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Developing new drugs that activate the protective arm of the renin–angiotensin system as a potential treatment for respiratory failure in COVID-19 patients

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COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has reached pandemic proportions with negative impacts on global health, the world economy and human society. The clinical picture of COVID-19, and the fact that Angiotensin converting enzyme 2 (ACE2) is a receptor of SARS-CoV-2, suggests that SARS-CoV-2 infection induces an imbalance in the renin–angiotensin system (RAS). We review clinical strategies that are attempting to rebalance the RAS in COVID-19 patients by using ACE inhibitors, angiotensin receptor blockers, or agonists of angiotensin-II receptor type 2 or Mas receptor (MasR). We also propose that the new MasR activator BIO101, a pharmaceutical grade formulation of 20-hydroxyecdysone that has anti-inflammatory, anti-fibrotic and cardioprotective properties, could restore RAS balance and improve the health of COVID-19 patients who have severe pneumonia.

Introduction

The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in Wuhan, China in December 2019 [1]. The vast majority (around 80%) of patients infected with SARS-CoV-2 are asymptomatic or display only mild illness. Nevertheless, the approximately 20% of patients who have more severe COVID-19 illness may require hospitalization, sometimes in an intensive care unit (ICU). COVID-19 mortality occurs mainly in elderly patients and/or in patients with underlying comorbidities such as hypertension, cardiovascular diseases or diabetes at an estimated rate of between 26% and 62% [2]. Severe COVID-19 illness and fatal outcomes are associated with acute respiratory disease syndrome (ARDS), myocardial injury, cardiac dysfunction, arrhythmias and renal alterations [3]. Excessive expression of inflammatory cytokines and mediators (cytokine storm) also contribute to lung dysfunction and shock in COVID-19 patients [4]. As SARS-CoV-2 is transmitted between humans aerially and because only a limited fraction of the world population has been infected to date, the numbers of COVID-19-positive cases and associated deaths are expected to increase in the months and even years to come. Unfortunately, despite intensive research efforts, we are still lacking effective treatment modalities that can substantially reduce mortality in patients suffering from severe forms of COVID-19. Therapeutic alternatives that can be used to treat this devastating disease are thus urgently required. Here, we review clinical attempts to restore the balance of the renin-angiotensin system (RAS), which is altered following SARS-CoV-2 infection.

SARS-CoV-2 and the renin–angiotensin system

SARS-CoV-2 infects human cells through the cellular receptor angiotensin-converting enzyme 2 (ACE2), a key element of the RAS [4,5]. ACE2 is expressed to varying degrees in nearly all human organs, but the preeminent infection of the lungs by SARS-CoV2 is closely related to the propagation of the virus via aerosols and to the high levels of ACE2 expression in airway epithelial cells, endothelial cells and alveolar epithelial type II cells [4,6]. More-

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over, ACE2 expression in the brain, gut, heart, or kidney can also explain both the broad tissue tropism of SARS-CoV-2 and the variety of clinical manifestations observed in COVID-19 patients [7].

Angiotensin-I (Ang-I) is converted into Angiotensin-II (Ang-II) by ACE. The ACE/Ang-II/Ang-II receptor type 1 (AT1R) axis is usually referred to as the 'harmful' or classical arm of the RAS. Ang-II binds a second receptor (AT2R), the effects of which mainly oppose those of AT1R. AT2R is part of the 'protective' arm of the RAS and can also be activated by Angiotensin-(1–9) and Angiotensin-(1–7), which are formed by ACE2 from Ang-I and Ang-II, respectively. Although AT2R has been demonstrated to be upregulated under pathological conditions and to counteract the effects of AT1R (thereby protecting tissues against inflammation, apoptosis and oxidative stress) [8], its expression declines after birth and it is present at much lower expression levels than AT1R in adult tissues. Thus AT1R rather than AT2R is predominantly activated by Ang-II. Fortunately, the 'protective' arm of RAS also involves activation of the highly expressed Mas receptor (MasR) by Ang-(1–7). Ang-(1–7) is able to counteract the effects of Ang-II and shows anti-inflammatory, anti-oxidative and vasodilatory properties [9]. The ACE2/Ang-(1–7)/MasR axis is thus the major 'protective' arm of the RAS (Fig. 1) [6].

