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A geroscience perspective on immune resilience and infectious diseases: a potential case for metformin

Jamie N. Justice ⬤ · **Sriram Gubbi · Ameya S. Kulkarni · Jenna M. Bartley · George A. Kuchel · Nir Barzilai**

Abstract We are in the midst of the global pandemic. Though acute respiratory coronavirus (SARS-COV2) that leads to COVID-19 infects people of all ages, severe symptoms and mortality occur disproportionately in older adults. Geroscience interventions that target biological aging could decrease risk across multiple age-related diseases and improve outcomes in response to infectious disease. This offers hope for a new host-directed therapeutic approach that could (i) improve outcomes following exposure or shorten treatment regimens; (ii) reduce the chronic pathology associated with the infectious disease and subsequent comorbidity, frailty, and disability; and (iii) promote development of immunological memory that protects against relapse or improves response to vaccination. We review the possibility of this approach by examining available evidence in metformin: a generic drug with a proven safety record that will be used in a large-scale multicenter clinical trial. Though rigorous translational research and clinical trials are needed to test this empirically, metformin may improve host immune defenses and confer protection against long-term health consequences of infectious disease, age-related chronic diseases, and geriatric syndromes.

Keywords Metformin · Aging · Geroscience · Immunity · COVID-19

J. N. Justice (✉)
Sticht Center for Healthy Aging and Alzheimer's Prevention, Internal Medicine – Gerontology and Geriatric Medicine, Wake Forest School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157, USA
e-mail: jnjustic@wakehealth.edu

S. Gubbi
Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20814, USA

A. S. Kulkarni · N. Barzilai
Department of Medicine, Division of Endocrinology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

A. S. Kulkarni · N. Barzilai
Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY 10461, USA

J. M. Bartley · G. A. Kuchel
Center on Aging, University of Connecticut School of Medicine, Farmington, CT 06030, USA

J. M. Bartley
Department of Immunology, University of Connecticut School of Medicine, Farmington, CT 06030, USA

Geroscience in a time of global pandemic

The promise of geroscience is that interventions that target biological aging could fortify organismal resilience and delay the onset or lessen the severity of multiple age-related diseases en masse. Never has this promise been more critical than now, in the midst of the global pandemic. Though acute respiratory coronavirus (SARS-COV2) infects people of all ages, severe symptoms and mortality occur disproportionately in older adults. In the USA, 8 out of 10 deaths occurred in persons over the age of 65 years [1], and in Britain,

persons above age 80 years were ~ 180 fold more likely to die that those in their 40s [2]. Severity and complications also increase nonlinearly with age. In China, the odds of hospitalization at ages 40–49 years were 4.3% but nearly doubled to 8.2%, for 50–59-year-olds, and continued to climb for each decade of advancing age up to 18.4% in persons over the age of 80 years [3, 4].

This trend is not unique to COVID-19. It is well known that older persons generally have greater susceptibility to infections than younger adults due to age-related changes in cell-mediated immunity, underlying chronic disease, and geriatric syndromes [5, 6]. Moreover, the pattern of exponential increase in mortality rate with age in COVID-19 exhibits a doubling time estimated between 6.6 to 9 years (USA, China, Italy); this is mirrored by the exponential increase in age-related risk of mortality from all causes (doubling time of ~ 9 years, USA), and cause specific which ranges from 4.7 years (chronic obstructive pulmonary disease, COPD) to 7.1 years (diabetes mellitus, DM) [7]. This suggests contributions of age-related declines in immune and inflammatory responses in harmony with systemic age-related physiologic dysregulation and comorbidity [3, 8]. Chronically elevated inflammation and dysregulated cell–cell communication constitute examples of biological hallmarks of aging and represent both contributors to and consequences of varied chronic conditions that are independently associated with increased COVID-19 vulnerability. For example, the presence of multiple chronic conditions or comorbidities such as diabetes, chronic kidney disease, and heart failure are associated with proinflammatory cytokines and other biomarkers of biological hallmarks of aging [9–11], and have been shown to be independently associated with severe COVID-19 illness and death [12–14] [15, 16].

Therefore, while virus-specific treatments and vaccines are critically needed, other complementary strategies are also needed to address increased vulnerability to severe COVID-19 during this window of vulnerability as vaccines and antiviral agents are being developed. To that end, treatment of the biological underpinnings which render older adults more vulnerable may not only improve outcomes during this COVID-19 pandemic, but could also offer benefits in terms of future pandemics involving other pathogens, while also impacting positively the onset and progression of varied acute and chronic diseases [17]. Geroscience interventions that target biological aging could decrease risk across multiple age-related diseases and improve outcomes in response to infectious disease. This offers hope for a new host-directed therapeutic approach that could (i) improve outcomes following exposure or shorten treatment regimens; (ii) reduce the chronic pathology associated with the infectious disease and subsequent co-morbidity, frailty, and disability; and (iii) promote development of immunological memory that protects against relapse or improves response to vaccination [18, 19] (Fig. 1).

Translational interventions in geroscience

Preclinical interventions testing programs show that biological aging pathways can be therapeutically targeted. Perhaps, the most robust intervention is caloric restriction, which has been shown to prolong the lifespan of experimental animal models such as nematodes, flies, and mice [20, 21] and may improve immune response [22]. In addition, promising pharmacological and nutraceutical candidates have been identified. In most cases, median lifespan extension is accompanied by improved health and ability of preclinical models to withstand both chronic and acute challenges [23, 24]. A select subset of these have intriguing effects in humans on overall health and immune function, including acarbose [25], and mTORC1 inhibitors rapamycin, everolimus, and RTB101 [26]. For example, mice treated with 6 weeks of rapamycin mounted a greater response to influenza vaccine than age-matched control mice [27], and two phase II studies of mTORC inhibitors demonstrated improved antibody responses to an influenza vaccine and decreased rates of upper respiratory tract infections by varied pathogens in healthy community–dwelling older adults [26, 28]. Phase III trial of mTORC1 inhibitor RTB101 did not meet its primary endpoint for prevention of respiratory illness. However, two new phase I-II trials with mTOR inhibitor sirolimus are now in progress in hospitalized patients with COVID-19 pneumonia (NCT04341675 and NCT04371640) and those with RTB101 in the nursing home setting (NCT04409327).

