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*Published in:*
Trends in Pharmacological Sciences

*DOI:*
10.1016/j.tips.2020.07.003

*Publication date:*
2020

Trends in Pharmacological Sciences

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Opinion

# Can Activation of NRF2 Be a Strategy against COVID-19?

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**Acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 is largely the result of a dysregulated host response, followed by damage to alveolar cells and lung fibrosis. Exacerbated proinflammatory cytokines release (cytokine storm) and loss of T lymphocytes (leukopenia) characterize the most aggressive presentation. We propose that a multifaceted anti-inflammatory strategy based on pharmacological activation of nuclear factor erythroid 2 p45-related factor 2 (NRF2) can be deployed against the virus. The strategy provides robust cytoprotection by restoring redox and protein homeostasis, promoting resolution of inflammation, and facilitating repair. NRF2 activators such as sulforaphane and bardoxolone methyl are already in clinical trials. The safety and efficacy information of these modulators in humans, together with their well-documented cytoprotective and anti-inflammatory effects in preclinical models, highlight the potential of this armamentarium for deployment to the battlefield against COVID-19.**

## Highlights

The host inflammatory response is a crucial determinant of disease outcome and correlates with disease severity in SARS-CoV-2-induced infection, for which there is no treatment to date.

Activation of transcription factor nuclear factor erythroid 2 p45-related factor 2 (NRF2) promotes resolution of inflammation and, in parallel, restores cellular redox and protein homeostasis, and facilitates tissue repair.

NRF2 can be activated by pharmacological inducers that target Kelch-like ECH-associated protein 1 (KEAP1), the principal negative regulator of NRF2.

The available information on pharmacokinetics, pharmacodynamics, safety, and efficacy for the NRF2 activators sulforaphane and bardoxolone methyl (currently in advanced clinical trials for other disease indications) in humans makes them excellent candidates for testing in randomized clinical trials in COVID-19.

## Exacerbated Inflammation in Severe COVID-19 Pathology

Numerous clinical observations during the outbreaks of coronaviruses – severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), and most recently SARS-CoV-2 – convincingly show that, in addition to virus propagation, the host inflammatory response is a crucial determinant of disease outcome [1]. A parallel can be drawn with influenza, for which lethality is not associated with the cytolytic action of the pathogen but instead with the inflammatory response orchestrated by the host immune system [2]. In the most severe cases of the disease, a cytokine storm (excess production of cytokines) [3] is associated with T cell depletion, pulmonary inflammation, and lung damage. Patients showing **acute respiratory distress syndrome** (ARDS; see Glossary) and other types of virus-induced pneumonia also present features of **macrophage activation syndrome** (MAS) [3]. There is also evidence of leukopenia, with a ~two-fold decrease in T cell number [4], which may be the result of pyroptosis, a form of cell death that mainly affects cells of the immune system [5]. On the other hand, **granulocytosis** might be partly responsible for the strong burst of superoxide [2], a type of **reactive oxygen species** (ROS) [6], and the additional production of proinflammatory cytokines [7].

To comprehensively manage the symptoms of COVID-19 (the disease caused by SARS-CoV-2), it is crucial to understand the most appropriate context for introducing an anti-inflammatory therapy to complement an antiviral therapy. Such therapy must control inflammation without altering the ability of the host to mount an efficient adaptive immune response against the virus. We propose that boosting endogenous cellular defenses by targeting the cytoprotective transcription factor NRF2 (gene name *NFE2L2*) will promote the resolution of COVID-19 associated

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 https://doi.org/10.1016/j.tips.2020.07.003

inflammation and also restore redox homeostasis and facilitate tissue repair. It should be noted that the protein under discussion is distinctly separate from the identically abbreviated nuclear respiratory factor 2 (also known as GA binding protein transcription factor subunit β, gene name *GABPB1*), a completely different transcription factor involved in mitochondrial biogenesis [8].

## Overview of NRF2 and Its Anti-Inflammatory Roles

NRF2 is a cap'n'collar (CNC) transcription factor that heterodimerizes with small musculoaponeurotic fibrosarcoma (sMAF) proteins, K, G, or F [9], or with transcription factors C-JUN and JUND [10], to bind to **antioxidant response elements** (AREs), and regulates the transcription of target genes, including those encoding proteins involved in cellular redox homeostasis, detoxification, macromolecular damage repair, and metabolic balance [11]. Under basal conditions, NRF2 interacts with the E3 ligase substrate adapter Kelch-like ECH-associated protein 1 (KEAP1) that targets the transcription factor for ubiquitination and proteasomal degradation [11–13] (Figure 1, Key Figure). **Electrophiles** and ROS (collectively termed inducers) inactivate KEAP1 by modifying specific sensor cysteine residues [14], resulting in NRF2 accumulation and enhanced target gene transcription.

NRF2 activity is frequently dysregulated in disease states, including diabetes, liver disease, and inflammatory bowel disease [15], and declines with aging [16]. Some of these disease states (e.g., diabetes) and older age are risk factors associated with SARS-CoV-2-induced ARDS [17]. Importantly, activation of NRF2 has been shown to be involved in preserving lung architecture in response to inflammatory cues, and therapeutic effects of NRF2 activation have been reported in animal models of several lung disorders, including respiratory infections and ARDS [18]. Moreover, single-nucleotide polymorphisms (SNPs) located in the promoter region of *NFE2L2* (encoding NRF2) have been implicated in lung disease susceptibility in humans, hence reinforcing NRF2 as therapeutic target for pulmonary diseases [19,20].

NRF2 also plays a role in both the execution and the resolution of inflammation [12] by repressing proinflammatory genes such as *IL6* and *IL1B* [21]. This is particularly prominent in lipopolysaccharide (LPS)-stimulated macrophage cells, where the anti-inflammatory immunometabolite itaconate, that accumulates during metabolic reprogramming of these cells, activates NRF2 [22]. Moreover, NRF2 also induces the transcription of several macrophage-specific genes that participate in tissue repair. These include macrophage receptor with collagenous structure (MARCO), a receptor required for bacterial phagocytosis, cluster of differentiation 36 (CD36), a scavenger receptor for oxidized low-density lipoproteins (LDL) [24], and IL-17D [25], which confer protection against viral infections [26]. Similarly, NRF2 activation restores redox homeostasis by upregulating **glutathione** (GSH), NADPH, thioredoxin, thioredoxin reductase, and peroxiredoxin that protect against **oxidative stress** and favor alternative wound healing versus classical proinflammatory activation of macrophages and other immune cells [27].

### NRF2 in Viral Infections

The role of NRF2 in viral infections has been investigated in the context of both DNA and RNA viruses. In general, viruses can benefit from either activating or inhibiting NRF2 in host cells [28]. This might be dependent on factors such as the stage of infection [29] or the specific mechanisms of viral propagation – that favor either death of the infected cells and lytic release of virions, or survival of the infected cells with reduction of the inflammatory response to help viral propagation [30]. For human coronavirus HCoV-229E, which is associated with the common cold and pulmonary disease [31], deficiency in expression of the NRF2 target gene glucose-6-phosphate dehydrogenase (*G6PDH*) increases ROS production and enhances viral gene expression and particle production [32]. Crucially, the NRF2 pathway has been found to be suppressed in lung

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biopsies from COVID-19 patients; conversely, pharmacological inducers of NRF2 inhibit the replication of SARS-CoV2 and the inflammatory response [33].

Interestingly, there is reciprocal crosstalk between NRF2 and NF-κB in inflamed tissues, where innate immune cells are recruited [34–36]. Following infection with SARS-CoV, NF-κB is activated in lungs of mice and in human monocyte macrophages *in vitro*; conversely, inhibition of NF-κB decreases inflammation and improves survival after SARS-CoV infection in mice [7,37]. Thus, pharmacological activation of NRF2 might also limit NF-κB-mediated inflammatory processes inflicted in the lung by SARS-CoV-2 infection.

