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Insights into the modulation of the interferon response and NAD^+ in the context of COVID-19

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ABSTRACT
The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in dramatic worldwide mortality. Along with developing vaccines, the medical profession is exploring new strategies to curb this pandemic. A better understanding of the molecular consequences of SARS-CoV-2 cellular infection could lead to more effective and safer treatments. This review discusses the potential underlying impact of SARS-CoV-2 in modulating interferon (IFN) secretion and in causing mitochondrial NAD^+ depletion that could be directly linked to COVID-19's deadly manifestations. What is known or surmised about an imbalanced innate immune response and mitochondrial dysfunction post-SARS-CoV-2 infection, and the potential benefits of well-timed IFN treatments and NAD^+ boosting therapies in the context of the COVID-19 pandemic are discussed.

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first emerged in Wuhan, China in December 2019 and rapidly spread as a pandemic, leading to increased global morbidity and high mortality [1, 2]. The clinical manifestations of COVID-19 range from an asymptomatic course to severe lethal pneumonia or acute respiratory distress syndrome (ARDS) [3]. Although the name suggests that the first and main target of SARS-CoV-2 is the lung, basic investigations and clinical data document multiple organ damage that includes the heart, blood vessels, and brain [2–4]. Clinical reports demonstrate that all age groups are susceptible to infection by SARS-CoV-2, but exhibit different degrees of symptoms, with the elderly or those having comorbidities such as hypertension, cardiovascular diseases, diabetes, and lung diseases being more vulnerable to developing severe symptoms [5, 6].

Although multiple proteins can serve as a gateway to SARS-CoV-2, evidence indicates that angiotensin-converting enzyme 2 (ACE2) is the main attachment protein for entry into human cells [7, 8]. Besides inducing cell death, SARS-CoV-2 can trigger an imbalanced innate inflammatory response characterized by stimulation of pro-inflammatory cytokine release and inhibition of interferon (IFN)-I and IFN-III production, exacerbating thusly disease severity [9]. In addition to suppression of the IFN response, novel "attack" mechanisms that are mediated by enhanced mitochondrial dysfunction and nicotinamide adenine dinucleotide (NAD^+) depletion are being suggested in the context of SARS-CoV-2 infection [10, 11].

Response of the innate immune system to SARS-CoV-2

Accumulating preclinical and clinical investigations highlight the pivotal role of the innate immune system, which serves as the first line of defense against pathogens, in SARS-CoV-2 progression and development. The innate immune response senses viral infection by recognizing their pathogen-associated

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molecular patterns (PAMPs) with pattern recognition receptors (PRRs). These include the retinoic acid-inducible gene I-like receptors (RLRs), Toll-like receptors (TLRs), the nucleotide oligomerization domain-like receptors (NLRs), and cytosolic DNA sensors [12, 13]. The contribution of PRRs in the context of SARS-CoV has been investigated in a genetic study indicating increased susceptibility to viral infection in the absence of RIG-I and melanoma differentiation-associated protein 5 (MDA-5), cytosolic RNA sensors, and their specific signaling pathways [14]. Although SARS-CoV-2 replicates in the cytosol, strong evidence suggests that the replicated viral genome can be recognized by cytosolic RNA sensors, including melanoma differentiation-associated protein 5 (MDA-5) and RIG-1, and endosomal sensors such as TLR3 and TLR7 [14, 15]. A study by Li et al. indicated that both MDA-5 and RIG-I are involved in sensing coronavirus mouse hepatitis virus (MHV) infection, consequently inducing production of IFNs so as to inhibit viral replication [14]. Furthermore, Cervantes-Barragan et al. and Scheuplein et al. reported that TLR7 plays a central role in the recognition of coronaviruses such as murine hepatitis virus and subsequent induction of IFN-α [15, 16]. Similarly, TLR4 deficient mice were shown to be more susceptible to SARS-CoV infection [17, 18]. Recent published evidence in the context of COVID-19 demonstrated that the binding of the SARS-CoV-2 spike protein to TLR4 could be involved in the initiation of the cytokine storm, resulting in the development and progression of COVID-19 [19]. It has been reported in an in *silico* study performed by Choudhury and Mukherjee that the spike protein has the strongest interaction with TLR4 compared to other TLRs [20]. Additionally, the interaction of SARS-CoV-2 with TLR4 has been observed to cause dysregulation in the TLR4 signaling pathway, leading to the activation of the pro-inflammatory MyD88-dependent pathway rather than the TIR domain-containing adaptor-inducing interferon-β/TRIF-related adaptor molecule (TRIF/TRAM)-dependent anti-inflammatory and interferon pathways [19].

Viral recognition by the innate immune system stimulates activation of NF-κB-mediated secretion of pro-inflammatory cytokines and IFN regulatory factor (IRF), such as IRF-3 and IRF-7, mediated production of type I IFNs (IFN-α and IFN-β) and type III IFNs (IFN-λ1, IFN-λ2, IFN-λ3, and IFN-λ4) [13, 21]. With respect to the pro-inflammatory response, it has been observed that following viral infection PRRs, including TLRs and RIG-Like receptors, recognize PAMPs. Upon binding to PAMPS, TLRs recruit myeloid differentiation primary response gene 88 (MyD88) and Toll/IL-1 receptor (TIR), whereas RIG-like receptors exert an effect via the MAVS pathway [22–24]. Consequently, these molecules recruit TNF-receptor associated factor (TRAF), catalyzing the synthesis of K63-linked polyubiquitin chains, subsequently recruiting and activating Ikappa B kinase complex (IKK), phosphorylating and degrading thereafter the inhibitory molecule IκB [25, 26]. The destruction of IκB activates the NF-κB dimer, leading to translocation to the nucleus and release of pro-inflammatory cytokines that constitute an anti-viral innate immune response [27]. The secreted IFNs, on the other hand, activate cell membrane receptors that couple to JAK/STAT signaling and nuclear translocation of IRFs, e.g., IRF-9. The latter activate genes containing interferon-stimulated response elements (ISREs) in their promoters, which causes expression of a set of IFN-stimulated genes (ISGs) that establish an antiviral state [21, 28, 29]. This innate immune response mediated by the release of IFNs in the context of SARS-CoV-2, however, seems to be deficient leading to development of deadly manifestations, essentially fatal respiratory failure. Accumulating evidence demonstrates that SARS-CoV-2 leads to dysregulated immune response and delayed IFN production, contributing therefore to the development and progression of COVID-19 [30, 31]. The underlying pathological mechanism, however, is yet to be fully elucidated. It has been reported that SARS-CoV-2 RNA stimulates RIG-I-MAVS-dependent IFN signaling pathway [32]. The accessory protein ORF9b has been shown to be upregulated within 24 hours following COVID-19 infection, during which the delayed IFN-β secretion was seen, suggesting a major role of ORF9b in the response to SARS-CoV-2 [30, 31]. A study performed by Jing et al., indicated that ORF9b accumulates in primary human pulmonary alveolar epithelial cells and antagonizes the antiviral effect of the type I IFN response following SARS-CoV-2 infection [32].