A critical obstacle for larger clinical trials and widespread clinical application of any drug targeting aging is safety, tolerability, and expense. So far, only a select couple of drugs can overcome these translational barriers. One such drug is metformin. Though metformin offers modest improvements in lifespan in preclinical
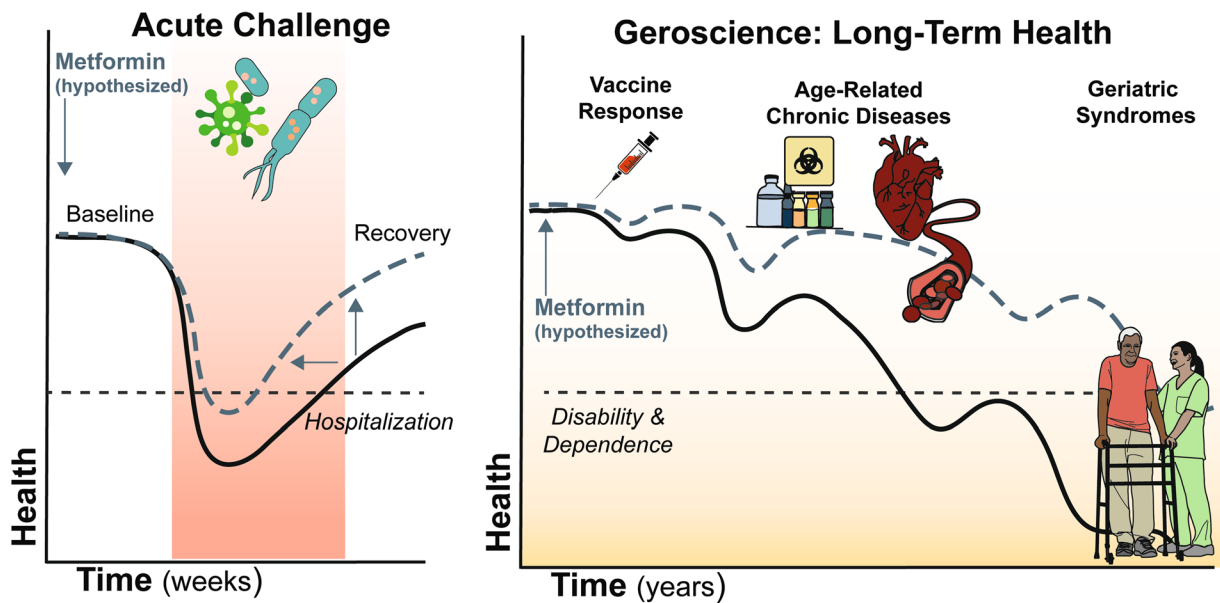
Fig. 1 Geroscience and immune resilience: acute and long-term health. Metformin use (blue line) initiated prior to acute illness like COVID-19 (left most panel) may improve resilience resulting in fewer events like hospitalization (dotted line), briefer recovery time, and improved return to baseline health compared to non-use (black line). Greater long-term health effects could be observed as improved response to vaccine and prevention or delay of age-related diseases and geriatric syndromes

interventions testing [29], it targets molecular and cellular drivers of aging which may exert positive effects more broadly on health span. Moreover, metformin (i) is a generic and relatively inexpensive medication, (ii) has an excellent safety record for over 5 decades, and (iii) can be generally well tolerated with long-term use in a large population of aging adults. Metformin will be tested in a first-of-its-kind trial designed to create a regulatory pathway for aging as a target for drug development, the phase III multicenter randomized double-blind placebo-controlled trial Targeting Aging with MEtformin (TAME) [30, 31]. With recruitment start coinciding with what is anticipated to be the waning of the COVID-19 global pandemic, TAME provides an excellent platform to probe preventive effects on age-related chronic disease and long-term consequences of infectious disease and vaccine response in older persons. In light of the recent global pandemic, we provide a discussion of metformin in immune aging and infectious disease focusing on clinical evidence.

A brief history of metformin and aging

Metformin is a biguanide and is currently the most widely prescribed oral hypoglycemic medication for type 2 diabetes treatment or prevention prescribed worldwide. However, its history dates back to the seventeenth century, when metformin-like guanidine compounds found in traditional herbal medicine *Galega officinalis* were used to treat conditions like plague, fever, and snake bites. Metformin was rediscovered in the 1940s, in a search for antimalarial agents, and in 1949, metformin was found to be helpful in treating a local influenza outbreak in the Philippines [32, 33]. Its immune effects were accompanied by the beneficial side effect of lowered blood glucose in some of the influenza patients. In the 1950s, the physician Dr. Jean Stern translated this glucose-lowering potential into diabetes treatments, though metformin was not finally approved by the Food and Drug Administration (FDA) as an antidiabetic until 1995.

Interest in metformin's clinical effects beyond glucose regulation originated from epidemiologic studies, which observed a reduction in cardiovascular risk and all-cause mortality in individuals with diabetes treated with metformin in the Diabetes Prevention Program (DPP) and UK Prospective Diabetes Study (UKPDS) [34, 35]. Since then, there is growing interest in metformin's clinical benefits beyond diabetes (reviewed in [30]) in diseases ranging from gestational diabetes, polycystic ovary syndrome (PCOS), and human immunodeficiency virus (HIV)–associated lipodystrophy, to dementia-type

neurodegenerative disorders and certain cancers in individuals with and without type 2 diabetes.

Metformin attenuates hallmarks of biological aging

Metformin has emerged as a leading candidate in clinical trials in aging and age-related acute and chronic diseases given its cellular effects which are distinct from its effects on glucose metabolism in diabetics. Kulkarni et al. [36] recently reviewed metformin's effects on the biological hallmarks of aging, with supporting evidence from *C. elegans*, drosophila, and rodents. Metformin acts through a few widely accepted cellular and molecular mechanisms that are highly interdependent and implicated metformin's downstream effects on immune response, chronic diseases, and geriatric syndromes (Table 1). Chief among these cellular mechanisms are metformin's actions on the mitochondrion as a weak inhibitor of complex I of the mitochondrial electron transport chain, activation of the energy sensor AMP–activated protein kinase, inhibition of mTORC1, and regulation of inflammatory cytokine signaling [30, 36]. Converging evidence also implicates lysosomes [37], gut microbiome [38], and epigenetic modulation as mediators of metformin's actions on biological aging [39, 40]. Importantly, metformin is not proposed to exert action by any one single or distinct mechanism but through multiple mechanisms. Moreover, it is precisely this plurality of pleiotropic cellular effects that makes metformin a valuable tool to achieve downstream effects on immunoprotection, and multiple age-related diseases and geriatric conditions.