## SARS-CoV-2 Biology and Potential Crosstalk with NRF2

The SARS-CoV-2 genome encodes non-structural proteins (nsp) that are required for replication, structural proteins including spike (S), envelope (E), membrane (M), and nucleocapsid (N), and accessory proteins ORF3, 6, 7a, 7b, 8, and 9b that interact with the host cells [38]. The receptor-binding domain (RBD) located in the S protein of SARS-CoV-2 interacts with the **angiotensin-converting enzyme 2** (ACE2) of host cells to allow viral entry (Figure 1) [39]. The use of ACE inhibitors/angiotensin-receptor blockers, which are widely prescribed to patients with cardiovascular pathologies [40], is currently being considered for COVID-19 (clinical trial numbers[i] NCT04311177 and NCT04312009 for the use of losartan) because angiotensin II, the target of ACE inhibitors, has vasoconstrictive, proinflammatory, pro-oxidative, and prothrombotic effects [41]. However, these inhibitors alter the balance ACE/ACE2 and increase ACE2 levels, thus potentially increasing the number of docking sites for viral entry [42]. NRF2 deficiency is known to upregulate ACE2, whereas its activator oltipraz reduces ACE2 levels [43], suggesting that NRF2 activation might reduce the availability of ACE2 for SARS-CoV-2 entry into the cell (Figure 1).

By analogy with other coronaviruses, SARS-CoV-2 is expected to modulate the host translational machinery to favor the generation of its own proteins (Figure 1) [44]. Host countermeasures to this step include inactivation of eukaryotic initiation factor 2 (eIF2) by two of the three cellular eIF2α kinases, protein kinase R (PKR) and PKR-like endoplasmic reticulum kinase (PERK), which are known to be activated in response to SARS-CoV infection [45]. Interestingly, PKR also has the potential to upregulate the autophagy cargo protein p62, which competes with NRF2 for binding to KEAP1 [46] and further promotes the autophagic degradation of KEAP1 [47], thus activating NRF2 transcriptional activity (Figure 1). Moreover, it has been observed in SARS-CoV infection that host-induced blockade of translation of coronavirus proteins, including the S protein, triggers the **unfolded protein response** (UPR), activating PERK [48] that phosphorylates and activates NRF2 [49]. This may thus be one step at which NRF2 can be modulated to reduce the potential of SARS-CoV-2 infection of host cells.

Cells infected with RNA viruses recognize viral molecular patterns, especially nucleic acids, by cytoplasmic and endosomal receptors, such as the RNA sensors retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5) [50], and the DNA sensor cyclic GMP-AMP synthase (cGAS), which signals through the adaptor protein **stimulator of interferon genes** (STING) to mediate an appropriate immune response [51]. These innate detection systems activate interferon regulatory factor 3 (IRF3)-mediated transcription of type I and III interferons (IFNs) [51]. Coronavirus infections (including SARS-CoV) have been shown to antagonize STING-mediated host immune systems [52,53]. Although type I IFNs are crucial for restricting viral replication and spread by activating autocrine and paracrine type I IFN receptor signaling, excessive release of IFNs by infected pulmonary alveolar cells or resident macrophages may exacerbate the pulmonary infiltration of additional monocyte-derived macrophages, further

## Glossary

**Acute respiratory distress syndrome (ARDS):** a type of respiratory failure characterized by rapid onset of widespread inflammation in the lungs which impairs their ability to exchange oxygen and carbon dioxide.
**Angiotensin-converting enzyme 2 (ACE2):** a transmembrane receptor protein that is found in lungs, arteries, heart, kidney, and intestine, and which serves as the main entry point into cells for SARS- CoV and SARS-CoV2.
**Antioxidant response elements (AREs):** specific DNA sequences in the promoter regions of NRF2-dependent genes.
**Bioavailability:** the proportion of a drug or other substance which, when introduced into the body, enters the circulation and is thus able exert biological effects.
**Electrophile:** a chemical species that is attracted to an electron-rich center. Electrophiles are chemically reactive and, by accepting an electron pair, binds to nucleophiles.
**Glutathione (GSH):** the most abundant thiol in animal cells; >90% of total glutathione is in the reduced form (GSH), and the remainder is in the disulfide form (GSSG). An increased GSSG/GSH ratio is indicative of oxidative stress.
**Granulocytosis:** an increase in number of granulocytes (basophils, eosinophils, and neutrophils) in the peripheral blood.
**Macrophage activation syndrome (MAS):** a potentially fatal complication of rheumatic diseases; MAS is characterized by high fever and can be associated with hemorrhage, damage to the liver, kidney, and the central nervous system, and may lead to multiple organ failure.
**NAD(P)H:quinone oxidoreductase 1 (NQO1):** a homodimeric FAD-binding protein that catalyzes the obligatory two-electron reduction of quinones to hydroquinones, thus preventing redox cycling and glutathione depletion. NQO1 is a classical NRF2-regulated gene and is used as a marker of NRF2 transcriptional activity.
**Nonsteroidal anti-inflammatory drugs (NSAIDs):** inhibitors of the activity of cyclooxygenases (COX-1 or COX-2) that catalyze the biosynthesis of prostaglandins (involved in inflammation) and thromboxanes (involved in blood clotting).

potentiating inflammatory damage [54]. NRF2 downregulates IFN production, in part by downregulating STING expression [55,56] (Figure 1). Therefore, NRF2 may attenuate the inflammatory response to viral infection by preventing excessive production of IFNs.

In addition, upregulation of the NRF2-transcriptional target heme oxygenase 1 (HO-1, gene name *HMOX1*) has been linked to an antiviral response against many viruses including HIV, hepatitis C virus (HCV), hepatitis B virus (HBV), enterovirus 71 (E71), influenza virus, respiratory syncytial virus (RSV), Dengue virus (DENV), and Ebola virus (EBOV) [57]. HO-1 can mediate antiviral responses by forming a heterodimeric complex with IRF3 [58]. With this interaction, IRF3 is phosphorylated and translocated into the nucleus where it induces the expression of type I IFNs (Figure 2).

Several other mechanisms have been described for the control of viral infection by HO-1, which can be extrapolated to some extent to SARS-CoV-2. HO-1 catalyzes the degradation of heme into three products, biliverdin, $Fe^{2+}$, and CO, each with putative anti-SARS-CoV-2 activity. Coronaviruses produce viral proteases – 3C-like proteinase (3CL-pro) and papain-like protease (PLpro) – that process the viral polyproteins and are essential for viral replication [59]. Both 3CL-Pro and PLpro share high homology with other viral proteases [60] that are known to be inhibited by biliverdin [61] (Figure 2). Biliverdin is then expected to inhibit both SARS-CoV-2 3CLpro and PLpro. Free $Fe^{2+}$ binds to the highly conserved divalent metal-binding pocket of RdRp of HCV, inhibiting its enzymatic activity (Figure 2) [62,63]. Because this binding pocket is highly conserved in SARS-CoV-2 [64] a similar mechanism may confer NRF2/HO-1-mediated antiviral activity against COVID-19. Furthermore, CO elicits antiviral responses against positive-sense single-stranded RNA (+ssRNA) viruses such as E71 [65] and bovine viral diarrhea virus (BVDV9) [66], and this effect is phenocopied by the CO donor, CO-releasing molecule 2 (CORM-2), through a mechanism that depends on the activation of soluble guanylyl cyclase (sGC), which increases the local levels of cGMP and activates protein kinase G (PKG) (Figure 2). In turn, PKG inhibits NADPH oxidase (NOX) [67], preventing an increase in ROS levels (Figure 2) that otherwise would contribute to inflammation. If these mechanisms are also mirrored in the context of COVID-19, activation of the NRF2/HO-1 pathway holds promise for mitigating SARS-CoV-2 infection.