SARS-CoV-2 infection, by downregulating ACE2 expression and activity [10], reduces the conversion of Ang-II to Ang-(1–7), resulting in significantly higher levels of Ang-II in COVID-19 patients [11,12]. Importantly, these excessive levels of Ang-II are linearly associated with SARS-Cov-2 viral load and severity of lung injury during COVID-19 [13,14]. In addition, the plasma levels of Ang-(1–7) and potentially those of Ang-1–9 [15,16] are significantly lower in COVID-19 patients than in healthy controls, and these levels are particularly low in COVID-19 patients who are admitted to ICUs. As a consequence, a general imbalance between the 'harmful' and 'protective' arms of the RAS, resulting from excessive activation of AT1R and limited activation of AT2R and MasR, has been proposed, and this hypothesis is supported by the clinical picture reported in COVID-19 patients [12]. Therefore, it has been suggested that restoration of the balance of the RAS could be a particularly relevant way to treat patients who are infected with SARS-CoV-2.

Rebalancing the RAS using ACE inhibitors and/or angiotensin receptor blockers in COVID-19 patients

The initial proposal to restore an adequate balance of the RAS involves inhibiting its 'harmful' ACE/Ang-II/AT1R arm, either with angiotensin converting enzyme inhibitors (ACEIs) that limit the formation of Ang-II or by using angiotensin receptor blockers