Mechanisms: metformin in immune response and inflammation

Metformin also exerts actions in immune modulation and inflammatory response, lending resilience to infectious diseases and acute respiratory illnesses (Fig. 2, Table 2). Metformin's interference with key immunomodulatory mechanisms are both dependent and independent of its effects on improving in metabolic parameters. Several mechanisms may underlie metformin's effects on immune resilience and responses to infectious disease; however, it is important to note that the effects of metformin on the immune system are dependent on the pathological context (reviewed in [41]). In the context of some cancers, metformin has been shown to be immunostimulatory with a shift favoring M1 macrophage polarization over the more antiinflammatory M2 [42, 43] and increased antigen-specific T cell responses coupled with decreased immunosuppressive T regulatory cells [44, 45]. However, this shift towards an immunostimulatory phenotype seems specific to pathological immunosuppressive conditions such as cancer. Conversely, in most other situations, metformin is able to quell aberrant inflammation and promote antiinflammatory pathways. Indeed, in the context of wound healing, metformin induces M2 polarization [46]. Additionally, during autoimmune diseases [47], graft-versus-host disease [48] and others more proinflammatory conditions, metformin is able to increase T regulatory cells and decrease T helper (Th)17 cells [47–49], an effect likely partially mediated by AMPK activation [50]. Moreover, metformin treatment in uninfected mice increases in vitro T cell IFN-γ responses as well, indicating that metformin treatment has the ability to modulate immune cells independent of current infections and potentially improve responses to future pathogens [51]. For coronaviruses, the timing of type I IFN production is critical to infection outcome: an early burst of type I IFN leads to protection, but a delay in IFN production causes an inability to control viral replication or pathogen spread, leading to cellular damage of airway epithelia and the lung parenchyma and an eventual lethal inflammatory cytokine storm [52]. Thus, metformin has the potential to improve the initial type I IFN response that is crucial for coronavirus immune responses and prevent or reduce cellular damage from exaggerated, prolonged inflammatory responses.

Indeed, metformin's effect on immune responses to chronic pathogen exposures or challenges, as well as general states with elevated inflammation, tends to be overall immunosuppressive and antiinflammatory (Fig. 2). This dysregulated immune signaling is particularly relevant to SARS-CoV-2, which causes exaggerated and aberrant host immune responses that are uniquely challenging in persons of advanced age [53]. This 'cytokine storm' of perpetuating proinflammatory signaling loops is thought to lead to acute respiratory distress syndrome and poorer outcomes, especially in older adults [54, 55]. Metformin improves the immune response and reduces inflammation by promoting the formation of M2 macrophages and T regulatory and CD8 memory T cells [56], and may prevent the differentiation of monocytes to macrophages via an AMPK-dependent inhibition of STAT3 [57]. In the face of chronic infection or disease, metformin also reduces

Table 1 Summary of metformin's mechanisms in attenuating hallmarks of aging. Metformin has been postulated to exert gerotherapeutic effects through several molecular pathways related to biological hallmarks of aging [36]

Aging hallmarks	Metformin's effects on key targets and pathways
Altered intercellular communication	• Antiinflammatory response and immunomodulation ↓ pro-inflammatory cytokines (IL-6, IL-1β, CXCL1/2) [59] ↓ NF-κB and IKKα/β signaling [111] • Regulation of the gut microbiota by altering microbial folate Anti-inflammatory response via ↑ *Akkermansia*, ↑ *Bacteroides*, ↑ *Butyricimonas* and ↑ *Parabacteroides* genera [112, 113] Methionine restriction [114]
Deregulated nutrient sensing	• Direct targeting of key energy sensors and modulation of nutrient sensing pathways ↑ AMPK [37, 115, 116], ↑ SIRT1 [117, 118] ↓ mTORC1 (via ↓ Rad-GTPase, ↑ TSC2, ↑ REDD1) [119–122] ↓ Insulin and IGF-1 signaling [123]
Genomic instability	• Genome protective effects with ↑ DNA-damage-like response and ↑ DNA repair and regulation of ATM-protein kinases [124–126]
Loss of proteostasis	• Increased autophagy and rescue of protein misfolding ↑ LAMP-1 and ↑ Beclin-1 [127, 128] ↑ CEBPD-mediated autophagy [129]
Mitochondrial dysfunction	• Inhibition of mitochondrial complex I of the electron transport chain • Lowering mitochondria-induced oxidative stress [130–132] ↓ Endogenous production of ROS [130–133] • Improved mitochondrial biogenesis via ↑ PGC-1α [134]
Stem cell exhaustion	• Improved stem cell rejuvenation capacity [135–137] • Delayed stem cell aging [138]
Epigenetic alterations	• Transcriptional regulation via histone modifications, DNA methylation and miRNAs [40] Phosphorylation of Histone Acetyl Transferases (HATs) ↓ Class II Histone Deacetylases (HDACs) ↑ DICER1 [139]
Telomere attrition	• Reduction of telomere shortening and ↑ telomeric repeat containing RNA (TERRA) [140]
Cellular senescence	• Suppression of senescence-associated secretome ↓ p16, ↓ p21 and RNA-levels of SASP hallmarks [111, 139, 141] ↓ β-gal activity and ↓proinflammatory senescence [142]

genes encoding for cytokines associated with inflammation response by suppression of tumor necrosis factor-α (TNF-α) dependent NF-κB signaling and expression of interleukin (IL)-6 (IL-6), IL-β (IL-1β), MCP-1 (CCL2 chemokine ligand 2), CXCL5, and IP-10 (CXCL10) [51, 58, 59]. Collectively, this results in lower levels of circulating proinflammatory cytokines, and in a recent report in older persons with T2DM, the reduction in proinflammatory signaling was associated 5-year all-cause mortality risk [60]. Furthermore, metformin treatment diminished LPS-induced acute lung injury [61], suggesting that metformin may be able to reduce the cytokine storm-like response and prevent exaggerated lung damage due to SARS-CoV-2 as well. In addition, metformin can induce autophagy and facilitate phagosome-lysosome fusion and induces phagocytosis of neutrophils, which points to a role for metformin in killing or containing pathogens, controlling inflammation, and activating innate and adaptive immune response in the host [62].

Metformin may also reduce inflammation through gut microbiota [38, 62]. Multiple studies have shown that the glucose lowering ability of metformin is partly mediated through the gut microbiota changes [63–65]. Others have further shown that the antiinflammatory effects of metformin may also be partly mediated through the gut microbiota, even in nondiabetic conditions where the pattern of gut microbe changes with metformin are negatively correlated with a variety of inflammatory diseases [66]. While the mechanism of effect on the microbiota is not known, it seems that metformin may reduce dysfunctional gut permeability ("leaky gut") by increasing goblet cell mass and mucin production [67], and may alleviate gut dysbiosis by