Production and potential modulation of IFN response by SARS-CoV-2

Substantial evidence documents that interferons exert a central importance in the innate immune response to viral infection [33]. It has been reported that IFN-I and IFN-III are induced following viral recognition by the innate immune system [13, 34]. IFN-I has been shown to be widely expressed and to induce a potent inflammatory antiviral effect, leading to the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-17 [35]. IFN-III, however, is mainly secreted by epithelial cells; it exerts a local antiviral effect, thus eliciting less inflammatory damage [36, 37]. Despite the powerful anti-viral response in the setting of SARS-CoV-2, mainly mediated by activation of the innate immune system, SARS-CoV-2 remains highly pathogenic. In light of this observation, recent evidence demonstrates that SARS-CoV-2 can interfere with the following processes of the innate response: (1) innate sensing, (2) ISG function, and (3) IFN production and signaling pathways, increasing thereby the severity of the disease [13] (Figure 1).

To begin with, coronaviruses such as SARS-CoV have been shown to be replicated in interior double membrane vesicles increasing therefore the virulence of the virus [38]. Chean et al. demonstrated that SARS-CoV nonstructural protein (Nsp) 14 has guanine-N7-methyltransferase activity that can mimic this cap on viral RNA [39]. Additionally, Nsp16 has been observed to further modify the viral cap with its 2′-O-methyl-transferase activity, preventing eventually viral recognition by MD5A [40]. This observation was further substantiated in a study performed by Menachery et al. showing that in a mouse model of Middle East respiratory syndrome coronavirus (MERS-CoV) infection, mutating Nsp16 attenuated viral virulence and disease severity [41]. Although this evasion strategy is likely used by SARS-CoV-2, since Nsp16 of SARS-CoV and SARS-CoV-2 share 92% amino acid sequence homology [42], recent published investigations indicated that SARS-CoV-2 stimulates the RIG-I-MAVS-dependent IFN signaling pathway, but leads to the accumulation of ORF9b, an accessory protein, consequently antagonizing the host-virus response mediated by IFN release [32].

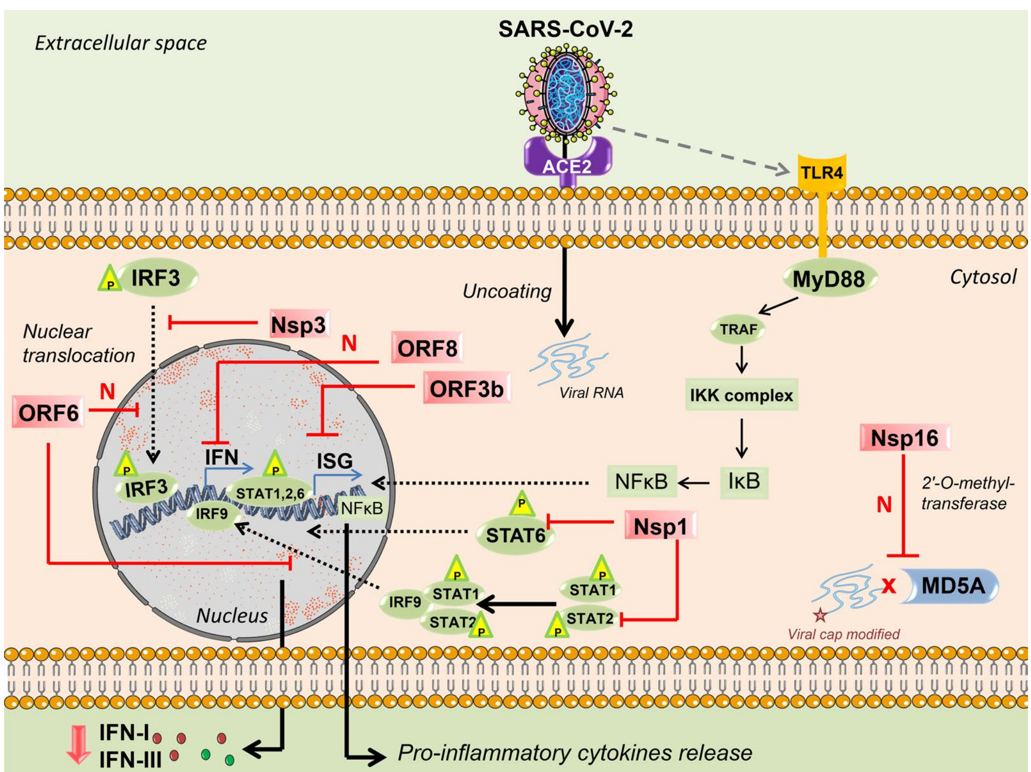

Figure 1. Diagram depicting the impact of SARS-CoV-2 on the innate immune system mediated by INF secretion. Various ways that SARS-CoV-2 evades INF signaling are schematically represented, including: SARS-CoV-2 RNA recognition by the cytoplasmic RNA sensor MDA5 which leads to IFN induction and subsequent IFN signaling in surrounding cells, resulting in the expression of ISGs. ORFs in association with nucleocapsid proteins of SARS-CoV-2 inhibit the expression of IFN and the activation of ISGs. SARS-CoV-2 proteins that have been reported to interfere with these pathways are indicated in red rectangles. Furthermore, SARS-CoV-2 was reported to induce the TLR4 signaling pathway leading to the activation of the pro-inflammatory MyD88-dependent pathway and NF-κB activation triggering the gene expression of pro-inflammatory cytokines. ACE2, Angiotensin Converting Enzyme2; IFN, interferon; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; MDA5, melanoma differentiation-associated gene 5; N, nucleocapsid protein; Nsp, nonstructural protein; ORF, open reading frame; P, phosphate; RIG-I, retinoic acid-inducible gene 1; SARS-CoV, severe acute respiratory syndrome coronavirus; STAT, signal transducer and activator of transcription; TLR4, toll like receptor 4; MyD88, myeloid differentiation primary response gene 88; TRAF, TNF-receptor associated factor; IKK, Ikappa B kinase complex; NF-kB, Nuclear Factor kappa.