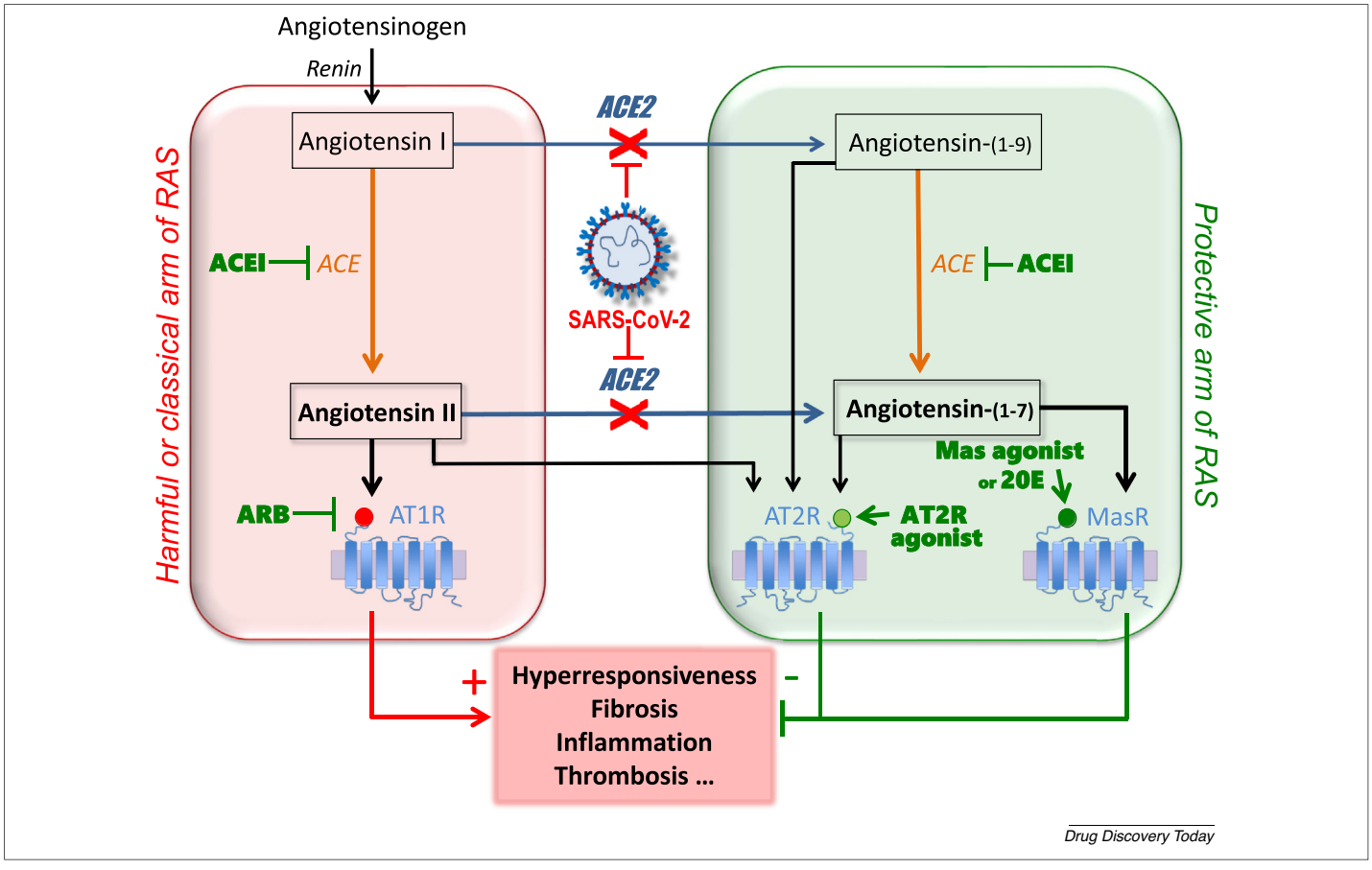

FIGURE 1
Therapeutic strategies targeting the 'harmful' or classical (red box) and 'protective' (green box) arms of the renin–angiotensin system (RAS), and the potential beneficial role of 20-hydroxyecdysone in addressing lung injury in patients with COVID-19. 20E, 20-hydroxyecdysone; ACE, angiotensin converting enzyme 1; ACE2, angiotensin converting enzyme 2; AT1R, angiotensin-II receptor type 1; AT2R, angiotensin II receptor type 2; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor AT1R blocker; MasR, Mas receptor; RAS, renin–angiotensin system; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(ARBs) to inhibit AT1R activation (Fig. 1). Notably, one of the most preeminent COVID-19-associated comorbidities is hypertension, meaning that many SARS-CoV2-infected patients are already taking ACEI or ARB medication.

The risks of using ACEIs and ARBs in the context of Sars-CoV2 infection have been much debated. Indeed, it was feared that ACEI and ARBs might increase the expression level of ACE2 and consequently facilitate the infection of hosts cells by SARS-CoV-2 [3,17]. However, a large-scale study of *ACE2* gene expression in the lungs of ACEIs and ARBs users has been performed and did not support this hypothesis [18]. Indeed, it was shown that ACEI use was associated with significantly lower *ACE2* expression, whereas ARBs did not affect *ACE2* expression. Interestingly, the use of ARBs was associated with increased expression of *ACE* and decreased expression of *AGTR1* (the gene coding for AT1R) [18]. Jointly, the Heart Failure Society of America, the American College of Cardiology, and the American Heart Association recommend continuation of RAS antagonists for patients infected with SARS-CoV-2 and suffering of heart failure, hypertension, or ischemic heart disease, for which these agents are the standard care [19]. This confirms the safety of using ACEIs and ARBs such as losartan as an approach to treat COVID-19 patients through inhibition of the harmful arm of the RAS.

As previously described in SARS-CoV infection, ACEI and ARBs can control the stimulation of the ACE/Ang-II/AT1R axis, thereby

TABLE 1

Ongoing clinical trials using angiotensin receptor AT1R blockers (ARBs) to inhibit the ACE/Ang-II/ATR1 axis of the renin–angiotensin system (RAS).