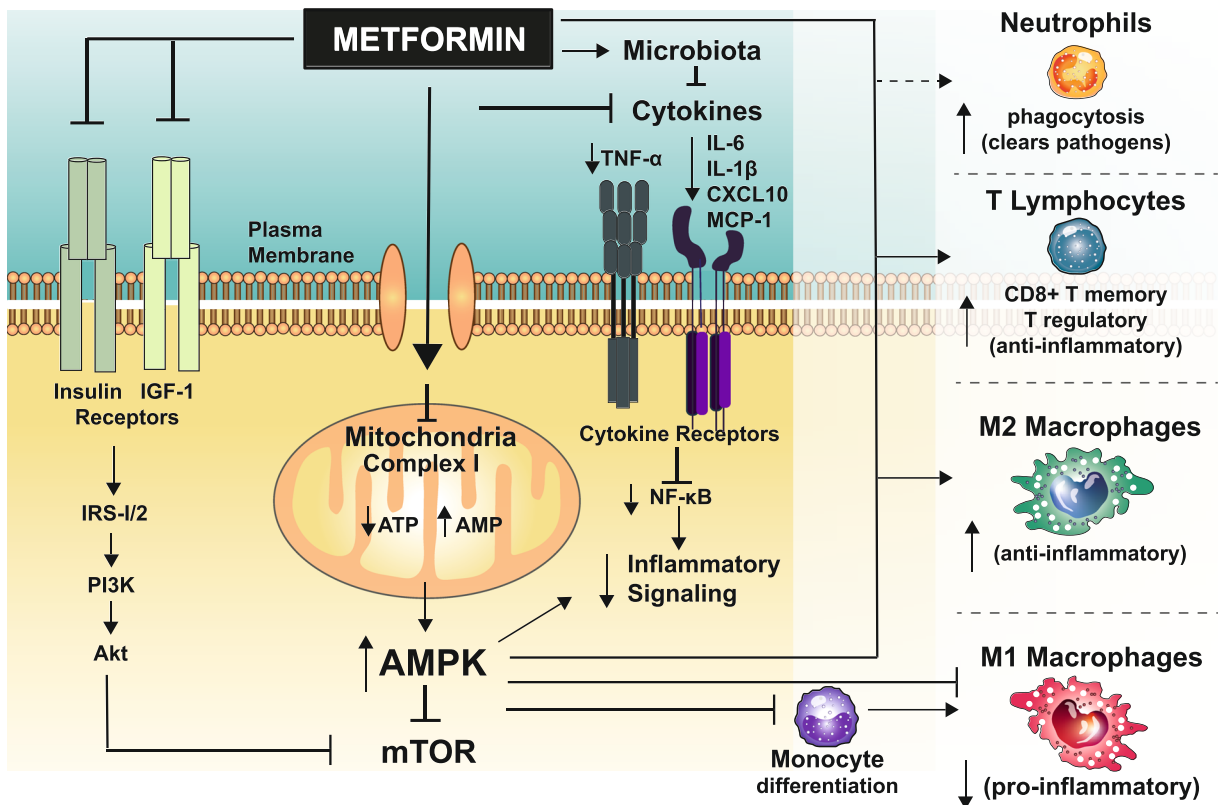## Metformin Mitigates Chronic Pro-inflammatory Immune Response

Fig. 2 Metformin alleviates chronic proinflammatory immune signaling and restores immune response. Metformin's cellular mechanisms include weak inhibition of complex I of the mitochondrial electron transport chain, activation of the energy sensor AMP-activated protein kinase, inhibition of the heteromultimeric protein kinase mTORC1, and suppression of elevated proinflammatory cytokines production. Converging evidence also implicates the gut microbiome which further alleviates inflammation and phagosome-lysosome fusion which induces phagocytosis of neutrophils to reduce pathogen burden. The collective result is a dampened broad proinflammatory cytokine signaling and improved immune cell activation

increasing *Akkermansia muciniphila* and short-chain fatty acid-producing bacteria, such as *Bacteroides* and *Butyricimonas* [68]. Of particular interest, these alterations were still observed in aged obese mice despite the presence of preexisting age-related microbiota changes and were correlated with lower IL-6 and Il1β [67] [68]. This suggests that metformin may also be able to mitigate exaggerated inflammatory responses via changes in gut microbiota.

Metformin and resilience to severe infectious disease

COVID-19

The origin and pathogenesis of the acute respiratory disease caused by the novel coronavirus SARS-CoV2

is increasingly well understood [54]. SARS-CoV2 is an enveloped nonsegmented positive-sense RNA virus. The virus gains entrance to host cells through angiotensin-converting enzyme 2 (ACE2) receptors, and upon entry the virion releases its RNA in the cytoplasm. Translation and replication occur and new virions are then released from the host cell [69, 70]. However, key to COVID-19 pathogenesis is the attendant explosive and deleterious host immune response and immunopathogenesis [71]. This cytokine storm, or cytokine release syndrome, may occur approximately 5–8 days after symptom onset and is associated with dismal outcomes including ICU care, mechanical ventilation, prolonged hospital care, and in some cases, death. Inflammatory factors elevated during this storm include IL-6, IFNγ, TNFα, IL-1β, IL-8, MCP-1, (CXCL10), and lower T cell counts (CD8+ and CD4+

Table 2 Metformin treatment in experimental models of infectious disease or acute respiratory illness

Disease or illness	Design, species	Pathogen or exposure	Metformin dosing	Relevant result	Ref.
Acute or chronic lung illness					
ARDS	In vivo, mouse	LPS-induced lung injury model in BALB/c mice	Prophylactic 50 mg/kg from 7 days prior to LPS injury	- Partially reverse pulmonic injury (Pulmonary edema, vascular exudation, and neutrophil accumulation) and inflammatory cytokines -reduce LPS-induced death	[143]
ARDS	In vivo, mouse	LPS-induced lung injury model in BALB/c mice	With exposure 250 mg/kg (i.p.) 0.5 h prior to LPS injury	- Suppress LPS-induced lung injury and inhibit markers of oxidative stress	[144]
ARDS	Ex vivo, human	BAL and hMDM from ARDS patients	With exposure 500 µmol/L metformin added to culture of ARDS hMDMs + BAL	- Increase in uptake of apoptotic neutrophils and neutrophil extracellular trap (NET) engulfment	[145]
Lung fibrosis	In vivo, mouse	Bleomycin lung fibrosis model	Treatment 65 mg/kg; (i.p.) for 18 days, initiated 10-day post-bleomycin lung injury	- Significant reductions in profibrotic markers total lung hydroxyproline, α-SMA expression. Collagen - Promotes fibrosis resolution up to 3 weeks post-exposure	[146]
Bacterial infections					
L. pneumophila	In vitro, mouse, human	- Bone marrow–derived macrophages - RAW cells (mouse) - U937 cells (human)	Treatment 2 mM metformin 6- or 24-h postinfection	- Suppress bacterial growth in time and concentration dependent manner	[86]
L. pneumophila	In vivo, mouse	L. pneumophila pneumonia model	Prophylactic 5 mg/ml metformin from 7-days prior to infection	- Improve survival and significant reduction in bacterial number in the lung	[86]
M. Tuberculosis	In vitro, human	Healthy human peripheral blood mononuclear cells (PBMC) infected with m. tuberculosis	Treatment 3–3000 µM metformin incubation for 24 h	- Enhanced cellular metabolism - Inhibited p70S6K and 4EBP1 - Decreased cytokine production, cellular proliferation - Increased phagocytosis activity	[147]
M. Tuberculosis	In vitro, human	Lung epithelial cells and macrophages	With exposure 2 mM and 4 mM metformin for 48-h, initiated with infection	- Reduce bacillary loads in macrophages and lung epithelial cells	[148]
M. Tuberculosis	In vitro	Human monocytic cell line THP-1 and hMDMs	With exposure 2 mM metformin in culture initiated with infection	- Restrict mycobacterial growth by inducing mitochondrial reactive oxygen species production	[51]
M. Tuberculosis	In vivo, mouse	Acute and chronic M. tuberculosis	Adjuvant treatment 500 mg/kg starting 7 or 42 days post-infection (± isoniazid or ethionamide)	- Enhance the efficacy of conventional anti-TB drugs -improve TB-induced tissue pathology and immune response	[51]
M. Tuberculosis	In vivo, mouse	BALB/c mice infected with M. tuberculosis	Treatment 250 mg/kg for up to 6-months, initiated 6-weeks post-infection	- No effect on lung bacillary burdens or microbiological relapse	[149]