## Armamentarium of Available NRF2 Activators for Potential Anti-Inflammatory Therapy of COVID-19

An important limitation for the development of effective therapies against SARS-Cov-2 is the poor reproducibility of COVID-19 in animal models, most of which do not share relevant physiology, do not mount an appropriate immune response, or do not present relevant clinical symptoms [68]. Nevertheless, genetic or pharmacological NRF2 activation has consistently demonstrated anti-inflammatory and antiviral effects in other pathologies in animals and in humans. The most physiologically and pharmacologically relevant mechanism of NRF2 regulation is by targeting specific cysteine sensors within KEAP1 [11]. A comprehensive review on the use of NRF2 activators against viral infections has been published recently [69]. Given that changes in redox homeostasis in infected cells and lung inflammation are hallmarks of infections caused by respiratory viruses [70], the information obtained from viruses that affect the airways may be relevant for extrapolation to COVID-19. Indeed, experimental evidence is beginning to emerge, and it was recently demonstrated that the NRF2 activators dimethyl fumarate (DMF) and 4-octyl itaconate (4-OI), a cell-permeable analog of the endogenous anti-inflammatory metabolite itaconate [22], suppress the inflammatory response to SARS-CoV2 in human cells, including peripheral blood mononuclear cells (PBMCs) from COVID-19 patients [33].

**Oxidative stress:** imbalance between oxidants and antioxidants in favor of the oxidants, leading to disruption of cellular redox signaling and damage to proteins, lipids, and DNA.
**Reactive oxygen species (ROS):** unstable oxygen-containing molecules formed by redox reactions or by electronic excitation.
**Stimulator of interferon genes (STING):** an adaptor protein associated with the endoplasmic reticulum, which is essential for transcription of host defense genes, including type I interferons (IFNs) and proinflammatory cytokines, following recognition of aberrant DNA species or cyclic dinucleotides (CDNs) in the cytosol.
**Unfolded protein response (UPR):** a homeostatic signaling network that is activated following endoplasmic reticulum stress and results in functional recovery of the organelle.

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## Key Figure

### Putative Viral Cycle of SARS-CoV2 Highlighting Points of Potential Crosstalk with NRF2 Activation

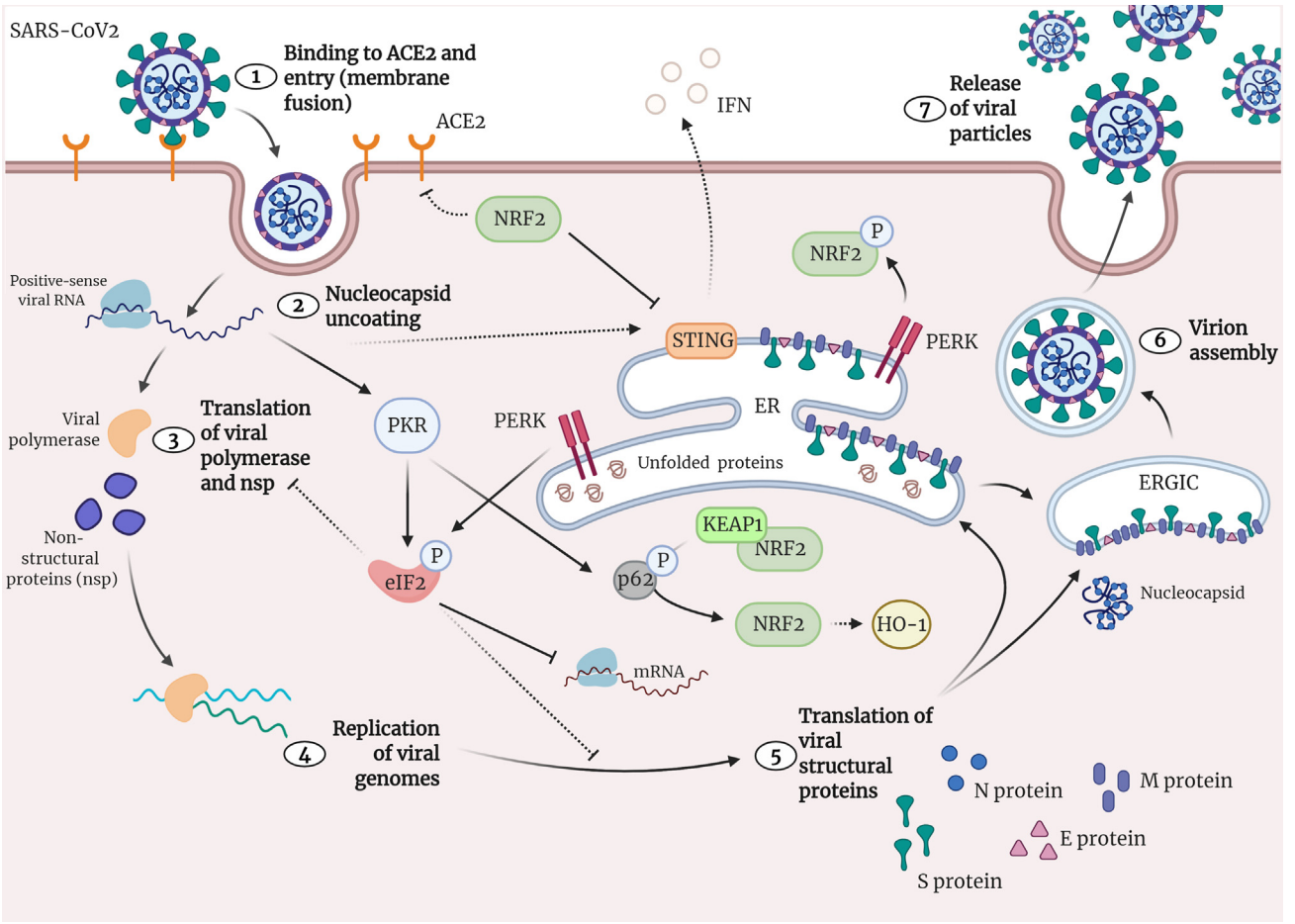



Figure 1. Steps 1–7 depict the different steps of the viral cycle. (1) Binding of the viral spike (S) protein to ACE2 leads to virion entry. NRF2 has been shown to repress *ACE2* gene expression in rats [43]. (2) The viral nucleocapsid is uncoated in the cytoplasm of the host cell. (3) Translation of the viral positive-sense single-stranded RNA (+ssRNA) and cleavage of the translation product into specific viral proteins. Viral RNA inside the host cell activates the DNA/RNA sensor cGAS, which signals through the adaptor STING [117], and induces the expression of type I and III interferons (IFNs). NRF2 represses IFN production by downregulating STING expression [56]. (4) Replication of the viral genome. NRF2 induces the expression of HO-1, generating $Fe^{2+}$ that can bind to the divalent metal-binding pocket of the RNA-dependent RNA polymerase (RdRp) of SARS-CoV2 and inhibit its catalytic activity [63,64]. (5) Translation of structural proteins. Host defense is conducted by double-stranded RNA-activated protein kinase R (PKR), which phosphorylates eIF2 and inhibits protein translation. PKR also phosphorylates p62, thus activating NRF2 upon removal of its repressor KEAP1 by autophagy [118]. Inhibition of protein translation in turn activates the unfolded protein response (UPR). PERK, a crucial Ser/Thr protein kinase in UPR signaling, phosphorylates NRF2, resulting in its stabilization and increased transcriptional activity [49]. (6) Virion assembly. (7) Release of viral particles. Abbreviations: ACE2, angiotensin-converting enzyme 2; eIF2, eukaryotic initiation factor 2; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; HO-1, heme oxygenase 1; IFN, interferon; KEAP1, Kelch-like ECH-associated protein 1; NRF2, nuclear factor erythroid 2 p45-related factor 2; PERK, PKR-like endoplasmic reticulum kinase; P, phosphorylation; PKR, protein kinase R; STING, stimulator of interferon genes. Figure generated with Biorender (https://biorender.com/).

