellular matrix deposition in human kidney fibroblasts.[62] Furthermore, opaganib reduced fibrosis and decreased inflammatory cell infiltration in the kidneys of mice subjected to unilateral ureteral obstruction (RedHill Biopharma unpublished data). Therefore, opaganib may suppress AKI and subsequent renal failure in patients with severe Covid-19.

Finally, the roles of sphingolipids in thrombosis have been studied since 1995, when Yatomi et al demonstrated that S1P promotes platelet aggregation.[123] Interestingly, genetic knockout of SK2, but not SK1, markedly reduced S1P levels in platelets, and strongly attenuated platelet aggregation in vitro and in vivo thrombus formation in ferric chloride-treated mice.[124] This is consistent with preliminary studies that demonstrate that opaganib administration reduces in vivo thrombus formation in the ferric chloride model (RedHill Biopharma unpublished data). Additionally, tissue factor-mediated coagulation following SARS-CoV-2 infection has been linked to stimulated sphingolipid metabolism.[125] Consequently, opaganib treatment may provide an anticoagulant benefit to Covid-19 patients resulting in a lower risk of thrombosis.

## Conclusion

Clinical experience with opaganib demonstrates that it can be safely administered to severely compromised patients with cancer or Covid-19. Data from the completed Phase 2/3 clinical trial indicate efficacy of opaganib in a subset of severe Covid-19 patients. Antiviral and anticancer therapy usually involve multiple drugs targeting different key proteins to achieve clinical benefit. Opaganib appears to be uniquely situated to simultaneously inhibit three sphingolipid-metabolizing enzymes in human cells, ie SK2, DES1 and GCS (Figure 2). While additional studies are needed to elucidate which of these enzymes mediate its antiviral activity, opaganib has the potential to suppress a range of viruses, including SARS-CoV-2. Because of this tripartite targeting, it is unlikely that viral resistance to opaganib will be encountered either through adaptive mutation during therapy or by random mutation to generate additional viral variants.

Currently, the primary treatments available for patients with a score of 5 on the WHO Ordinal Scale for Clinical Recovery are dexamethasone and remdesivir. Dexamethasone is an anti-inflammatory medication that was shown in the RECOVERY study to be most effective for patients who are on mechanical ventilation, with a smaller effect for hospitalized patients on oxygen but not mechanically ventilated.[126] Remdesivir is an anti-viral nucleoside analogue that was initially shown to be most effective in time to recovery in patients with the equivalent of WHO 4 status with little to no effect in patients with the equivalent of WHO 5.[2] In the larger WHO SOLIDARITY trial, there was a small effect on mortality in the combined WHO 4 and 5 groups receiving remdesivir.[3] It is also important to note that remdesivir is only given by intravenous infusion. Opaganib would present an oral alternative with both anti-viral and anti-inflammatory properties for patients in the WHO 5 category. In addition, in a prespecified substrata analysis, when given on top of dexamethasone and remdesivir standard of care, opaganib was nominally superior to placebo. Overall, opaganib may provide an important oral drug for the treatment of patients with severe Covid-19.

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Apogee Biotechnology Corporation holds patents on opaganib and related compounds, which have been licensed to RedHill Biopharm LTD. Both companies have provided funding for laboratory and clinical research discussed in this manuscript.

## Disclosure

Charles D. Smith, Lynn W. Maines and Staci N. Keller are current employees and own stock in Apogee Biotechnology Corporation. In addition, Dr Charles D Smith has patents (7,338,961; 8,063,248; 8,557,800) licensed to RedHill Biopharma LTD. Dr Lynn W Maines reports patents (8324237; 8685936) issued to RedHill Biopharma. Vered Katz Ben-Yair, Reza Fathi, Terry F. Plasse and Mark L. Levitt are currently paid consultants to RedHill Biopharma LTD. The authors report no other conflicts of interest in this work.

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