Table 2 (continued)

Disease or illness	Design, species	Pathogen or exposure	Metformin dosing	Relevant result	Ref.
P. aeruginosa	In vitro	P. aeruginosa infection across airway epithelial (Calu-3) cultures	With exposure 0.02 or 1 mM metformin 18-h prior to inoculation	- Reduce hyperglycemia-induced P. aeruginosa growth through airway epithelial tight junction modulation	[84]
P. aeruginosa	In vivo, mouse	Streptozotocin-induced diabetes	Prophylactic 4 mg/ml (i.p.) at day −2, −1, 0 of infection	- Reduce airway glucose and bacterial load (without change in blood glucose)	[150]
P. aeruginosa	In vivo, C. elegans, mouse	- P. aeruginosa PA14 infection	With exposure 1–100 mM metformin initiated with infection	- Dose-dependent increase resistance to P. aeruginosa PA14 infection; - Increase levels of active PMK-1; - p38/PMK-1-mediated innate immunity conserved from worms to mammals	[151]
S. aureus	In vitro	H441 epithelial cells	With exposure 1 mM 18-h prior to infection	- Increase transepithelial resistance; - reduce glucose-dependent bacterial growth	[152]
S. aureus	In vitro, human	Airway epithelial cells	With exposure 0, 0.03, 0.3, or 1 mM metformin 18 h prior to inoculation	- Reduced paracellular flux across murine tracheas.	[153]
S. aureus	In vivo, mouse	Wild-type, C57BL/6 or db/db,	Prophylactic 40 mg/kg metformin 2 days prior to infection	- Modify glucose flux across the airway epithelium; - Limit hyperglycemia-induced bacterial growth	[153]
Trypanosoma cruzi	In vivo, mouse	CD-1 mice on high fat diet (HFD)	Prophylactic 50 mg/kg metformin by gavage (± HFD) 20 days prior to infection	- Reduce mortality from 20% on HFD to 3% on HFD + metformin	[82]
Parasitic infection					
Malaria P. berghei	In vitro	Huh7 cells	Treatment 0.04–1.25 mM metformin	- Reduce of total parasite load (dose-dependent)	[154]
Malaria P. berghei	In vivo, mouse	C57BL/6	Prophylactic 500 mg/kg/day metformin, 7 days prior to infection	- Reduce total burden of P. berghei liver infection and lessened disease severity	[155]
Malaria P. yoelii	In vivo, mouse	C57BL/6	Treatment 5 mg/ml metformin initiated with infection or 7 days post-infection	- Reduce of parasitemia	[156]
Viral infections					
HCV	In vitro	Huh7.5 cells infected with HCV particles or primary human hepatocytes	With exposure 2–10 mM metformin + 2 μM simvastatin	- Inhibited cell growth and HCV infection	[157]
HCV	In vitro	Huh-7.5 cells (HCV-susceptible subclone of Huh7 cells)	With exposure 0–10 mM metformin for 20 h incubation	- Anti-HCV effects in AMPK dependent and independently manner	[158]
HCV			Adjuvant treatment		[159]

Table 2 (continued)

Disease or illness	Design, species	Pathogen or exposure	Metformin dosing	Relevant result	Ref.
	In vivo, human	Patients with genotype 1 chronic HCV and insulin resistance	1275–2550 mg/day metformin for 48 weeks with antiviral PEG-IFN and RBV	- Improved sustained virological response (SVR) in females only (not overall)	[160]
HCV	In vivo, human	Patients with genotype 1 chronic HCV and insulin resistance	Adjuvant treatment 1500 mg/day metformin for 48 weeks with PEG-IFN and RBV	- Metformin adjuvant improved insulin sensitivity and increased the SVR rate	[161]
HCV	In vivo, human	Treatment-naïve chronic HCV patients	Adjuvant treatment 1500 mg/day for 6-months with PEG-IFN and RBV	- SVR rate in the metformin group was 75% versus 79% in controls (intention-to-treat) which was not significantly different.	
HBV	In vitro	HBV-producing human hepatoma cell line HepG2.2.15	Treatment 0–4 mM metformin for 6 days initiated 24 h post-infection	- Enhanced the inhibitory effects of interferon-α2b on HBsAg expression and HBV replication	[89]
HBV	In vitro	HepG2 and PLC/PRF/5 cells	Combined treatment 0-200 μM metformin +20 nmol/L rapamycin for 12–48-h	- Decrease cell viability at 12 h in Sir+Met - No change in Sir or Met monotherapy until 24 h	[162]
HBV	In vivo, human	Hepatocellular carcinoma (HCC) related to HBV infection in T2DM	Combined treatment 1700 mg/day metformin + 2 mg/day sirolimus postoperative	- Survival in the Sir+Met group was significantly longer compared with control and monotherapy	[162]
HBV	In vivo, mouse	HBV × antigen transgenic (HB × Tg) mice	Treatment 250 mg/kg/day metformin (drinking water), 2–18 months of age	- No effect on incidence of HCC, but slightly increased hepatic cellular retinol-binding protein-I	[163]
HBV	Ex vivo, human	Tumor and normal liver specimens in persons with T2DM and HBV associated HCC	Prophylactic metformin as prescribed for T2DM	- Inhibitory on HBV-associated tumorigenesis (HCC) by inhibiting tumor cell viability and promoting apoptosis - Lower recurrence rates of HBV-associated HCC	[164]
HIV	In vitro	Latently HIV-infected monocytic (THP-p89 cells) and lymphocytic (J1.1 T cell) model - acute infection models of X4- and R5-tropic viruses on Jurkat and Magi cells	Combined treatment 100 mM metformin with bryostatin	- Cotreatment modulates latent HIV-1 infection by purging latent virus from cellular reservoirs	[165]
Influenza (vaccine)	Ex vivo, human	B cells isolate (T2DM)	Prophylactic 2000 mg /day metformin >3 years	- Reduce B cell-intrinsic inflammation and increase antibody responses	[166]