The NRF2 activators targeting KEAP1 that are under clinical development have been recently described [11,71]. We discuss below DMF, the only drug approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) that targets the NRF2/KEAP1 axis [72], as well as two types of NRF2 activators that have been tested in advanced clinical trials

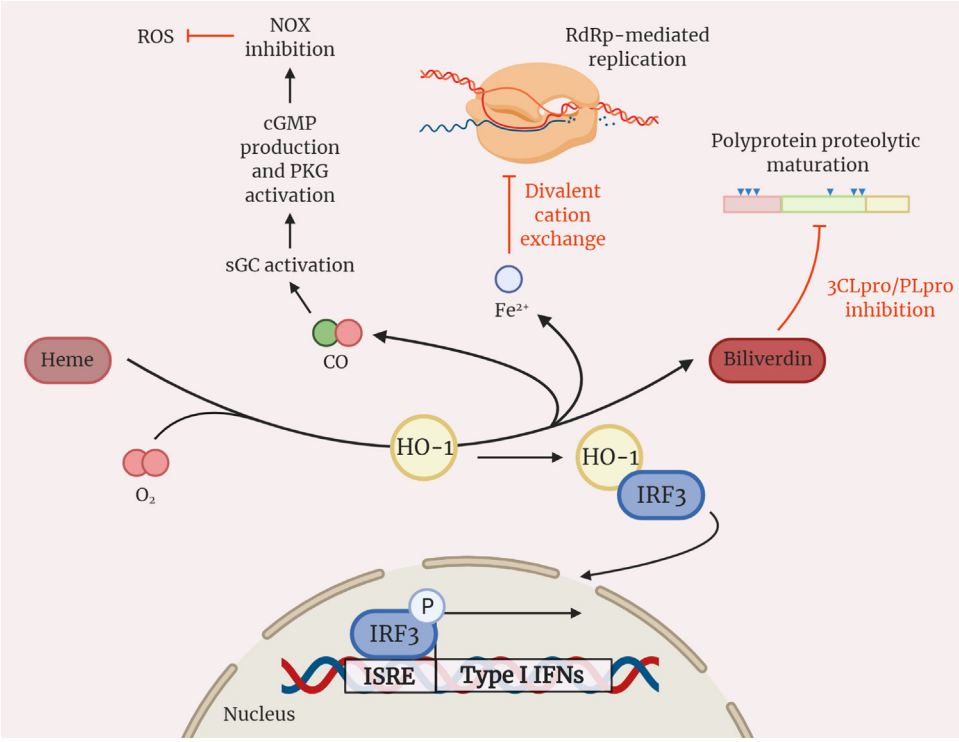



Figure 2. Antiviral Activity of HO-1, a Target of NRF2. HO-1 catalyzes the degradation of heme into carbon monoxide (CO), $Fe^{2+}$, and biliverdin. Free $Fe^{2+}$ is expected to bind to the highly conserved divalent metal-binding pocket of the viral RNA-dependent RNA polymerase (RdRp). Carbon monoxide activates soluble guanylyl cyclase (sGC) to generate cGMP, thus activating protein kinase G (PKG), which inhibits NAPDH oxidases (NOX), preventing an increase in reactive oxygen species (ROS). By inhibiting the SARS-CoV-2 proteases 3CLpro and PLpro, biliverdin is expected to suppress the proteolytic maturation of viral polypeptides. Heterodimerization of HO-1 with IRF3 facilitates the phosphorylation and nuclear translocation of IRF3 and the induction of type I IFN gene expression. Abbreviations, HO-1, heme oxygenase 1; IFN, interferon; ISRE, interferon-sensitive response element; IRF3, interferon regulatory factor 3; NRF2, nuclear factor erythroid 2 p45-related factor 2; P, phosphorylation. Figure generated with Biorender (https://biorender.com/).

(Table 1), and thus can be immediately expedited to clinical trials to examine their therapeutic efficacy in patients with COVID-19.

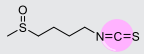
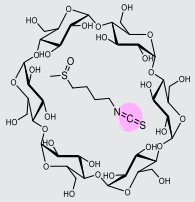
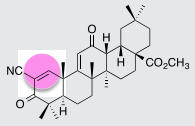
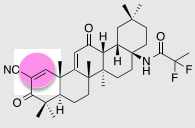
### Dimethyl Fumarate

DMF is used in the treatment of multiple sclerosis (MS) and psoriasis, and may have beneficial effect in lung diseases [73]. Consistent with its role in preventing demyelination, DMF protects against Theiler's murine encephalomyelitis virus (TMEV), a +ssRNA virus that causes a MS-like disease, via enhancing the NRF2 antioxidant and anti-inflammatory responses as well as suppressing IL-17A [74]. However, a well-characterized off-target effect, not related to the NRF2/KEAP1 axis, is leukopenia that occurs in a subset of MS patients [75]. Considering that leukopenia is a hallmark of severe cases of COVID-19, the potential use of DMF in this setting should be considered with caution.

### Sulforaphane

The isothiocyanate sulforaphane (SFN), originally isolated from broccoli, a cruciferous vegetable, as an inducer of the classical NRF2 target, **NAD(P)H:quinone oxidoreductase 1** (NQO1) [76], is the most potent naturally occurring NRF2 activator, with well-documented antioxidant and anti-inflammatory effects [77]. The high **bioavailability** of SFN and its stabilized α-cyclodextrin-

Table 1. Selected Clinical Trials of Relevance to Inflammation and Infection of Sulforaphane and Its Encapsulated Variant Sulfodarex (SFX-01), and Bardoxolone-Methyl and Its Structural Analog Omaveloxolone (RTA-408), Four NRF2 Activators That Target KEAP1

| Compound[a] | Disease | Clinical trial | Clinical trial identifier[i] |
|---|---|---|---|
| Sulforaphane  | Healthy | Phase I | NCT01008826 |
| | | Phase I | NCT02023931 |
| | Chronic obstructive pulmonary disease (COPD) | Phase II | NCT01335971 |
| | Asthma | Phase I | NCT01845493 |
| | | Phase I | NCT01845493 |
| | | Phase I/II | NCT01183923 |
| | Aging | Phase II | NCT03126539 |
| | Allergic rhinitis | Phase II | NCT02885025 |
| | *Helicobacter pylori* infection | Phase IV | NCT03220542 |
| | Type 2 diabetes mellitus | Phase II | NCT02801448 |
| Sulforadex (SFX-01)  | Subarachnoid hemorrhage | Phase II | NCT02614742 |
| | | | |
| Bardoxolone methyl (CDDO-Me)  CDDO-Me R = $CO_2CH_3$ | Pulmonary hypertension | Phase III | NCT03068130 |
| | | Phase III | NCT02657356 |
| | Renal insufficiency<br>Type 2 diabetes mellitus | Phase II | NCT01053936 |
| | Type 2 diabetic nephropathy<br>Chronic kidney disease | Phase II | NCT00811889 |
| | IgA nephropathy<br>Chronic kidney disease associated with type 1 diabetes mellitus<br>Focal segmental glomerulosclerosis<br>Autosomal dominant polycystic kidney disease | Phase II | NCT03366337 |
| | Chronic renal insufficiency<br>Type 2 diabetes mellitus | Phase III | NCT01351675 |
| | Liver disease | Phase I/II | NCT00550849 |
| | Hepatic impairment<br>Healthy | Phase I | NCT01563562 |
| | Alport syndrome | Phase II/III | NCT03019185 |
| Omaveloxolone (RTA-408)  RTA-408 R = $NHCOCF_2CH_3$ | Inflammation and pain following ocular surgery | Phase II | NCT02065375 |
| | Corneal endothelial cell loss<br>Ocular pain<br>Ocular inflammation<br>Cataract surgery | Phase II | NCT02128113 |
| | Radiation dermatitis in breast cancer | Phase II | NCT02142959 |

[a]The pink circles on the chemical structures indicate the electrophilic carbon that undergoes nucleophilic attack by cysteine 151 of KEAP1.

encapsulated version sulforadex (SFX-01) makes it an excellent candidate for alleviating excessive anti-inflammatory responses and protecting the lungs. SFN has been found to be protective in animal models of respiratory disease, including an ARDS model in rabbits [78] and a hyperoxia-induced pulmonary injury model in mice [79]. It also limits RSV replication and virus-induced inflammation in the lungs of wild-type, but not NRF2-null, mice [80]. In HIV-1 transgenic rats, SFN increased GSH levels and the expression of NQO1, and restored the tight junctions between the alveolar epithelial cells [81]. In an *in vitro* model of influenza A infection, SFN reduced both viral cell entry and replication [82]. In addition, SFN suppresses HCV replication [83] and reduces HSV-1 virion production [29]. Interestingly, SFN inhibits nucleotide-binding oligomerization domain (NOD)-, leucine-rich repeat (LRR)-, and pyrin domain-containing protein (NLRP) 1 and 3 inflammasomes (crucial innate immune components that shape host immune homeostasis) as well as pyroptosis, partly in an NRF2-independent manner [84]. Moreover, an interesting study conducted in smokers (a patient cohort with higher risk of lung infections, damage etc.) showed that SFN increased the expression of NQO1 in cells of nasal lavage fluid and, upon infection with live attenuated influenza virus, lowered the levels of IL-6 and viral load [85].