With respect to the inhibition of IFN-I and IFN-III production, Siu et al. reported that the membrane protein M of SARS-CoV has the ability to sequester innate sensors in membrane-associated cytoplasmic compartments, evading therefore the innate response [43]. Multiple studies report that MERS M protein and SARS-CoV nucleocapsid (N) protein, Nsp3, and papain-like protease domain can inhibit the phosphorylation and nuclear translocation of IRF-3, thus disrupting IFN-I anti-viral response [44–46]. These findings were further strengthened by a study performed by Blanco-Melo et al. highlighting that low levels of IFN-I and IFN-III are the hallmark of SARS-CoV-2 [47].

Lastly, SARS-CoV Nsp1 has been shown to block phosphorylation of the transcription factor of ISGs, STAT6 [48]. Additionally, SARS-CoV accessory protein ORF3b inhibits transcription of ISGs, resulting in decreased IFN-I and IFN-III levels [45]. A cell culture experiment showed that the open reading frame 6 (ORF6), ORF8, and nucleocapsid (N) proteins of SARS-CoV-2 are able to inhibit expression of IFN-β and activation of ISGs [21]. These proteins exhibited strong inhibition of the type I IFN (IFN-β) promoter and NF-κB element. All three were able to inhibit the ISRE after infection of cells with Sendai virus, while ORF6 and ORF8 could inhibit the ISRE after treatment with IFN-β. SARS-CoV-2 ORF6 was found to block STAT1 and STAT2 nuclear translocation so as to impair transcriptional induction of ISGs [49]. Of note, however, SARS-CoV-2 ORF6 was reported to interfere less efficiently than SARS-CoV ORF6 with human IFN induction and IFN signaling [50]. Xia et al. identified multiple SARS-CoV-2 proteins that antagonize the IFN-I response [51]. Three proteins antagonize IFN-I production: ORF6 binds importin Karyopherin α 2 (KPNA2) to inhibit IRF3 nuclear translocation; Nsp6 suppresses IRF3 phosphorylation by binding TANK binding kinase 1 (TBK1); and Nsp13 binds and blocks TBK1 phosphorylation. Two groups of proteins were found to antagonize IFN-I signaling by blocking STAT1/STAT2 phosphorylation or nuclear translocation. In this case, Nsp1 and Nsp6 of SARS-CoV-2 more efficiently suppressed IFN-I signaling than their counterparts from SARS-CoV or MERS-CoV [51]. Collectively, SARS-CoV-2 proteins interfere with the innate immune system leading to delayed anti-viral response mediated by IFNs secretion and thus facilitate virus replication [2]. These findings would be helpful to unveil the molecular pathways that may be targeted in the setting of SARS-CoV-2.

Consistent with this observation, the serum of SARS-CoV-2 patients showed a similar trend with marked increase in pro-inflammatory cytokine release and evidence for inhibition of IFN-I and IFN-III production [52]. Other investigations suggested a delayed release of IFNs rather than complete absence. For example, in an animal model of SARS-CoV, IFN-I was undetectable in the lung until several hours after the viral load peak [53].

Despite the well-established anti-viral role of IFNs, contradicting findings are reported in the settings of coronavirus infections. Cameron et al. reported a positive correlation between increased plasma IFN-α levels and disease severity [54]. Similarly, in a clinical study of patients diagnosed with SARS-CoV-2, elevated IFN-α levels were accompanied by increased viral load, and thus enhanced disease progression and development [55].

Fine tuning mitochondrial innate immune response in the setting of viral infection

Besides the well-established role of mitochondria in regulating multiple metabolic processes, including redox reactions and regulation of NAD^+ and cellular metabolism [56], it is becoming increasingly recognized that mitochondria are fundamentally implicated in the innate immune response following viral infection including by HIV, hepatitis B, and herpes simplex [57–60] (Figure 2). These viruses cause a robust inflammatory response mediated by the release of mtDNA, resulting in enhanced tissue damage [61–63]. Mitochondrial DNA triggers an innate immune response by inducing cyclic GMP-AMP formation that activates in turn the endoplasmic reticulum (ER)-resident protein, stimulator of interferon genes (STING). STING subsequently induces TANK-binding kinase-1 phosphorylation of IRF-3 and STAT6, which dimerize and induce the transcription of hundreds of ISGs, all of which exert a pivotal role in the inhibition of viral replication [64–67]. In addition to the induction of ISGs, circulating mtDNA has been observed to play a critical role in the stimulation of pro-inflammatory response in COVID-19 patients via its ability to activate cGAS and TLR-9 [57]. The release of mtDNA from the cell has been shown to occur during formation of the neutrophil extracellular trap, a pathogenic step implicated in lung tissue injury and blood clotting formation in COVID-19 patients

Additionally, it has been shown that mice deficient in mitochondrial antiviral signaling protein (MAVS)