ARDS Acute respiratory distress syndrome, *LPS* lipopolysaccharide, *i.p.* intraperitoneal, *BAL* bronchoalveolar lavage, *hMDM* human monocyte–derived macrophages, *NET* neutrophil extracellular trap, *PBMC* peripheral blood mononuclear cells

Legionella pneumophila (*L. pneumophila*); *Mycobacterium tuberculosis* (*M. Tuberculosis*); tuberculosis (TB); *Pseudomonas aeruginosa* (*P. aeruginosa*); *Staphylococcus aureus* (*S. aureus*); high fat diet (HFD); *Plasmodium berghei* (*P. berghei*); *Plasmodium yoelii* (*P. yoelii*); hepatitis C virus (HCV); pegylated interferon (PEG-IFN); ribavirin (RBV); hepatitis B virus (HBV); hepatocellular carcinoma (HCC); human immunodeficiency virus (HIV)

T lymphocytes) in older adults with severe COVID-19 cases [71–76]. Importantly, elevated levels of the pro-inflammatory factors are associated severity of COVID-19 and unfavorable outcomes like acute respiratory distress syndrome, multiple-organ failure [73, 76]. Interestingly, the list proinflammatory cytokines implicated in the cytokine storm are nearly identical to those suppressed with metformin administration.

Intriguing epidemiologic evidence indicates a potential benefit of metformin on COVID-19 health outcomes (Table 3). In a retrospective analysis of 283 T2DM patients from Wuhan, China, with confirmed COVID-19, investigators found no difference in the length of stay in hospital, but persons taking metformin had significantly lower in-hospital mortality (3 of 104, 2.9%) than those not taking metformin (22 of 179, 12.3%) [77]. Upon hospital admission, both groups were under strict glucose control and all received antiviral and supportive treatments including antibacterial and anticoagulants, with no significant differences in treatment. This report is further supported by an in-review analysis of de-identified claims data from UnitedHealth of 6256 persons hospitalized with COVID-19 [78]. Women who had at least 90 days of metformin claims in the 12 months before hospitalization were found to have ~21% lower odds of mortality compared with persons with T2DM but without metformin. This association was not observed for men. Though difficult to quantify, metformin's immunoprotective effects in the context of COVID-19 may be masked if fewer persons taking metformin are being admitted to hospital.

Importantly, these findings are relevant only in the context of long-term treatment with metformin prior to infection. Therefore, these epidemiologic studies do not generalize to potential actions of metformin administered in hospitalized patients with active infection as a treatment adjuvant. While metformin use is generally safe, side effects and gastrointestinal discomfort do occur in a proportion of the population, and it is unknown what the additional risk of initiating metformin in hospital for those with comorbid or critical conditions upon admission would be. Basic research coupled with carefully controlled clinical trials would be required prior to translation to the clinic. Moreover, the protective effects of metformin on health outcomes due to acute infectious disease must be replicated and confirmed with rigorous prospective studies with and without overt T2DM. Finally, emerging evidence supports close investigation of

both potential sexual dimorphism and mediating effects of metformin on proinflammatory components of the cytokine storm also require close investigation.

Other respiratory-related infectious disease

Evidence of metformin as a therapeutic strategy in other infectious diseases and respiratory illnesses is emerging from a range of experimental designs, model systems, and pathogens or exposures (Table 2). In a screen of 13 FDA-approved autophagy activators and AMPK modulators, metformin (i) displayed direct antimycobacterial effects; (ii) controlled the growth of drug-resistant bacterial strains, (iii) increased production of mitochondrial reactive oxygen species (mROS), and (iv) facilitated phagosome-lysosome fusion [51, 79]. In mice, use of metformin monotherapy enhanced ameliorated lung pathology and enhanced host immune responses, and in combination therapy, metformin enhanced the specific immune response and the efficacy of conventional antimycobacterial drugs [51]. Collectively, this confers host protection and lessens burden of active infection. For example, retrospective analyses of tuberculosis patients who were on metformin had fewer pulmonary cavities, less advanced disease, and lower mortality rate than those not on metformin [51, 80]. Moreover, in 2416 persons being treated for tuberculosis in Taiwan, coexisting metformin use was significantly associated with decreased mortality during treatment; strikingly, metformin users had similar mortality as patients without T2DM [80].

Laboratory studies using cells or model organisms suggest metformin could be effective against numerous other pathogens, including *Trypanosoma cruzi*, *Trichinella spiralis*, *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) [81, 82]. Of relevance to COVID-19, bacterial infections of the respiratory system by *S. aureus* and *P. aeruginosa* are associated with local (vs. systemic) hyperglycemia, which may be associated with respiratory tract infections in conditions like chronic obstructive pulmonary disease [83]. Interestingly, metformin's effects on airway epithelial tight junctions [84] and bacterial load are independent of effects on blood glucose but use an AMPK- and PKCζ-mediated pathway [84, 85]. Moreover, in a murine *Legionella pneumophila* pneumonia model, metformin treatment improved survival of mice, which was associated with a significant reduction in bacterial number in the lung,

Table 3 Epidemiologic studies of metformin use in infectious disease

Reference	Population	Disease status	Comparator	Follow-up	Outcome	Effect size (95% CI)
COVID-19						
Luo [77]	China $N = 283$	COVID-19 +T2DM	Non-use	Retro	In hospital mortality	OR 0.23
Bramante [78]	USA, women $n = 3302$	COVID-19 +T2DM	Non-use	Retro	In hospital mortality	OR 0.792 (0.640, 0.979)
	US, All $N = 6256$	COVID-19 +T2DM	Non-use	Retro	In hospital mortality	OR 0.904 (0.782, 1.045)
Other respiratory illness						
Degner [80]	Taiwan $N = 2416$	TB +T2DM	Non-use		All-cause mortality	HR 0.56 (0.39–0.82)
Mortensen [87]	USA, VA $N = 7424*$	Pneumonia +T2DM	Non-use	Case-control	In hospital mortality	OR 0.80 (0.72–0.88)
Yen [88]	Taiwan $N = 20,644*$	COPD +T2DM	Non-use	Case-control 5 years	Hospitalized pneumonia	HR 1.17 (1.11–1.23)
Nonrespiratory infectious disease						
Chen [167]	$N = 135$	Chronic HCV +HCC	Non-use	Retro	5-year survival rate	HR 0.24 (0.07–0.80)
Chen [70]	Taiwan $N = 71,824$	HBV	Non-use	9 years	All-cancer	HR 0.82 (0.75–0.90)
Chen [70]	Taiwan $N = 71,824$	HBV	Non-use	9 years	All-cause mortality	HR 0.56 (0.39–0.82)
Nkontchou [168]	France $N = 100$	HCV Cirrhosis	Non-use	5 years	Incident HCC	HR 0.19 (0.05–0.99
Nkontchou [168]	France $N = 100$	HCV Cirrhosis	Non-use	5 years	Liver-related mortality	HR 0.22 (0.05–0.99)
Romero-Gomez [159]	Spain $N = 123$	Chronic HCV	Placebo	72 weeks	SVR	52.5% (v. 42.2% Pl)
Sharifi [161]	Iran $N = 140$	Chronic HCV	Placebo	RCT, 24–48 weeks	SVR	75% (v. 79% Pl)
Yu [160]	China $N = 98$	Chronic HCV	Placebo	72 weeks	SVR	59.2% (v. 38.8% Pl)