Sources of sulforaphane, including standardized broccoli extracts, dietary supplements, and encapsulated stabilized sulforaphane (Prostaphane and SFX-01) have been in numerous clinical trials for indications that range from lung disease to inflammatory diseases which are closely related to COVID-19 pathophysiology. These include chronic obstructive pulmonary disease (COPD), asthma, allergy, rhinitis, aging, diabetes mellitus, *Helicobacter pylori* infection, and subarachnoid hemorrhage (Table 1). The clinical trials provide extensive pharmacokinetics, pharmacodynamics, safety, and efficacy information [77] that can be extrapolated to COVID-19. Notably, most of these trials have recommended cruciferous-free diets during the study period to minimize baseline noise and accurately detect the plasma and urinary levels of sulforaphane and its metabolites [86].

### Bardoxolone Methyl (CDDO-Me) and Omaveloxolone (RTA-408)

Semisynthetic pentacyclic triterpenoids derived from the natural product oleanolic acid represent the most potent NRF2 activators known to date, with activities in the sub- to low-nanomolar concentration range [87]. An early study highlighted that the anti-inflammatory and NRF2-inducer potencies of 18 triterpenoids correlated linearly over six orders of magnitude of concentration, suggesting that the two processes are mechanistically linked [88]. Some triterpenoids isolated from *Ganoderma lucidum* have been found to be potential inhibitors of the NS2B-NS3 protease of DENV [89]. A study conducted in 2003 after the SARS outbreak [90] found that glycyrrhizin, a triterpenoid from liquorice roots, inhibited the replication of SARS-CoV in two clinical isolates of coronaviruses from patients with SARS. The potential effect of glycyrrhizin on NRF2 was not studied; however, glycyrrhizin has been shown to activate NRF2 in other settings [77,91], and it is plausible that at least part of the observed antiviral effect was due to NRF2 activation.

Bardoxolone methyl (CDDO-Me), a semisynthetic pentacyclic triterpenoid, has been shown to possess a broad-spectrum anti-inflammatory activity against both DENV and Zika virus (ZIKV) [92]. In preclinical models, bardoxolone methyl alleviated LPS-induced acute lung injury through NRF2-dependent suppression of inflammation and oxidative stress [93]. The antiviral properties of bardoxolone methyl have also been shown in cell culture models of HBV, HCV, and HSV1 infections, where it reduced intracellular HBV RNA pre-genome levels, suppressed HCV genome replication [94], and inhibited the production of HSV1 virions [29]. Bardoxolone methyl and a closely related analog, omaveloxolone (RTA-408), are currently in clinical trials for various indications where inflammation and oxidative stress

underlie disease pathogenesis, including pulmonary hypertension, pulmonary arterial hypertension, and ocular inflammation (Table 1) [95].

It is important to note that, even though NRF2 is the primary mediator, additional factors contribute to the anti-inflammatory effects of SFN, bardoxolone methyl, and omaveloxolone (RTA-408). Thus, SFN inhibits NF-κB [96], inhibitor of NF-κB kinase subunit β (IKKβ), and STAT3 [98], whereas bardoxolone methyl inhibits IKKβ [99] and STAT3 and STAT5 activation [100]. Overall, the combined NRF2-activating and anti-inflammatory effects of these compounds culminate in highly robust cytoprotection. These compounds potentially warrant exploration for the management of COVID-19 symptoms.

## NRF2 Activators versus Other Anti-Inflammatory Approaches to COVID-19

The exacerbated inflammation observed in COVID-19 patients could potentially be treated with anti-inflammatory drugs such as corticosteroids and **nonsteroidal anti-inflammatory drugs** (NSAIDs). Indeed, the RECOVERY (randomised evaluation of COVID-19 therapy) trial, a randomized multicenter clinical trial in COVID-19 patients from National Health Service (NHS) hospitals in the UK, found that low-dose dexamethasone, a corticosteroid, reduced mortality in ventilated patients and in patients receiving oxygen only, although it had no effect in patients not receiving respiratory support [101].

Although results with NSAIDs are not conclusive in people with COVID-19 [102], ibuprofen, an NSAID, has been shown to impair neutrophil function, their recruitment to the inflammatory site, and the resolution of inflammatory processes in patients with pneumonia [103]. However, ibuprofen is associated with higher rates of nephrotoxicity [104], cardiovascular disease, and stroke [105], and appears to increase the risk for these outcomes in ARDS [106]. A significant difference between NSAIDs and NRF2 activators is that NRF2 elicits a much more integrated regulation of the inflammatory response because it is necessary for both execution and resolution. Furthermore, by regulating the endogenous cytoprotective systems, NRF2 may have a more physiological role in achieving a balance between the beneficial and adverse effects of inflammation.

Another alternative to conventional anti-inflammatory drugs that holds great promise in COVID-19 is the use of drugs that target cytokines involved in the cytokine storm in COVID-19. This includes targeting IL-6 and IL-1 signaling [107]. Tocilizumab (a humanized monoclonal antibody against the IL-6 receptor) and anakinra (a recombinant human IL-1 receptor antagonist) are being repurposed and studied in COVID-19 [108,109]. Use of NRF2 activators represents an excellent alternative or parallel to these approaches because it is known that NRF2 inhibits IL-6 and IL-1β gene expression [21].

## Concluding Remarks and Future Perspectives

In the past few months, COVID-19, a disease caused by a novel coronavirus SARS-CoV-2, has had a tremendous health and socioeconomic impact at a global scale. We propose here a potential anti-inflammatory therapy based on pharmacological targeting of transcription factor NRF2. We envisage that the benefits of pharmacological activation of NRF2 in the context of SARS-Cov-2 infection will be threefold: (i) increasing fitness and providing protection to the host cell; (ii) promoting the anti-inflammatory phenotype during macrophage activation, thus preventing uncontrolled production of proinflammatory cytokines and pyroptosis; and (iii) inhibiting viral propagation. Notably, unlike direct antioxidants such as vitamin C, that are short-lived (minutes to hours) and are consumed in the process of ROS scavenging, the antioxidant and cytoprotective effects of NRF2 activation are long-lasting and persist for several days after

### Outstanding Questions

How does SARS-CoV-2 downregulate NRF2 in the host cells, and does this depend on the stage of the viral infection?

Does NRF2 contribute to metabolic reprogramming and adaptation of macrophages and T cells, and does it affect their anti-inflammatory functions during the course of COVID-19?

Can pharmacological activation of NRF2 decrease SARS-CoV-2 entry into host cells?

Do all NRF2 activators reduce replication and virion production, or do they affect other stages of propagation of SARS-CoV-2?

If they do, does this effect depend on NRF2 and/or HO-1?

inducer elimination [110,111]. This is because they are mediated by enzymes that, in contrast to small molecules, have long half-lives [11] and are not consumed, and are instead regenerated during the reactions which they catalyze [112].