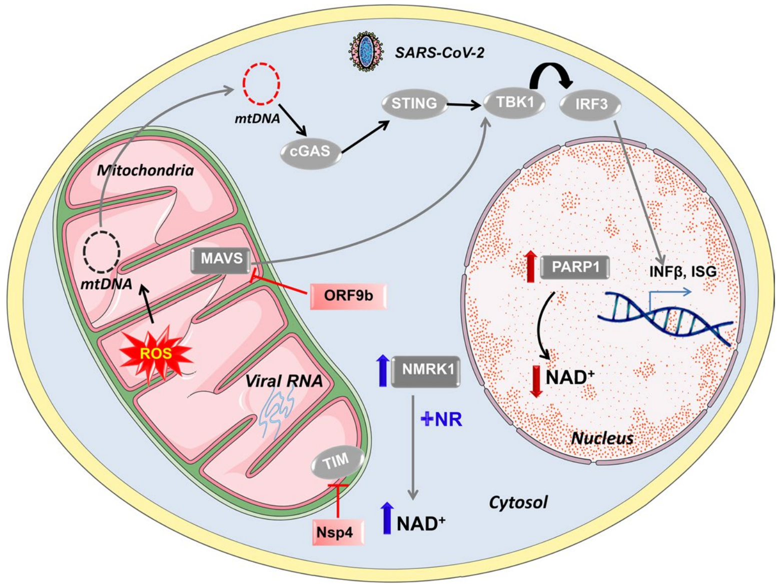

Figure 2: Putative mechanisms of mitochondria-related immune functions controlled by SARS-CoV-2 and their effect on NAD^+ levels. SARS-CoV-2 encodes a virulence factor known as ORF-9b, which localizes to mitochondria and triggers MAVS degradation. SARS-CoV-2 localizes to mitochondria and increases ROS production. As a result, after SARS-CoV-2 infection, PARP1 is activated to repair DNA fragmentation. PARP-1 activation leads to decreased NAD^+ levels. On the other hand, the expression of NMRK-1, an NAD biosynthetic enzyme, is upregulated. Red rectangles represent viral proteins. Please see the text for additional details. cGAS, cyclic GMP-AMP Synthase; IFN, interferon; ISG, IFN-stimulated gene; MAVS, mitochondrial antiviral signaling protein; Nsp, nonstructural protein; ORF, open reading frame; P, phosphate; STING, stimulator of interferon genes; SARS-CoV, severe acute respiratory syndrome coronavirus; TANK, TRAF family member associated NF-κB activator; TBK1, TANK-binding kinase 1; ROS, reactive Oxygen Species; NMRK1, nicotinamide riboside kinase 1; NAD^+, nicotinamide adenine dinucleotide; NR, nicotinamide riboside; mt DNA, mitochondrial DNA.

after being infected by vesicular stomatitis virus (VSV), a negative-strand RNA virus, exhibit a diminished ability to produce IFN-I, resulting in higher susceptibility to viral infection [68]. A study done by Cao et al. highlighted that methylcrotonyl-CoA carboxylase, an enzyme located in mitochondria, interacts with MAVS, increasing IFN-I levels and consequently enhances the anti-viral response [69]. It has also been documented that following the recognition of viral RNA by RIG-1/MDA5, a signaling complex is triggered on the outer membrane of the mitochondria, defined as MAVS/TRAF3/TRAF6/TOM70, resulting in the induction of a potent anti-viral response [70, 71]. Furthermore, activation of PRR in the setting of viral infection has been shown to switch cell metabolism from oxidative phosphorylation to glycolysis, leading thusly to the stimulation of IFN-α secretion and enhancing eventually an anti-viral defense mechanism [72, 73]. Notably, NAD^+, a central metabolic coenzyme that exerts a key role in mitochondrial function and longevity, has been reported to regulate innate immune cell function, including that of macrophages [59]. Additionally, NAD^+ is involved in ATP generation that has been shown to be implicated in the regulation of the immune system [74].

Modulation of mitochondria by SARS-CoV-2

SARS-CoV-altered mitochondria-associated mechanisms implicated in the innate immune response have been proposed in multiple investigations, yet the exact mechanism is still poorly understood. For instance, it has been reported that SARS-CoV encodes a virulence factor known as open reading frame (ORF)-9b, which localizes to mitochondria, induces mitochondria elongation, and triggers MAVS degradation (Figure 2). This severely suppresses the innate immune response by inhibiting production of IFNs [75]. Although the number of basic investigations reporting SARS-CoV-2-induced mitochondrial manipulation are relatively scant, Bojkova et al. demonstrated that SARS-CoV-2 downregulates MAVS in human colon epithelial carcinoma cells CaCo-2 [30]. Additionally, recent computational work by Wu et al. indicated that SARS-CoV-2 localizes to mitochondria, resulting potentially in increasing reactive oxygen species (ROS) production and thereby suppressing mitochondrial function [76]. These findings were further supported by Gordon et al. who showed that SARS-CoV-2 interacts with the TIM complex, a component of the mitochondria import machinery, and Nsp8 interferes with mitochondria ribosome [77]. It has also been shown that SARS-CoV-2 upregulates genes implicated in the process of NF-κB regulation and ROS production and downregulates genes involved in mitochondria organization and respiration [78].

Interest in NAD^+ in the setting of SARS-CoV-2 infection is expanding. SARS-CoV-2 triggers a maladaptive immune response and aggravates DNA damage. As a result, the innate immune response is stimulated to fight the infection and poly (ADP-ribose) polymerase 1 (PARP-1), an NAD^+ consuming enzyme, is activated to repair DNA fragmentation and inhibit viral replication [79, 80]. PARP's anti-viral effect, however, is opposed by the ADP-ribosylhydrolase macrodomain of viral Nsp3, whose activity is necessary for virulence [81].

Recent evidence demonstrated disturbed NAD^+ metabolism and NAD^+ biosynthetic and consuming enzymes post-SARS-CoV-2 infection [11]. For instance, expression of nicotinamide riboside-1 (NMRK-1), an enzyme involved in NAD^+ biosynthesis was upregulated, whereas the expression of nicotinamide N-methyltransferase (NNMT), an enzyme eliminating nicotinamide from cells, was downregulated, suggesting a compensatory mechanism in infected cells to maintain NAD^+ levels [11, 80]. Additionally, it has been demonstrated that SARS-CoV-2 increases expression of several PARPs, leading to NAD^+ depletion and dysregulation of the immune response in the lungs of patients diagnosed with SARS-CoV-2 [11]. Taken together, replenishing NAD^+ levels through NMRK-1 may restore PARP function to support the innate immune response in fighting the viral infection.

Potential therapeutic strategies for COVID-19

Potential contribution of IFN therapy

A growing body of evidence supports IFNs as a potential therapy for the SARS-CoV-2 pandemic. Multiple clinical trials are currently underway to evaluate the impact of IFN alone or in combination with other anti-viral drugs on SARS-CoV-2 infection (Table 1). The potential beneficial effect of IFNs, however, should be assessed in terms of the timing. For instance, IFN-β-1b was administrated early after infection, highlighting that the timing of IFN-I is a crucial factor [82]. This observation is in line with previous clinical studies pointing out that IFN-I should be administered at an early stage of MERS-CoV infection [83, 84]. To avoid the ineffectiveness or potential adverse effects of late administration of IFN-I including malaise, fatigue, and fever [85], new therapeutic strategies are being evolved in the clinical setting. For instance, an aerosol formulation of recombinant IFN has been used to deliver the cytokine inside the lungs. In fact, studies have shown that the pulmonary concentration is higher following inhalation than with systemic administration, minimizing therefore the adverse effects of IFN [86, 87]. Additionally, Zhou et al. performed a clinical study on 77 SARS-CoV-2 patients in Wuhan. In this study, patients who received nebulized IFN-α-2b alone or in combination with Umifenovir, an anti-viral agent, showed a marked decrease in viral load in the upper respiratory tract [88].