Study title	Phase	Interventions	Sponsor	ClinicalTrials.gov identifier
Inhibition of the harmful arm of the RAS with ARBs				
Study of Open Label Losartan in COVID-19	I	Losartan	University of Kansas Medical Center, USA	NCT04335123
Chloroquine + Losartan Compared to Chloroquine Alone for the Treatment of COVID-19 Pneumonia	II	Chloroquine phosphate plus losartan vs. chloroquine phosphate	Hospital Universitario José E. Gonzalez Monterrey, Nuevo Leon, Mexico	NCT04428268
Early Treatment with Ivermectin and LosarTAN for Cancer Patients With COVID-19 Infection (TITAN)	II	Ivermectin plus losartan vs. placebo	Instituto do Cancer do Estado de Sao Paulo Sao Paulo, Brazil	NCT04447235
Telmisartan for Treatment of COVID-19 Patients	II	Standard care plus telmisartan vs. standard care	Laboratorio Elea Phoenix S.A., Argentina	NCT04355936
Losartan for Patients With COVID-19 Requiring Hospitalization	II	Losartan vs. placebo	University of Minnesota, USA	NCT04312009
Losartan for Patients With COVID-19 Not Requiring Hospitalization	II	Losartan vs. placebo	University of Minnesota, USA	NCT04311177
Pilot Clinical Trial of the Safety and Efficacy of Telmisartan for the Mitigation of Pulmonary and Cardiac Complications in COVID-19 Patients	II	Telmisartan vs. placebo	University of Hawaii, USA	NCT04360551
COVID MED Trial – Comparison of Therapeutics for Hospitalized Patients Infected With SARS-CoV-2 (COVIDMED)	II/III	Losartan vs. lopinavir/ritonavir vs. hydroxychloroquine sulfate vs. placebos	Bassett Healthcare, USA	NCT04328012
Coronavirus Response – Active Support for Hospitalised Covid-19 Patients (CRASH-19)	III	Standard of care vs. aspirin vs. losartan vs. simvastatin	London School of Hygiene and Tropical Medicine, UK	NCT04343001
Efficacy of Hydroxychloroquine, Telmisartan and Azithromycin on the Survival of Hospitalized Elderly Patients With COVID-19 (COVID-Aging)	III	Standard of care vs. hydroxychloroquine vs. azithromycin vs. telmisartan	University Hospital, Strasbourg, France	NCT04359953
Treatments to Decrease the Risk of Hospitalization or Death in Elderly Outpatients with Symptomatic SARS-CoV-2 Infection (COVID-19) (COVERAGE)	III	Dietary supplement: vitamins vs. imatinib vs. telmisartan	University Hospital, Bordeaux, University of Bordeaux, France	NCT04356495
Do Angiotensin Receptor Blockers Mitigate Progression to Acute Respiratory Distress Syndrome With SARS-CoV-2 Infection	IV	Losartan	Sharp HealthCare, La Mesa, California, USA	NCT04340557
Valsartan for Prevention of Acute Respiratory Distress Syndrome in Hospitalized Patients With SARS-COV-2 (COVID-19) Infection Disease	IV	Valsartan vs. placebo	Radboud University, The Netherlands	NCT04335786
Controlled evaLuation of Angiotensin Receptor Blockers for COVID-19 resplraTorY Disease (CLARITY)	IV	Standard of care plus angiotensin receptor blockers vs. standard of care	The George Institute, Australia	NCT04394117

TABLE 2

Ongoing clinical trials using angiotensin-converting enzyme inhibitors (ACEIs) to inhibit the ACE/Ang-II/ATR1 axis of the renin–angiotensin system (RAS).

Study title	Phase	Interventions	Sponsor	ClinicalTrials.gov Identifier
Inhibition of the harmful arm of the RAS with ACEI				
Ramipril for the Treatment of COVID-19 (RAMIC)	II	Ramipril vs. placebo	University of California, San Diego, USA	NCT04366050
Efficacy of Captopril in Covid-19 Patients with Severe Acute Respiratory Syndrome (SARS) CoV-2 Pneumonia (CAPTOCOVID)	II	Captopril vs. standard of care	Assistance Publique – Hôpitaux de Paris, France	NCT04355429
Angiotensin Converting Enzyme Inhibitors in Treatment of COVID 19	III	Captopril or enalapril vs. choloroquine	Tanta University, Egypt	NCT04345406

reducing the production of pro-inflammatory cytokines including IL-1, IL-6 and TNF-α, and can modulate the activation of innate and adaptive immunity [20]. In addition, ACEI and ARBs, by reducing the formation of Ang-II, are expected to inhibit the vasoconstrictive and prothrombotic actions of Ang-II. Numerous clinical trials to test the efficacy of maintaining versus stopping ACEI and ARB treatments in COVID-19 patients who have hypertension are actively recruiting [21]. Lists of clinical trials using ARBs and ACEIs to inhibit the 'harmful' arm of the RAS are presented in Tables 1 and 2, respectively. Interestingly, it has been reported that the antithrombotic effects of the ACEI captopril and the ARB losartan were indirectly mediated by Ang-(1–7), and were abolished by the MasR antagonist A-779 [22]. The stimulated formation of Ang-(1–7), a protective anti-inflammatory metabolite, in response to ACEIs and ARBs is mediated by ACE2. This suggests an indirect beneficial effect of ACEI and ARBs by activating the 'protective' arm of the RAS [3], which could be exploited for the treatment of cardio-respiratory failure in COVID-19 patients.