Most NRF2 inducers, including DMF, sulforaphane, and bardoxolone methyl, are electrophiles that modify cysteine sensors of KEAP1 and inactivate its repressor function. Concerns in using electrophilic NRF2 activators include: (i) possible toxicity at high doses – electrophiles react with GSH and thus, at high doses, may cause GSH depletion; indeed an electrophilic metabolite is responsible for the hepatotoxicity of high doses of acetaminophen [113]. (ii) Possible perturbations of redox signaling owing to persistent NRF2 activity. Both concerns can be resolved by careful selection of dose and dosing regimen in the design of clinical trials, such as including periods of NRF2 activation only during times of active disease with the aim of restoring redox balance and resolving inflammation. Moreover, experimental evidence suggests that the maximally tolerated dose of sulforaphane (and other electrophiles) is not necessarily the most effective [114]. This realization is driving research that aims to identify non-electrophilic NRF2 activators, which inhibit binding to KEAP1, and where promising candidates are emerging [71,115,116].

An area of potential interest for developing COVID-19 candidate drugs in the NRF2 pathway would be to identify inhibitors of the transcriptional repressor BTB domain and CNC homolog 1 (BACH1) that shows increased levels as NRF2 activation declines with age (a risk factor in COVID-19) [18].

Collectively, the research discussed strongly suggests that NRF2 activation holds promise as a strategy against COVID-19. However, before implementing this strategy it is desirable to address several important issues (see Outstanding Questions). For example, the finding that SARS-CoV-2 inhibits NRF2 [33] indicates that the virus deprives the host cells of an essential cytoprotective pathway, and it will be crucial to determine how and when during the process of the viral infection this takes place, and the underlying mechanism. It is presently unclear whether NRF2 can contribute to metabolic reprogramming and adaptation of macrophages and T cells, and whether it affects their effector functions during the course of COVID-19. It will also be important to establish whether pharmacological activation of NRF2 can suppress the entry of SARS-CoV-2 into the host cell, how NRF2 activators reduce the replication of SARS-CoV-2, and whether this effect depends on NRF2, HO-1, or the broader network of proteins regulated by NRF2.

Nonetheless, the wealth of safety and efficacy information for NRF2 activators such as sulforaphane and bardoxolone methyl, that are already in advanced clinical trials for other indications, provides a clear route for their testing in randomized clinical trials in patients with COVID-19. If successful, this therapeutic strategy could be rapidly mobilized to improve recovery and decrease the need for mechanical ventilation in patients with severe COVID-19, relieving the enormous strain that is currently being experienced by intensive care units worldwide.

**Acknowledgments**

We are grateful to the Biotechnology and Biological Sciences Research Council (BB/L01923X/1), Tenovus Scotland (T17/14), Cancer Research UK (C20953/A18644), the Autonomous Community of Madrid (grant B2017/BMD-3827), the 'Tatiana de Guzman el Bueno Foundation' (P-024-FTPGB 2018), and the European Regional Development Fund, Competitiveness Operational Program 2014–2020 (P_37_732/2016; REDBRAIN) for funding our research.

**Disclaimer Statement**

A.T.D-K. is a member of the Scientific Advisory Board of Evgen Pharma and is a consultant for Aclipse Therapeutics and Vividion Therapeutics. A.C. is a consultant for Aclipse Therapeutics.

## Resource

[i]https://www.clinicaltrials.gov/

## References


1. Guan, W.J. *et al.* (2020) Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382, 1861–1862
2. Galani, I.E. and Andreakos, E. (2015) Neutrophils in viral infections: current concepts and caveats. *J. Leukoc. Biol.* 98, 557–564
3. Huang, C. *et al.* (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506
4. Qin, C. *et al.* (2020) Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin. Infect. Dis.* Published online March 12, 2020. https://doi.org/10.1093/cid/ciaa248
5. Fung, S.Y. *et al.* (2020) A tug-of-war between severe acute respiratory syndrome coronavirus 2 and host antiviral defence: lessons from other pathogenic viruses. *Emerg. Microbes Infect.* 9, 558–570
6. Sies, H. and Jones, D.P. (2020) Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 21, 363–383
7. DeDiego, M.L. *et al.* (2014) Inhibition of NF-kappaB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. *J. Virol.* 88, 913–924
8. Jornayvaz, F.R. and Shulman, G.I. (2010) Regulation of mitochondrial biogenesis. *Essays Biochem.* 47, 69–84
9. Otsuki, A. and Yamamoto, M. (2020) *Cis*-element architecture of Nrf2–sMaf heterodimer binding sites and its relation to diseases. *Arch. Pharm. Res.* 43, 275–285
10. Venugopal, R. and Jaiswal, A.K. (1998) Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 17, 3145–3156
11. Cuadrado, A. *et al.* (2019) Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat. Rev. Drug Discov.* 18, 295–317
12. Cuadrado, A. *et al.* (2018) Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach. *Pharmacol. Rev.* 70, 348–383
13. Hayes, J.D. and Dinkova-Kostova, A.T. (2014) The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. *Trends Biochem. Sci.* 39, 199–218
14. Dinkova-Kostova, A.T. *et al.* (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11908–11913
15. Rabbani, P.S. *et al.* (2019) Dysregulation of Nrf2/Keap1 redox pathway in diabetes affects multipotency of stromal cells. *Diabetes* 68, 141–155
16. Schmidlin, C.J. *et al.* (2019) Redox regulation by NRF2 in aging and disease. *Free Radic. Biol. Med.* 134, 702–707
17. Wu, C. *et al.* (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern. Med.* 180, 1–11
18. Liu, Q. *et al.* (2019) Role of Nrf2 and its activators in respiratory diseases. *Oxidative Med. Cell. Longev.* 2019, 7090534
19. Acosta-Herrera, M. *et al.* (2015) Common variants of NFE2L2 gene predisposes to acute respiratory distress syndrome in patients with severe sepsis. *Crit. Care* 19, 256
20. Hua, C.C. *et al.* (2010) Functional haplotypes in the promoter region of transcription factor Nrf2 in chronic obstructive pulmonary disease. *Dis. Markers* 28, 185–193
21. Kobayashi, E.H. *et al.* (2016) Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nat. Commun.* 7, 11624
22. Mills, E.L. *et al.* (2018) Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* 556, 113–117
24. Harvey, C.J. *et al.* (2011) Targeting Nrf2 signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. *Sci. Transl. Med.* 3, 78ra32
25. Seelige, R. *et al.* (2017) The ancient cytokine IL-17D is regulated by Nrf2 and mediates tumor and virus surveillance. *Cytokine* 91, 10–12
26. Saddawi-Konefka, R. *et al.* (2016) Nrf2 induces IL-17D to mediate tumor and virus surveillance. *Cell Rep.* 16, 2348–2358
27. Brune, B. *et al.* (2013) Redox control of inflammation in macrophages. *Antioxid. Redox Signal.* 19, 595–637
28. Deramaudt, T.B. *et al.* (2013) Regulation of oxidative stress by Nrf2 in the pathophysiology of infectious diseases. *Med. Mal. Infect.* 43, 100–107
29. Wyler, E. *et al.* (2019) Single-cell RNA-sequencing of herpes simplex virus 1-infected cells connects NRF2 activation to an antiviral program. *Nat. Commun.* 10, 4878
30. Halder, U.C. *et al.* (2011) Cell death regulation during influenza A virus infection by matrix (M1) protein: a model of viral control over the cellular survival pathway. *Cell Death Dis.* 2, e197
31. Gorse, G.J. *et al.* (2009) Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. *J. Infect. Dis.* 199, 847–857
32. Wu, Y.H. *et al.* (2008) Glucose-6-phosphate dehydrogenase deficiency enhances human coronavirus 229E infection. *J. Infect. Dis.* 197, 812–816
33. Olagnier, D.P. *et al.* (2020) Identification of SARS-CoV2-mediated suppression of NRF2 signaling reveals a potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. *Nat. Commun.* Published online May 27, 2020. http://dx.doi.org/10.21203/rs.3.rs-31855/v1
34. Cuadrado, A. *et al.* (2014) Transcription factors NRF2 and NF-kappaB are coordinated effectors of the Rho family, GTP-binding protein RAC1 during inflammation. *J. Biol. Chem.* 289, 15244–15258
35. Lastres-Becker, I. *et al.* (2014) Fractalkine activates NRF2/NFE2L2 and heme oxygenase 1 to restrain tauopathy-induced microgliosis. *Brain* 137, 78–91
36. Boutten, A. *et al.* (2011) NRF2 targeting: a promising therapeutic strategy in chronic obstructive pulmonary disease. *Trends Mol. Med.* 17, 363–371
37. Dosch, S.F. *et al.* (2009) SARS coronavirus spike protein-induced innate immune response occurs via activation of the NF-kappaB pathway in human monocyte macrophages in vitro. *Virus Res.* 142, 19–27
38. Zhu, N. *et al.* (2020) A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733
39. Chen, Y. *et al.* (2020) Structure analysis of the receptor binding of 2019-nCoV. *Biochem. Biophys. Res. Commun.* 525, 135–140
40. Lopes, R.D. *et al.* (2020) Continuing versus suspending angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: impact on adverse outcomes in hospitalized patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – the BRACE CORONA trial. *Am. Heart J.* 226, 49–59
41. Mehta, P.K. and Griendling, K.K. (2007) Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am. J. Physiol. Cell Physiol.* 292, C82–C97
42. Magrone, T. *et al.* (2020) Focus on receptors for coronaviruses with special reference to angiotensin-converting enzyme 2 as a potential drug target – a perspective. *Endocr. Metab. Immune Disord. Drug Targets.* Published online April 27, 2020. https://doi.org/10.2174/1871530320666200427112902
43. Zhao, S. *et al.* (2018) Nrf2 deficiency upregulates intrarenal angiotensin-converting enzyme-2 and angiotensin 1–7 receptor expression and attenuates hypertension and nephropathy in diabetic mice. *Endocrinology* 159, 836–852
44. Nakagawa, K. *et al.* (2016) Viral and cellular mRNA translation in coronavirus-infected cells. *Adv. Virus Res.* 96, 165–192
45. Krahling, V. *et al.* (2009) Severe acute respiratory syndrome coronavirus triggers apoptosis via protein kinase R but is resistant to its antiviral activity. *J. Virol.* 83, 2298–2309