IFN-III could also be a promising therapy for multiple different pathogens including respiratory syncytial virus, influenza B virus, influenza A virus, and SARS-CoV [89]. For instance, IFN-III was shown to be more effective than IFN-I in decreasing viral

Table 1. A list of COVID-19 clinical studies that are assessing the efficacy and safety of IFN treatment.

Identifier number	Study desire	Enrollment	Drug of interest
ChiCTR2000029387	Single study, randomized, open labeled, prospective clinical trial	101 Patients	• RBV + IFN-α • LPV/r + IFN-αRVB + LVP/r + IFN-α
NCT04276688	Prospective randomized open-label controlled clinical trial	127 Patients	• LPV/r • RBV • IFN-β-1b
NCT04315948	Multi center, adaptive, randomized, open clinical study	3100 Patients	• Remdesivir • LPV/r • IFN-β-1a • Hydroxychloroquine • Standard care
IRCT20100228003449N28	Randomized clinical trial	79 Patients	• IFN-β-1a • Hydroxychloroquine + LPV/r • Hydroxychloroquine + ATZ/r
NCT04492475	Randomized, controlled clinical trial	1000 Patients	• Remdesivir + IFN-β-1a
ChiCTR2000030262	Open label, non-randomized clinical trial	33 Patients	• IFN-κ + TFF2
IRCT20100228003449N28	Randomized clinical trial	92 Patients	• IFN-β-1a

LPV/r, Lopinavir/Ritonavir; RBV, Ribavirin; ATZ/r, Atazanavir/Ritonavir.

replication in intestinal epithelial cells [90]. Of note, it has been observed that SRAS-CoV-2 morbidity is linked to increased IFN-I and IFN-III expression levels in the lung [91]. Additionally, in mice exposed to synthetic viral RNA, IFN-III released by dendritic cells in the lung has been correlated with enhanced lung epithelium damage, consequently exacerbating disease severity [91]. Complicating this picture, a study performed by Hadjadj et al., indicated diminished type I IFN levels in peripheral blood immune cells from severe COVID-19 patients [92]. Taken together, these findings suggest that in contrast to local, the systemic production of IFNs in the setting of COVID-19 may exert beneficial effects.

Potential contribution of NAD$^+$ boosting therapy

NAD$^+$ boosting therapy has recently become a potential treatment for SARS-CoV-2 infection. A randomized double-blinded case-control trial (ID# NCT04407390) is currently evaluating the efficacy of the NAD$^+$ precursor nicotinamide riboside (NR) (1 g/day) for COVID-19 patients. Additionally, a phase II clinical study was conducted to assess the impact of NR, in combination with hydroxychloroquine, on mild to moderate COVDI-19 patients. The findings revealed a 30% decrease in recovery time following NR administration when compared to patients receiving standard care [11]. PARP inhibitors have also been shown to exert protective effects in the context of SARS-CoV-2 infection by decreasing the cytokine storm mediated by excessive release of pro-inflammatory cytokines (IL-6 and TNF-α) and attenuating lung fibrosis [93].

Besides NAD$^+$ boosting therapy, silence information regulator-1 (SIRT-1), implicated in mitochondria biogenesis, has been proposed to exert beneficial effects post-SARS-CoV-2 infection. It has been indicated that SIRT-1 decreases viral replication and the hyper-inflammatory response mediated by IL-1β, IL-6, and TNF-α [94]. Given the above, NAD$^+$ boosting and mitochondria-targeted therapies may be critical players in suppressing the cytokine storm and potential treatments against SARS-CoV-2 viral attack. Further experimental investigations and clinical data, however, are needed to draw a firm conclusion about the efficacy and safety of NAD$^+$ boosting and mitochondria-targeted therapies in the context of SARS-CoV-2 infection.

Conclusion

Mounting evidence suggests that an imbalanced innate immune response and mitochondria alterations play a crucial role in COVID-19 progression and development. A particularly insidious aspect of SARS-CoV-2 infection is the inhibition of the innate immune response that is associated with release of IFNs, together with enhanced mitochondrial dysfunction and NAD$^+$ depletion. Additional evidence concerning how SARS-CoV-2 inhibits the innate immune system and enhances mitochondrial dysfunction is needed. Defining the mechanisms of IFN evasion and preventing NAD$^+$ depletion may open up novel therapeutic approaches. While an effective treatment for COVID-19 is still elusive, a better understanding of the molecular pathways that are altered following SARS-CoV-2 infection should expedite the development of therapeutic and preventive treatments for this newly emerged virus.

Author's contributions

NJH conceived the manuscript and initiated the literature search and writing. All authors contributed to the further writing and content of the manuscript. Both GWB and FAZ assisted with the editing.

Declaration of Interest

The authors have no conflicts of interest to declare.

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References

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727–733. doi:10.1056/NEJMoa2001017.
2. Booz GW, Altara R, Eid AH, et al. Macrophage responses associated with COVID-19: A pharmacological perspective. *Eur J Pharmacol*. 2020;887:173547. doi:10.1016/j.ejphar.2020.173547.
3. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The*