Potential beneficial effects of restoring RAS balance through AT2R and MasR activation in COVID-19

Stimulating the protective axis of the RAS instead of blocking the harmful arm with ACEIs or ARBs is an alternative possibility to restore RAS equilibrium. The RAS 'protective' arm has a pleiotropic effect and controls many biological functions. Thus, activating this axis seems to be a particularly interesting alternative to attempts to activate more specific molecules that act on only one clinical feature of the disease, for instance anti-inflammatory drugs such as tocilizumab specifically targeting IL-6R to prevent the cytokine storm observed in severely ill COVID-19 patients.

In order to restore the balance of the RAS, the use of AT2R agonists to stimulate the protective arm can be proposed. However, as AT2R is weakly expressed in adults and even more so in elderly people, its activation by agonists might have a limited effect in restoring RAS balance. Nevertheless, both Ang-(1–9) and Ang-(1–7) stimulate AT2R formation, and elevated plasma levels of these two peptides have been found in survivors of ARDS [23], suggesting that they could indeed provide potential beneficial effects in COVID-19 patients. Supporting this idea, Ang-(1–9) ameliorates pulmonary arterial hypertension via AT2R [24]. However, it has been shown that some Ang-(1–9) can also be converted to Ang-II and can enhance arterial thrombosis in rats [25], which could be deleterious during COVID-19. This ambivalent activity may explain why no clinical attempt is underway to treat COVID-19 through administration of Ang-(1–9) itself. By contrast, a synthetic cyclized AT2R agonist demonstrated anti-inflammatory effects and anti-hypertrophic effects in a chronic lung injury animal model [26]. In addition, C21 (a non-peptide synthetic agonist) [27], showed beneficial effects in animal models of pulmonary hypertension [28,29]. A Phase 2 clinical trial evaluating the efficacy and safety of C21 in COVID-19 patients (ClinicalTrials #NCT04452435) has been completed, but the results have not yet been reported [30] (Table 3).

More than 35 years ago, it was shown that Ang-(1–7) is a key component of the RAS and not merely a degradation product of Ang-I and Ang-II [31]. Ang-(1–7) is part of the protective arm and counteracts the harmful arm of the RAS by binding to AT2R and MasR. As AT2R expression is limited, activation of the MasR by Ang-(1–7) may predominantly explain the effects of Ang-(1–7) in rebalancing the RAS. Accordingly, it has been proposed that Ang-(1–7), and MasR agonists more generally, could be used as a treatment for patients with severe COVID-19 illness [9]. In support of this hypothesis, many *in vitro* and *in vivo* studies have demonstrated that MasR is expressed in the epithelium and smooth muscles of the airways, in alveolar cells, and in the endothelium and smooth muscle cells of vascular tissues [32]. Thus stimulation of the ACE2/Ang-(1–7)/MasR axis has the potential to produce beneficial effects on respiratory functions, arterial oxygenation, and lung tissue [32]. This is notably the case in the context of pulmonary emphysema [33], lung fibrosis [34], pulmonary hypertension [35], lung inflammation [36], chronic asthma [32] and cigarette smoking [37]. Indeed, it has been shown that MasR activation by Ang-(1–7) plays an important role in opposing the pro-thrombotic and pro-inflammatory effects of Ang-II [38], by reducing the synthesis of pro-inflammatory cytokines and by inhibiting the migration of inflammatory cells to the lungs, leading to a better respiratory function [39]. Interestingly, in a murine model of ARDS, Ang-(1–7) has also been shown to reduce collagen deposition in the lungs [39]. In addition, the Ang-(1–7)/MasR axis inhibits pulmonary fibrosis [40] and induces apoptosis of neutrophils [41]. Moreover, the ACE2/Ang-(1–7)/Mas axis protects against thrombosis [42], as demonstrated in animals in which MasR had been knocked out, which show a shorter bleeding time and increased size of thrombi [43].