COVID-19 Novel coronavirus-19, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *T2DM* type 2 diabetes mellitus, *Retro* retrospective cohort, *PL* placebo, *SVR* sustained viral response, *RCT* randomized clinical trial

*Cases and controls matched

which was mediated by mitochondrial ROS production and AMPK signaling and enhanced macrophage bactericidal activity [86]. Epidemiologic evidence are conflicting (Table 3); some studies show a net benefit of metformin use on outcomes like 30-day mortality in US veterans hospitalized with pneumonia [87], whereas a population-based Taiwanese case-control study failed to demonstrate a preventive effect of metformin on hospitalization for pneumonia in persons with T2DM and COPD [88]. Clinical trials are needed to bridge the promising preclinical evidence and epidemiologic evidence. A planned small randomized crossover placebo-controlled trial will explore effects on airway glucose

and bacterial growth in nondiabetic persons with COPD (NCT03651895); however, trials with follow-up time sufficient to capture a broad range of respiratory-related outcomes may be necessary.

Chronic viral infections

Insights can also be drawn from metformin use in the context of chronic viruses, such as liver targeting hepatitis B virus (HBV) and hepatitis C virus (HCV), and human immunodeficiency virus (HIV). HBV and HCV are among the leading causes of liver disease. In HBV, approximately 90% of infections are acute, whereas

10% progress to chronic infection. Metformin-mediated decreases in hepatitis B surface antigen (HBsAg) levels in culture supernatants and in cell lysates and may work synergistically with other antivirals [89]. This will be tested in a randomized placebo-controlled trial in nondiabetic persons with chronic HBV: an investigative team in China will examine the addition of 24-week metformin to ongoing standard HBV treatment entecavir on cumulative rate of HBsAg loss (NCT04182321). Trials on long-term disease outcomes are have not been conducted, but a population-based cohort study of 71,824 HBV-infected patients in Taiwan showed that metformin use was chemoprotective; patients on metformin had a lower incidence of all-cancer, not just HBV-related liver cancer (adjusted hazard ratios, HR 0.75 (95% CI, 0.67–0.84)) [90]. This is interesting because with chronic viral exposure some of metformin's protective effects may extend beyond the direct long-term health consequences of HBV. HCV, like SARS-CoV 2, is a ribonucleic acid (RNA) virus. HCV poses a serious health threat across all ages, but those over the age of 65 years now account for a large portion of all chronic hepatitis C infections in the USA and have the highest rate of hepatitis C-related deaths [91]. In patients with HCV and T2DM, metformin's effects on sustained viral response (SVR) are mixed (reviewed in [81]). However, overall metformin is associated with benefit on liver injury and reduces the rate of incident hepatocellular carcinoma [92], possibly by activating type I INF signaling against HCV via activation of AMPK.

Metformin is a first-line medication for management of T2DM and insulin resistance in persons living with HIV, but metformin may have a role in HIV pathogenesis as well [93, 94]. Dysregulated metabolism is implicated in the relationship between viral proteins, immune activation, and inflammation in persons infected with HIV, and is hypothesized to dampen the protective innate and adaptive arms of immunity [95]. In persons living with HIV and T2DM in Botswana, metformin use is associated with improved CD4 lymphocyte count recovery [96]. An independent study in China suggests metformin inhibit NF-κB/p65 phosphorylation to suppress CD54 expression on CD4+ T cells, which is associated with disease progression in persons living with HIV [97]. In a recent 24-week pilot study involving 12 virally suppressed HIV-infected individuals without T2DM who were randomized to metformin or observation, metformin reduced CD4 T cell exhaustion as measured by negative immune checkpoint receptors (NCR)

such as programmed cell death protein-1 (PD1) and T cell immunoreceptor with Ig and ITIM domains (TIGIT) [98]. Other trials are planned including a pilot trial in nondiabetic HIV-infected persons to evaluate metformin's effect on various virological assays, inflammation, and disease outcomes (NCT02659306) [99, 100]; follow-up trials will be needed to uncover common mechanisms and best drug combinations [101]. Additionally, an emerging hypothesis posits that metformin may improve gut microbiota composition, which could reducing inflammation and mitigate risk of nonAIDS comorbidities [62, 102, 103].

Vaccine response

In 2014, it was reported that an mTORC1 inhibitor increased antibody titers to flu vaccination in 218 healthy elderly subjects [28]. Whether metformin improves vaccine response in nondiabetic older adults remains an active area of exploration. Recent reports are mixed. For example, a 2017 study measured in vivo and in vitro influenza vaccine responses in newly diagnosed treatment naïve T2DM patients and patients taking metformin for at least 3 years. Ongoing metformin use was associated with recovered B cell function and vaccine response compared with those who were just recently diagnosed with T2DM. Moreover, metformin administered in vitro reduced B cell intrinsic inflammation and increased antibody responses in B cells harvested from the recently diagnosed T2DM patients, resulting in B cell function similar to patients already taking metformin [104]. In contrast, a recent cross-sectional study or 67 older adults showed reduced antibody responses to influenza vaccination quantified by virus neutralizing Ab (VNA) titers and immunoglobulin (Ig) isotypes to H1N1 and H3N2 in the 11 metformin users compared with nonmetformin users [105]. Clinical trials in nondiabetic older adults are needed to resolve these seemingly discrepant results of observational studies in order to evaluate metformin as a potential adjuvant to boost response to vaccine. Two noteworthy studies on metformin and vaccine response in nondiabetic older adults are underway (Table 4). A pilot investigation is ongoing to determine whether metformin can improve the immune response to the pneumococcal conjugate vaccine (PCV-13) in older adults and if this effect is mediated by the gut microbiota (NCT03713801). An independent ongoing pilot trial will evaluate metformin's effect on both cellular and humoral influenza vaccine responses, in

Table 4 Randomized placebo controlled trials of metformin on vaccine response, infectious, or respiratory diseases