Lancet. 2020;395(10229):1054–1062. doi:10.1016/S0140-6736(20)30566-3.
4. Ouyang L, Gong J. Mitochondrial-targeted ubiquinone: a potential treatment for COVID-19. *Med Hypotheses*. 2020;144:110161. doi:10.1016/j.mehy.2020.110161.
5. Wang B, Li R, Lu Z, Huang Y. Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. *Aging (Albany NY)*. 2020;12(7):6049–6057. doi:10.18632/aging.103000.
6. Zheng Z, Peng F, Xu B, et al. Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. *J Infect*. 2020;81(2):e16–e25. doi:10.1016/j.jinf.2020.04.021.
7. Wu J, Deng W, Li S, Yang X. Advances in research on ACE2 as a receptor for 2019-nCoV. *Cell Mol Life Sci*. 2021;78(2):531–514. doi:10.1007/s00018-020-03611-x.
8. Ni W, Yang X, Yang D, et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit Care*. 2020;24(1):422–410. doi:10.1186/s13054-020-03120-0.
9. Sa Ribero M, Jouvenet N, Dreux M, Nisole S. Interplay between SARS-CoV-2 and the type I interferon response. *PLoS Pathog*. 2020;16(7):e1008737. doi:10.1371/journal.ppat.1008737.
10. de Las Heras N, Martín Giménez VM, Ferder L, Manucha W, Lahera V. Implications of oxidative stress and potential role of mitochondrial dysfunction in COVID-19: therapeutic effects of vitamin D. *Antioxidants*. 2020;9(9):897. doi:10.3390/antiox9090897.
11. Heer CD, Sanderson DJ, Voth LS, Alhammad YM, Schmidt MS, et al. Coronavirus infection and PARP expression dysregulate the NAD Metabolome: an actionable component of innate immunity. *J Biol Chem*. 2020;295(52):17986–17996. doi:10.1074/jbc.RA120.015138.
12. Thompson MR, Kaminski JJ, Kurt-Jones EA, Fitzgerald KA. Pattern recognition receptors and the innate immune response to viral infection. *Viruses*. 2011;3(6):920–940. doi:10.3390/v3060920.
13. Park A, Iwasaki A . Type I and type III interferons - induction, signaling, evasion, and application to combat COVID-19. *Cell Host Microbe*. 2020;27(6):870–878. doi:10.1016/j.chom.2020.05.008.
14. Li J, Liu Y, Zhang X. Murine coronavirus induces type I interferon in oligodendrocytes through recognition by RIG-I and MDA5. *J Virol*. 2010;84(13):6472–6482. doi:10.1128/JVI.00016-10.
15. Cervantes-Barragan L, ZüSt R, Weber F, et al. Control of coronavirus infection through plasmacytoid dendritic-cell-derived type I interferon. *Blood*. 2007;109((3):1131–1137. doi:10.1182/blood-2006-05-023770.
16. Scheuplein VA, Seifried J, Malczyk AH, et al. High secretion of interferons by human plasmacytoid dendritic cells upon recognition of Middle East respiratory syndrome coronavirus. *J Virol*. 2015;89(7):3859–3869. doi:10.1128/JVI.03607-14.
17. Khanolkar A, Hartwig SM, Haag BA, Meyerholz DK, Harty JT, Varga SM. Toll-like receptor 4 deficiency increases disease and mortality after mouse hepatitis virus type 1 infection of susceptible C3H mice. *J Virol*. 2009;83(17):8946–8956. doi:10.1128/JVI.01857-08.
18. Totura AL, Whitmore A, Agnihothram S, et al. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *MBio*. 2015;6(3):e00638–e00615. doi:10.1128/mBio.00638-15.
19. Aboudounya MM, Heads RJ. COVID-19 and toll-like receptor 4 (TLR4): SARS-CoV-2 may bind and activate TLR4 to increase ACE2 expression, facilitating entry and causing hyperinflammation.
20. Choudhury A, Mukherjee S. In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. *J Med Virol*. 2020;92(10):2105–2113. doi:10.1002/jmv.25987.
21. Li JY, Liao CH, Wang Q, et al. The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Res*. 2020;286:198074. doi:10.1016/j.virusres.2020.198074.
22. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol*. 2004;4(7):499–511. doi:10.1038/nri1391.
23. Seth RB, Sun L, Ea C-K, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell*. 2005;122(5):669–682. doi:10.1016/j.cell.2005.08.012.
24. Xu L-G, Wang Y-Y, Han K-J, Li L-Y, Zhai Z, Shu H-B. VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol Cell*. 2005;19(6):727–740. doi:10.1016/j.molcel.2005.08.014.
25. Chen ZJ. Ubiquitin signaling in the NF-kB pathway. *Nat Cell Biol*. 2005;7:758–765.
26. Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M . The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. *Cell*. 1997;91(2):243–252. doi:10.1016/s0092-8674(00)80406-7.
27. Zhao J, He S, Minassian A, Li J, Feng P. Recent advances on viral manipulation of NF-κB signaling pathway. *Curr Opin Virol*. 2015;15:103–111. doi:10.1016/j.coviro.2015.08.013.
28. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol*. 2005;5(5):375–386. doi:10.1038/nri1604.
29. Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. *Signal Transduct Target Ther*. 2020;5(1):84. doi:10.1038/s41392-020-0191-1.
30. Bojkova D, Klann K, Koch B, et al. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature*. 2020;583(7816):469–472. doi:10.1038/s41586-020-2332-7.
31. Lei X, Dong X, Ma R, et al. Activation and evasion of type I interferon responses by SARS-CoV-2. *Nat Commun*. 2020;11(1):3810–3812. doi:10.1038/s41467-020-17665-9.
32. Wu J, Shi Y, Pan X, et al. SARS-CoV-2 ORF9b inhibits RIG-I-MAVS antiviral signaling by interrupting K63-linked ubiquitination of NEMO. *Cell Rep*. 2021;34(7):108761. doi:10.1016/j.celrep.2021.108761.
33. Fensterl V, Sen GC. Interferons and viral infections. *Biofactors*. 2009;35(1):14–20. doi:10.1002/biof.6.

34. Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol*. 2011;1(6):519–525. doi:10.1016/j.coviro.2011.10.008.
35. Merad M, Martin JC. Author correction: pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat Rev Immunol*. 2020;20(7):448–448. doi:10.1038/s41577-020-0353-y.
36. Jewell NA, Cline T, Mertz SE, et al. Lambda interferon is the predominant interferon induced by influenza A virus infection in vivo. *J Virol*. 2010;84(21):11515–11522. doi:10.1128/JVI.01703-09.
37. Galani IE, Triantafyllia V, Eleminiadou E-E, et al. Interferon-λ mediates non-redundant front-line antiviral protection against influenza virus infection without compromising host fitness. *Immunity*. 2017;46(5):875–890.e6. doi:10.1016/j.immuni.2017.04.025.
38. Stertz S, Reichelt M, Spiegel M, et al. The intracellular sites of early replication and budding of SARS-coronavirus. *Virology*. 2007;361(2):304–315. doi:10.1016/j.virol.2006.11.027.
39. Chen Y, Cai H, Pan J, et al. Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel cap N7 methyltransferase. *Proc Natl Acad Sci USA*. 2009;106(9):3484–3489. doi:10.1073/pnas.0808790106.
40. Daffis S, Szretter KJ, Schriewer J, et al . 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature*. 2010;468(7322):452–456. doi:10.1038/nature09489.
41. Menachery VD, Gralinski LE, Mitchell HD, et al. Middle East respiratory syndrome coronavirus nonstructural protein 16 is necessary for interferon resistance and viral pathogenesis. *MSphere*. 2017;2(6):e00346-17. doi:10.1128/mSphere.00346-17.
42. Lokugamage KG, Hage A, de Vries M, et al. SARS-CoV-2 is sensitive to type I interferon pretreatment. *bioRxiv*. 2020; doi:10.1101/2020.03.07.982264.
43. Siu K-L, Kok K-H, Ng M-HJ, et al . Severe acute respiratory syndrome coronavirus M protein inhibits type I interferon production by impeding the formation of TRAF3.TANK.TBK1/IKKepsilon complex. *J Biol Chem*. 2009;284(24):16202–16209. doi:10.1074/jbc.M109.008227.
44. Yang Y, Zhang L, Geng H, et al. The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. *Protein Cell*. 2013;4(12):951–961. doi:10.1007/s13238-013-3096-8.
45. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, Baric RA, Palese P. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. *J Virol*. 2007;81(2):548–557. doi:10.1128/JVI.01782-06.
46. Devaraj SG, Wang N, Chen Z, et al. Regulation of IRF-3-dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus. *J Biol Chem*. 2007;282(44):32208–32221. doi:10.1074/jbc.M704870200.
47. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell*. 2020;181(5):1036–1045.e9. doi:10.1016/j.cell.2020.04.026.
48. Frieman M, Yount B, Heise M, Kopecky-Bromberg SA, Palese P, Baric RS. Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. *J Virol*. 2007;81(18):9812–9824. doi:10.1128/JVI.01012-07.
49. Miorin L, Kehrer T, Sanchez-Aparicio MT, et al. SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *Proc Natl Acad Sci USA*. 2020;117(45):28344–28354. doi:10.1073/pnas.2016650117.
50. Schroeder S, Pott F, Niemeyer D, et al. Interferon antagonism by SARS-CoV-2: a functional study using reverse genetics. *Lancet Microbe*. 2021;2(5):e210–e218. doi:10.1016/S2666-5247(21)00027-6.
51. Xia H, Cao Z, Xie X, et al. Evasion of Type I Interferon by SARS-CoV-2. *Cell Rep*. 2020;33(1):108234. doi:10.1016/j.celrep.2020.108234.
52. Blanco-Melo D, Nilsson-Payant B, Liu W, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19 Cell. *Cell*. 2020;181(5):1036–1045.
53. Channappanavar R, Fehr AR, Vijay R, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host Microbe*. 2016;19(2):181–193. doi:10.1016/j.chom.2016.01.007.
54. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J Virol*. 2007;81(16):8692–8706. doi:10.1128/JVI.00527-07.
55. Wei L, Ming S, Zou B, et al. Viral invasion and type i interferon response characterize the immunophenotypes during COVID-19 infection. Available at SSRN 3555695. 2020; 2020: 1–11.
56. Traggiai E, Volpi S, Walter J, Walker M, Sims K. Powering the immune system: mitochondria in immune function and deficiency. *J Immunol Res*. 2014;2014:164309. doi:10.1155/2014/164309.
57. Storci G, Bonifazi F, Garagnani P, Olivieri F, Bonafè M. The role of extracellular DNA in COVID-19: clues from inflamm-aging. *Ageing Res Rev*. 2021;66:101234. doi:10.1016/j.arr.2020.101234.
58. Galluzzi L, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. *Nat Rev Mol Cell Biol*. 2012;13(12):780–788. doi:10.1038/nrm3479.
59. Minhas PS, Liu L, Moon PK, et al. Macrophage de novo NAD + synthesis specifies immune function in aging and inflammation. *Nat Immunol*. 2019;20(1):50–63. doi:10.1038/s41590-018-0255-3.
60. Guzzi PH, Mercatelli D, Ceraolo C, Giorgi FM. Master regulator analysis of the SARS-CoV-2/human Interactome. *J Clin Med*. 2020;9(4):982. doi:10.3390/jcm9040982.
61. Lai JH, Wang MY, Huang CY, et al. Infection with the dengue RNA virus activates TLR9 signaling in human dendritic cells. *EMBO Rep*. 2018;19(8):e46182. doi:10.15252/embr.201846182.