A potential additional beneficial effect of Ang-(1–7) relates to the diaphragm dysfunction observed in some COVID-19 patients.

TABLE 3

Ongoing clinical trials using stimulation of the AT2R or ACE2/Ang-1–7/MasR axis of the renin–angiotensin system (RAS).

Study title	Phase	Interventions	Sponsor	ClinicalTrials.gov Identifier
Stimulation of the protective arm of the RAS with an angiotensin II receptor type 2 (AT2R) agonist				
Safety and Efficacy of C21 in Subjects With COVID-19	II	C21 vs. placebo	Vicore Pharma AB, Sweden	NCT04452435
Stimulation of the protective arm of the RAS with MasR agonists and activators				
Treatment of Angiotensin Peptide (1–7) for COVID-19	I	Standard of care plus Angiotensin peptide (1–7)-derived plasma vs. standard of care	Kanuni Sultan Suleyman Training and Research Hospital, Turkey	NCT04375124
TXA COVID-19 Clinical Trial	II	TXA127 vs. placebo	Columbia University, USA	NCT04401423
Angiotensin-(1,7) Treatment in COVID-19: the ATCO Trial	II/III	Angiotensin 1–7 vs. placebo	Erasme University Hospital Brussels, Belgium	NCT04332666
Testing the Efficacy and Safety of BIO101 for the Prevention of Respiratory Deterioration in COVID-19 Patients (COVA)	II/III	BIO101 vs. placebo	Biophytis, France	NCT04472728

Indeed, mechanical ventilation, the main supportive therapy for ARDS patients, induces ventilator-induced diaphragmatic dysfunction (VIDD) [44], which is characterized by a contractile dysfunction and a rapid muscular atrophy [45]. Muscular disorders may result from a RAS imbalance [46]. It has been shown that Ang-II induces diaphragm muscle wasting and respiratory muscle dysfunction [47], whereas Ang-(1–7) exerts a protective action in a rat model of VIDD [48] and could improve muscular functions in patients infected by SARS-CoV-2 [46].

Unfortunately, Ang-(1–7) has a very short half-life (less than one minute in human plasma) [49] and some studies point out a lack of specificity. Hence, there is a need to develop improved formulations or modified versions of Ang-(1–7) that have similar biological effects but a longer half-life than the endogenous peptide. For instance, hydroxypropyl-β-cyclodextrin–Ang-(1–7) complexes act as a long-lasting release system in which Ang-(1–7) is protected from inactivation by digestive tract enzymes, thus allowing oral administration [50]. This complex has shown many of the beneficial cardiovascular and metabolic effects of Ang-(1–7) in orally treated spontaneously hypertensive rats [43], as well as improvement of muscular function in a mouse model of hindlimb immobilization and Duchenne muscular dystrophy [51]. Similarly, intravenous infusion of cyclic Ang-(1–7), a form that is more resistant than Ang-(1–7) to enzymatic hydrolysis, has demonstrated long-term vasorelaxant effects in a rat model of myocardial infarction [52]. Moreover, continuous Ang-(1–7) infusion for 2 days resulted in improvement of endothelial cell functions in preeclamptic patients [53]. Other MasR agonists have similar pleiotropic protective effects. The two peptides CGEN-856 and CGEN-857 have been reported to have *ex vivo* vasorelaxant properties in mouse aortic rings or antihypertensive and cardioprotective effects in spontaneously hypertensive rats [54]. In addition, the orally active non-peptidic Mas receptor agonist AVE0991 was able to mimic the effects of Ang 1–7 on several organs, such as blood vessels [55], kidneys [56], and heart [57].

Interestingly, it has been reported that Mas stimulation in different animal models can increase AT2R expression, and that this relation is reciprocal. Co-administration of a MasR agonist and an AT2R agonist could be an approach to potentiate the beneficial effects of the protective RAS axis.

Overall, the possibility of using AT2R agonists, Ang-(1–7) or other Mas agonists could provide a valuable therapeutic strategy in COVID-19, and a few clinical trials to test this hypothesis with drug candidates are ongoing (Table 3).