Clinical trial	Population	Sample size	Group	Dose (per day)	Duration	Primary (1) secondary (2) endpoints	Status
Airway Glucose in COPD	40–75+ years nondiabetic	39	Placebo, metformin	1000 mg (1 × 1000 mg)	12 weeks	1) Sputum glucose concentration 2) Sputum bacterial load and inflammatory marker, quality of life, pulmonary function	Planned
Chronic HBV	18–55 years HBeAg-positive chronic HBV	60	Entecavir + placebo, metformin	1000 mg (2 × 500 mg XR)	36 weeks	1) Cumulative rate of HBsAg loss	Recruiting
VEME (pilot)	65+ years nondiabetic	26	Placebo, metformin	1500 mg (3 × 500 mg XR)	20 weeks	1) Cell-mediated immune responses to flu vaccine 2) Flu antibody titers, frailty phenotype, and T cell metabolic cellular profiles	Ongoing
Impact of Metformin on Immunity	63–89 years nondiabetic vaccine response impaired	50	Placebo, metformin	1500 mg (3 × 500 mg)	12 weeks	1) Change in antibody responses to PCV13 2) Change in immunophenotypes	Recruiting
Lilac Pilot	Nondiabetic persons living with HIV on ART	22	Metformin + ART	1700 mg (2 × 850 mg)	12 weeks	1) Size of the HIV reservoir 2) Safety, HIV reservoir in colon biopsies, frequency of CD4+ T cells harboring inducible HIV (TILDA), change in immune activation	Unknown

Airway Glucose in COPD: Metformin to reduce airway glucose in COPD patients, NCT03651895

Chronic HBV: Adding Metformin to the Standard Treatment for Patients With HBeAg-Negative Chronic Hepatitis B, NCT04182321, Beijing, China

VEME (pilot): Vaccination Efficacy with Metformin in Older Adults: A Pilot Study, NCT03996538, USA

Impact of Metformin on Immunity, NCT03713801, USA

Lilac Pilot: Metformin Immunotherapy in HIV Infection, NCT02659306, Canada

healthy nondiabetic, nonprediabetic older adults (NCT03996538). While many COVID-19 vaccines are under development, it is important to note that the response to vaccines is also diminished with aging. Thus, these trials will be important to elucidate potential mechanisms to enhance protective responses in older adults if/when a COVID-19 vaccine becomes available.

Geroscience: from acute care to long-term health and prevention

Evaluating intervention effects on comorbidity and resilience to acute challenge is even more critical in the aftermath of COVID-19. The lowered overall resilience of older COVID-19 patients is easily observed in the high incidence of fatalities, hospitalizations, and potential for long-term complications in patients with advanced ages. These outcomes are associated with or exacerbated the prevalence of age-related disease, comorbidity, frailty, and loss of endogenous protective and repair mechanisms.

Most importantly, once the immediate challenge of treating acute cases of COVID-19 have passed, the need to treat these newly acquired or exacerbated long-term chronic age-related diseases in our population will be greater than ever. Efforts to treat the infectious diseases presenting in our hospitals worldwide are essential. At the same time, we must also delve deeper to understand how molecular pathways inextricably linked to biological aging, such as mTOR signaling, cellular senescence, insulin/IGF-1 signaling, and AMPK activation, are linked to immune response. This focus could provide novel host, as opposed to pathogen-directed therapies to respond to the current pandemic crisis, but more importantly aid our ability to help those affected by COVID-19 and its attendant chronic health consequences. Understanding the interaction between aging biology, viral susceptibility and immune response, and progression to comorbid chronic disease, frailty, and disability remains one of the most important biomedical issues worldwide. Geroscience represents a major strategic advancement and new therapeutic approach to considering long-term options for prevention and mitigating the anticipated swell in long-term health complications that will be left in the wake of COVID-19.

Ample evidence links metformin to lower all-cause mortality and reduced rates of multiple disease of aging, even in nondiabetic populations [106] [107] and suggest a broad geroprotective role of metformin. Clinical trials exploring the effects of metformin in nondiabetic older adults on gerocentric outcomes are underway [108] or planned [30, 31]. For example, a placebo-controlled, double-blinded clinical trial of 2-year metformin treatment for the prevention of frailty in 120 older adults with prediabetes is ongoing at University of Texas Health Science Center at San Antonio [108]. The TAME trial will evaluate metformin's effects on incidence of any new age-related disease (cardiovascular disease, mild cognitive impairment/dementia, most cancers, or death) in 3000 older adults without diabetes [30, 31]. Both trials include collection of biospecimens and a variety of assessment measures to support ancillary investigations to complement the primary outcome. For example, in addition to an FDA-facing clinical disease composite, primary outcome TAME will include several indices of health span measures including measures of physical and cognitive function and frailty assessments, assays for key biomarkers, and biobanking of biofluids, cells, and feces for discovery and mechanistic investigations. While neither randomized controlled trial is designed to evaluate efficacy of metformin on COVID-19–related outcomes, they nonetheless represent unique and time-sensitive resources to the scientific community. These trials coincide with the pandemic, which provides an opportunity for long-term follow-up and deep phenotyping of older adults with and without exposure to COVID-19 in the course of the study and may include smaller subgroups who are symptomatic vs. asymptomatic. Fresh collection of peripheral blood mononuclear cells and tissues included in these trials provide an unprecedented opportunity for immunophenotyping; investigator-initiated studies could leverage ongoing trials to examine metformin's effects on immune cell activation and inflammatory cytokine production. Similar to other ongoing trials or large cohort studies [109, 110], TAME will include measures to query history, symptoms, and burden of COVID-19 as exploratory assessments. Moreover, TAME's large sample size could permit exploratory investigations on the effects of metformin on age-related diseases or functional decline in older adults with or without history of COVID-19, though such explorations would not bear the burden of proof necessary for a virus-related FDA indication. Other ancillary investigations could be envisioned to explore metformin's effects on immune response to vaccines in older adults once a vaccine against SARS-CoV2 is developed. Ultimately, these existing or upcoming clinical trials provides a

scaffolding for future investigations on long-term health effects of COVID-19 and metformin's potential to improve resilience following immune challenge.

Concluding remarks and future

In conclusion, metformin is an attractive tool or probe for clinical trials targeting aging and to improve host immune defense and resilience in COVID-19 and infectious disease. Its immunoprotective effects are hypothesized to (i) lessen severity of unfavorable health outcomes or death in the event of exposure to infectious disease; (ii) delay or prevent long-term chronic diseases or conditions which can stem from acute challenges or viral infections; and (iii) bolster immune response to vaccine. However, this hypothesis has yet to be rigorously tested. It is time for definitive geroscience trials of not only metformin but other promising geroprotective interventions like caloric restriction, mTOR inhibitors, and senolytics. Trials informed by geroscience will offer opportunity to investigate intervention effects on vaccine response and resilience to age-related chronic diseases and geriatric syndromes and provides a unique opportunity to advance study of host-immune defense with implication for recovery from the current pandemic and unforeseen future challenges.

Compliance with ethical standards

Conflict of interest ASK is now a Senior Scientist, Computational Genomics at AbbVie; his recent employment in Abbvie did not influence the literature reviewed. JNJ, SG, JMB, GAK, NB have no conflicts to report.

Code availability Not applicable.

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