46. Komatsu, M. *et al.* (2010) The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat. Cell Biol.* 12, 213–223
47. Taguchi, K. *et al.* (2012) Keap1 degradation by autophagy for the maintenance of redox homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* 109, 13561–13566
48. Chan, C.P. *et al.* (2006) Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. *J. Virol.* 80, 9279–9287
49. Cullinan, S.B. *et al.* (2003) Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol. Cell. Biol.* 23, 7198–7209
50. Loo, Y.M. *et al.* (2008) Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J. Virol.* 82, 335–345
51. Burdette, D.L. *et al.* (2011) STING is a direct innate immune sensor of cyclic di-GMP. *Nature* 478, 515–518
52. Sun, L. *et al.* (2012) Coronavirus papain-like proteases negatively regulate antiviral innate immune response through disruption of STING-mediated signaling. *PLoS One* 7, e30802
53. Chen, X. *et al.* (2014) SARS coronavirus papain-like protease inhibits the type I interferon signaling pathway through interaction with the STING–TRAF3–TBK1 complex. *Protein Cell* 5, 369–381
54. Acharya, D. *et al.* (2020) Dysregulation of type I interferon responses in COVID-19. *Nat. Rev. Immunol.* 20, 397–398
55. Olagnier, D. *et al.* (2017) Activation of Nrf2 signaling augments vesicular stomatitis virus oncolysis via autophagy-driven suppression of antiviral immunity. *Mol. Ther.* 25, 1900–1916
56. Olagnier, D. *et al.* (2018) Nrf2 negatively regulates STING indicating a link between antiviral sensing and metabolic reprogramming. *Nat. Commun.* 9, 3506
57. Espinoza, J.A. *et al.* (2017) Modulation of antiviral immunity by heme oxygenase-1. *Am. J. Pathol.* 187, 487–493
58. Koliaraki, V. and Kollias, G. (2011) A new role for myeloid HO-1 in the innate to adaptive crosstalk and immune homeostasis. *Adv. Exp. Med. Biol.* 780, 101–111
59. Bafna, K. *et al.* (2020) Structural similarity of SARS-CoV2 M(pro) and HCV NS3/4A proteases suggests new approaches for identifying existing drugs useful as COVID-19 therapeutics. *ChemRxiv* Published online April 21, 2020. http://dx.doi.org/10.26434/chemrxiv.12153615
60. Baez-Santos, Y.M. *et al.* (2015) The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. *Antivir. Res.* 115, 21–38
61. Zhu, Z. *et al.* (2010) Biliverdin inhibits hepatitis C virus nonstructural 3/4A protease activity: mechanism for the antiviral effects of heme oxygenase? *Hepatology* 52, 1897–1905
62. Fillebeen, C. *et al.* (2005) Iron inactivates the RNA polymerase NS5B and suppresses subgenomic replication of hepatitis C Virus. *J. Biol. Chem.* 280, 9049–9057
63. Fillebeen, C. and Pantopoulos, K. (2010) Iron inhibits replication of infectious hepatitis C virus in permissive Huh7.5.1 cells. *J. Hepatol.* 53, 995–999
64. Gao, Y. *et al.* (2020) Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science* 368, 779–782
65. Tung, W.H. *et al.* (2011) Enterovirus 71 induces integrin beta1/EGFR-Rac1-dependent oxidative stress in SK-N-SH cells: role of HO-1/CO in viral replication. *J. Cell. Physiol.* 226, 3316–3329
66. Ma, Z. *et al.* (2017) Carbon monoxide and biliverdin suppress bovine viral diarrhoea virus replication. *J. Gen. Virol.* 98, 2982–2992
67. Kalyanaraman, H. *et al.* (2018) Protein kinase G activation reverses oxidative stress and restores osteoblast function and bone formation in male mice with type 1 diabetes. *Diabetes* 67, 607–623
68. Cleary, S.J. *et al.* (2020) Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. *Br. J. Pharmacol.* Published online May 27, 2020. https://doi.org/10.1111/bph.15143
69. Lee, C. (2018) Therapeutic modulation of virus-induced oxidative stress via the Nrf2-dependent antioxidative pathway. *Oxidative Med. Cell. Longev.* 2018, 6208067
70. Komaravelli, N. and Casola, A. (2014) Respiratory viral infections and subversion of cellular antioxidant defenses. *J. Pharmacogenomics Pharmacoproteomics* 5, 1000141
71. Robledinos-Anton, N. *et al.* (2019) Activators and inhibitors of NRF2: a review of their potential for clinical development. *Oxidative Med. Cell. Longev.* 2019, 9372182
72. Linker, R.A. *et al.* (2011) Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* 134, 678–692
73. Grzegorzewska, A.P. *et al.* (2017) Dimethyl fumarate ameliorates pulmonary arterial hypertension and lung fibrosis by targeting multiple pathways. *Sci. Rep.* 7, 41605
74. Kobayashi, K. *et al.* (2015) Dimethyl fumarate suppresses Theiler's murine encephalomyelitis virus-induced demyelinating disease by modifying the Nrf2-Keap1 pathway. *Int. Immunol.* 27, 333–344
75. Mehta, D. *et al.* (2019) Effect of dimethyl fumarate on lymphocytes in RRMS: Implications for clinical practice. *Neurology* 92, e1724–e1738
76. Zhang, Y. *et al.* (1992) A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U. S. A.* 89, 2399–2403
77. Yagishita, Y. *et al.* (2019) Broccoli or sulforaphane: is it the source or dose that matters? *Molecules* 24, 3593
78. Sun, Z. *et al.* (2018) Protective mechanism of sulforaphane in Nrf2 and anti-lung injury in ARDS rabbits. *Exp. Ther. Med.* 15, 4911–4915
79. Cho, H.Y. *et al.* (2019) Sulforaphane enriched transcriptome of lung mitochondrial energy metabolism and provided pulmonary injury protection via Nrf2 in mice. *Toxicol. Appl. Pharmacol.* 364, 29–44
80. Cho, H.Y. *et al.* (2009) Antiviral activity of Nrf2 in a murine model of respiratory syncytial virus disease. *Am. J. Respir. Crit. Care Med.* 179, 138–150
81. Fan, X. *et al.* (2013) Activating the Nrf2-mediated antioxidant response element restores barrier function in the alveolar epithelium of HIV-1 transgenic rats. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 305, L267–L277
82. Kesic, M.J. *et al.* (2011) Nrf2 expression modifies influenza A entry and replication in nasal epithelial cells. *Free Radic. Biol. Med.* 51, 444–453
83. Yu, J.S. *et al.* (2016) Sulforaphane suppresses hepatitis C virus replication by up-regulating heme oxygenase-1 expression through PI3K/Nrf2 pathway. *PLoS One* 11, e0152236
84. Greaney, A.J. *et al.* (2016) Sulforaphane inhibits multiple inflammasomes through an Nrf2-independent mechanism. *J. Leukoc. Biol.* 99, 189–199
85. Noah, T.L. *et al.* (2014) Effect of broccoli sprouts on nasal response to live attenuated influenza virus in smokers: a randomized, double-blind study. *PLoS One* 9, e98671
86. Shapiro, T.A. *et al.* (2006) Safety, tolerance, and metabolism of broccoli sprout glucosinolates and isothiocyanates: a clinical phase I study. *Nutr. Cancer* 55, 53–62
87. Sporn, M.B. *et al.* (2011) New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress. *J. Nat. Prod.* 74, 537–545
88. Dinkova-Kostova, A.T. *et al.* (2005) Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc. Natl. Acad. Sci. U. S. A.* 102, 4584–4589
89. Bharadwaj, S. *et al.* (2019) Discovery of *Ganoderma lucidum* triterpenoids as potential inhibitors against Dengue virus NS2B-NS3 protease. *Sci. Rep.* 9, 19059
90. Cinatl, J. *et al.* (2003) Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet* 361, 2045–2046
91. Abo El-Magd, N.F. *et al.* (2018) Glycyrrhizin ameliorates high fat diet-induced obesity in rats by activating NrF2 pathway. *Life Sci.* 193, 159–170
92. Rothan, H.A. *et al.* (2019) Small molecule grp94 inhibitors block dengue and Zika virus replication. *Antivir. Res.* 171, 104590
93. Pei, X. *et al.* (2019) Bardoxolone treatment alleviates lipopolysaccharide (LPS)-induced acute lung injury through suppressing inflammation and oxidative stress regulated by Nrf2 signaling. *Biochem. Biophys. Res. Commun.* 516, 270–277