62. Singh KK, Chaubey G, Chen JY, Suravajhala P. Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesis. *Am J Physiol Cell Physiol*. 2020;319(2):C258–C267. doi:10.1152/ajpcell.00224.2020.
63. Burtscher J, Cappellano G, Omori A, Koshiba T, Millet GP . Mitochondria: In the cross fire of SARS-CoV-2 and immunity . *Iscience*. 2020;23(10):101631. doi:10.1016/j.isci.2020.101631.
64. Schoggins JW, MacDuff DA, Imanaka N, et al. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature*. 2014;505(7485):691–695. doi:10.1038/nature12862.
65. Storek KM, Gertsvolf NA, Ohlson MB, Monack DM. cGAS and Ifi204 cooperate to produce type I IFNs in response to Francisella infection. *J Immunol*. 2015;194(7):3236–45. doi:10.4049/jimmunol.1402764.
66. Watson RO, Bell SL, MacDuff DA, et al. The cytosolic sensor cGAS detects Mycobacterium tuberculosis DNA to induce type I interferons and activate autophagy. *Cell Host Microbe*. 2015;17(6):811–819. doi:10.1016/j.chom.2015.05.004.
67. Riley JS, Tait SW. Mitochondrial DNA in inflammation and immunity. *EMBO Rep*. 2020;21(4):e49799. doi:10.15252/embr.201949799.
68. Sun Q, Sun L, Liu H-H, et al. The specific and essential role of MAVS in antiviral innate immune responses. *Immunity*. 2006;24(5):633–642. doi:10.1016/j.immuni.2006.04.004.
69. Cao Z, Xia Z, Zhou Y, et al. Methylcrotonoyl-CoA carboxylase 1 potentiates RLR-induced NF-κB signaling by targeting MAVS complex. *Sci Rep*. 2016;6:33557. doi:10.1038/srep33557.
70. Kawai T, Akira S. Antiviral signaling through pattern recognition receptors. *J Biochem*. 2007;141(2):137–145. doi:10.1093/jb/mvm032.
71. Jiang H-w, Zhang H-n, Meng Q-f, et al. SARS-CoV-2 Orf9b suppresses type I interferon responses by targeting TOM70. *Cell Mol Immunol*. 2020;17(9):998–1000. doi:10.1038/s41423-020-0514-8.
72. Bajwa G, DeBerardinis RJ, Shao B, Hall B, Farrar JD, Gill MA. Cutting edge: critical role of glycolysis in human plasmacytoid dendritic cell antiviral responses. *J Immunol*. 2016;196(5):2004–2009. doi:10.4049/jimmunol.1501557.
73. Monlun M, Hyernard C, Blanco P, Lartigue L, Faustin B. Mitochondria as molecular platforms integrating multiple innate immune signalings. *J Mol Biol*. 2017;429(1):1–13. doi:10.1016/j.jmb.2016.10.028.
74. Weinberg SE, Sena LA, Chandel NS. Mitochondria in the regulation of innate and adaptive immunity. *Immunity*. 2015;42(3):406–417. doi:10.1016/j.immuni.2015.02.002.
75. Shi C-S, Qi H-Y, Boularan C, et al. SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *J Immunol*. 2014;193(6):3080–3089. doi:10.4049/jimmunol.1303196.
76. Wu KE, Fazal FM, Parker KR, Zou J, Chang HY. RNA-GPS predicts SARS-CoV-2 RNA residency to host mitochondria and nucleolus. *Cell Syst*. 2020;11(1):102–108.e3. doi:10.1016/j.cels.2020.06.008.
77. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*. 2020;583(7816):459–413. doi:10.1038/s41586-020-2286-9.
78. Singh K, Chen Y-C, Judy JT, Seifuddin F, Tunc I, Pirooznia M. Network Analysis and Transcriptome Profiling Identify Autophagic and Mitochondrial Dysfunctions in SARS-CoV-2 Infection. *bioRxiv*. 2020. doi:10.1101/2020.05.13.092536.
79. Fehr AR, Singh SA, Kerr CM, Mukai S, Higashi H, Aikawa M. The impact of PARPs and ADP-ribosylation on inflammation and host-pathogen interactions. *Genes Dev*. 2020;34(5-6):341–359. doi:10.1101/gad.334425.119.
80. Mehmel M, Jovanović N, Spitz U. Nicotinamide riboside—The current state of research and therapeutic uses. *Nutrients*. 2020;12(6):1616. doi:10.3390/nu12061616.
81. Alhammad YMO, Kashipathy MM, Roy A, et al. The SARS-CoV-2 conserved macrodomain is a mono-ADP-ribosylhydrolase. *J Virol*. 2021;95(3). doi:10.1128/JVI.01969-20.
82. Hung IF-N, Lung K-C, Tso EY-K, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *The Lancet*. 2020;395(10238):1695–1704. doi:10.1016/S0140-6736(20)31042-4.
83. Shalhoub S, Farahat F, Al-Jiffri A, et al. IFN-α2a or IFN-β1a in combination with ribavirin to treat Middle East respiratory syndrome coronavirus pneumonia: a retrospective study. *J Antimicrob Chemother*. 2015;70(7):2129–2132. doi:10.1093/jac/dkv085.
84. Al-Tawfiq JA, Momattin H, Dib J, Memish ZA. Ribavirin and interferon therapy in patients infected with the Middle East respiratory syndrome coronavirus: an observational study. *Int J Infect Dis*. 2014;20:42–46. doi:10.1016/j.ijid.2013.12.003.
85. Lam S, Lombardi A, Ouanounou A. COVID-19: A review of the proposed pharmacological treatments. *Eur J Pharmacol*. 2020;886:173451. doi:10.1016/j.ejphar.2020.173451.
86. Dhanani J, Fraser JF, Chan H-K, Rello J, Cohen J, Roberts JA. Fundamentals of aerosol therapy in critical care. *Crit Care*. 2016;20(1):269–216. doi:10.1186/s13054-016-1448-5.
87. Laube BL. The expanding role of aerosols in systemic drug delivery, gene therapy and vaccination: an update. *Transl Respir Med*. 2014;2(1):3. doi:10.1186/2213-0802-2-3.
88. Zhou Q, Chen V, Shannon CP, et al. Interferon-α2b treatment for COVID-19. *Front Immunol*. 2020;11:1061. doi:10.3389/fimmu.2020.01061.
89. Mordstein M, Neugebauer E, Ditt V, et al. Lambda interferon renders epithelial cells of the respiratory and gastrointestinal tracts resistant to viral infections. *J Virol*. 2010;84(11):5670–5677. doi:10.1128/JVI.00272-10.
90. Stanifer ML, Kee C, Cortese M, et al. Critical role of type III interferon in controlling SARS-CoV-2 infection in human intestinal epithelial cells. *Cell Rep*. 2020;32(1):107863. doi:10.1016/j.celrep.2020.107863.
91. Broggi A, Ghosh S, Sposito B, et al. Type III interferons disrupt the lung epithelial barrier upon viral recogni-

tion. *Science*. 2020;369(6504):706–712. doi:10.1126/science.abc3545.

92. Hadjadj J, Yatim N, Barnabei L, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science*. 2020;369(6504):718–724. doi:10.1126/science.abc6027.
93. Curtin N, Bányai K, Thaventhiran J, Le Quesne J, Helyes Z, Bai P. Repositioning PARP inhibitors for SARS-CoV-2 infection(COVID-19); a new multi-pronged therapy for acute respiratory distress syndrome?*Br J Pharmacol*. 2020;177(16):3635–3645. doi:10.1111/bph.15137.
94. Miller R, Wentzel A, Richards G. COVID-19: NAD + deficiency may predispose the aged, obese and type2 diabetics to mortality through its effect on SIRT1 activity. *Med Hypotheses*. 2020;144:110044. doi:10.1016/j.mehy.2020.110044.