94. Nio, Y. *et al.* (2019) Bardoxolone methyl as a novel potent antiviral agent against hepatitis B and C viruses in human hepatocyte cell culture systems. *Antivir. Res.* 169, 104537
95. Wang, Y.Y. *et al.* (2014) Bardoxolone methyl (CDDO-Me) as a therapeutic agent: an update on its pharmacokinetic and pharmacodynamic properties. *Drug Des. Devel. Ther.* 8, 2075–2088
96. Heiss, E. *et al.* (2001) Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J. Biol. Chem.* 276, 32008–32015
98. Hahm, E.R. and Singh, S.V. (2010) Sulforaphane inhibits constitutive and interleukin-6-induced activation of signal transducer and activator of transcription 3 in prostate cancer cells. *Cancer Prev. Res. (Phila.)* 3, 484–494
99. Ahmad, R. *et al.* (2006) Triterpenoid CDDO-Me blocks the NF-kappaB pathway by direct inhibition of IKKbeta on Cys-179. *J. Biol. Chem.* 281, 35764–35769
100. Liby, K. *et al.* (2006) The synthetic triterpenoid CDDO-imidazolide suppresses STAT phosphorylation and induces apoptosis in myeloma and lung cancer cells. *Clin. Cancer Res.* 12, 4288–4293
101. Mahase, E. (2020) Covid-19: demand for dexamethasone surges as RECOVERY trial publishes preprint. *BMJ* 369, m2512
102. Russell, B. *et al.* (2020) COVID-19 and treatment with NSAIDs and corticosteroids: should we be limiting their use in the clinical setting? *Ecancermedicalscience* 14, 1023
103. Voiriot, G. *et al.* (2019) Risks related to the use of non-steroidal anti-inflammatory drugs in community-acquired pneumonia in adult and pediatric patients. *J. Clin. Med.* 8, 786
104. Clave, S. *et al.* (2019) The invisible threat of non-steroidal anti-inflammatory drugs for kidneys. *Front. Pediatr.* 7, 520
105. Coxib *et al.* (2013) Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 382, 769–779
106. Wen, Y.C. *et al.* (2018) Risk of stroke associated with use of non-steroidal anti-inflammatory drugs during acute respiratory infection episode. *Pharmacoepidemiol. Drug Saf.* 27, 645–651
107. Costela-Ruiz, V.J. *et al.* (2020) SARS-CoV-2 infection: the role of cytokines in COVID-19 disease. *Cytokine Growth Factor Rev.* Published online June 2, 2020. https://dx.doi.org/10.1016/j.cytogfr.2020.06.001
108. Antwi-Amoabeng, D. *et al.* (2020) Clinical outcomes in COVID-19 patients treated with tocilizumab: an individual patient data systematic review. *J. Med. Virol.* Published online May 21, 2020. https://doi.org/10.1002/jmv.26038
109. González-García, A. *et al.* (2020) Successful treatment of severe COVID-19 with subcutaneous anakinra as a sole treatment. *Rheumatology (Oxford).* Published online June 22, 2020. hppt://dx.doi.org/10.1093/rheumatology/keaa318
110. Schmidt, H.H. *et al.* (2015) Antioxidants in translational medicine. *Antioxid. Redox Signal.* 23, 1130–1143
111. Gao, X. *et al.* (2001) Powerful and prolonged protection of human retinal pigment epithelial cells, keratinocytes, and mouse leukemia cells against oxidative damage: the indirect antioxidant effects of sulforaphane. *Proc. Natl. Acad. Sci. U. S. A.* 98, 15221–15226
112. Dinkova-Kostova, A.T. and Talalay, P. (2008) Direct and indirect antioxidant properties of inducers of cytoprotective proteins. *Mol. Nutr. Food Res.* 52, S128–S138
113. McGill, M.R. and Jaeschke, H. (2013) Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. *Pharm. Res.* 30, 2174–2187
114. Dinkova-Kostova, A.T. *et al.* (2007) Induction of the phase 2 response in mouse and human skin by sulforaphane-containing broccoli sprout extracts. *Cancer Epidemiol. Biomark. Prev.* 16, 847–851
115. Lazzara, P.R. *et al.* (2020) Isoquinoline Kelch-like ECH-associated protein 1-nuclear factor (erythroid-derived 2)-like 2 (KEAP1-NRF2) inhibitors with high metabolic stability. *J. Med. Chem.* 63, 6547–6560
116. Gorgulla, C. *et al.* (2020) An open-source drug discovery platform enables ultra-large virtual screens. *Nature* 580, 663–668
117. Ni, G. *et al.* (2018) cGAS and STING: at the intersection of DNA and RNA virus-sensing networks. *PLoS Pathog.* 14, e1007148
118. Vivarini, A.C. *et al.* (2017) Systems approach reveals nuclear factor erythroid 2-related factor 2/protein kinase R crosstalk in human cutaneous leishmaniasis. *Front. Immunol.* 8, 1127