20-Hydroxyecdysone (20E), a new MasR activator with potentially beneficial effects against COVID-19

20-hydroxyecdysone (20E) has been described recently as a new MasR activator, thanks to pharmacological and gene interference approaches [58]. Phytoecdysteroids are polyhydroxylated steroids analogous to arthropod molting hormones that are widely distributed in plants throughout the world and are present in significant amounts (up to >2% of dry weight) in 5–6% of randomly investigated plant species [59]. 20-hydroxyecdysone is the most frequently encountered and abundant representative. Ecdysteroids are present in numerous medicinal plants and have been found to have beneficial effects in mammals [59], opening new therapeutic opportunities in the context of the COVID-19 pandemic.

Of particular interest, 20E has shown anti-inflammatory effects *in vivo* in a mouse model of acute lung injury (ALI) [60]. Indeed, 20E treatment reduced the expression of inflammatory cytokines (TNF-α, IL-2, IL-6, IL-8) and increased the expression of anti-inflammatory cytokines (IL-4, IL-10) [60]. Modulation of inflammation by 20E was associated with a decrease in lung damage, as shown by histological examination of animal lungs. Similarly, two other studies in rat models of ALI demonstrated that 20E treatment protected the animals by increasing both the serum level of an anti-inflammatory cytokine (IL-10) and the expression of IL-10 mRNA in the lung tissue [61]. Moreover, Li and collaborators [62] demonstrated that 20E inhibited the TLR4 pathway, leading to the

promotion of surfactant protein A release by pneumocytes that finally improves respiratory functions.

Interstitial fibrosis of lung tissue is one of the hallmarks of COVID-19. Fibrosis in lung tissue is associated with organizing pneumonia, which eventually evolves into widespread fibrotic change leading to severe ALI. This progression has been shown to be linked with the development of ARDS [63]. Interestingly, 20E has demonstrated significant anti-fibrotic activity in heart and kidney by reversing the downstream effects of TGF-β1 on the activity of connective tissue growth factor (CTGF) [64], a profibrotic factor. In an animal model of Duchenne muscular dystrophy, we consistently observed that myocardial expression of the *CTGF* gene was reduced by 20E [65]. Thus, the antifibrotic properties of 20E could also be beneficial in COVID-19 patients.

In addition, thrombosis, resulting from coagulation abnormalities (coagulopathy) has been associated with the severe hypoxia observed in COVID-19 patients [66]. Accordingly, the administration of anticoagulants was proposed for COVID-19 patients with a high thrombotic risk [67]. MasR activation opposes the pro-thrombotic effects of Ang-II [43]. Moreover, MasR stimulation has been shown to maintain a healthy vascular homeostasis [42]. Thus, by activating MasR, 20E is expected to display similar anti-thrombotic effects. Accordingly, 20E has been shown to reduce hyper-coagulation induced by intensive physical exercise [68]. This effect of 20E deserves further confirmation in models closely related to COVID-19 pathology but holds additional promise for the amelioration of patients who are infected with SARS-CoV-2.

Clinical development of BIO101, a new drug candidate based on a pharmaceutical grade formulation of 20E to improve respiratory function in COVID-19 patients

Biophytis has developed BIO101, a pharmaceutical grade oral preparation of immediate-release 20E at ≥97% purity extracted from *Cyanotis* sp. plants. In the light of numerous studies demonstrating the beneficial effects of 20E on muscular function [69], BIO101 is currently being assayed for treating sarcopenia in a phase 2 clinical trial. (This is a double-blind, placebo controlled, randomized interventional clinical trial (SARA-INT), ClinicalTrials #NCT03452488.) We believe that BIO101 administration could also be helpful in the prevention of the muscle disuse atrophy, severe muscle weakness, deconditioning, fatigue, and impaired motility observed in COVID-19 patients who have been immobilized in the prone position during long-term hospitalization in ICUs [70]. BIO101 treatment could also be beneficial during a patient's rehabilitation.

In addition, by using nebulization of the powerful bronchoconstrictor methacholine in mice, we demonstrated that chronic oral administration of BIO101 prevents the decline of respiratory function in *mdx* mice, a murine model of Duchenne muscular dystrophy [71]. BIO101 improved breathing parameters (inspiratory and expiratory time and frequency) in the mice, as demonstrated by enhanced pause (Penh) measurements [71]. Interestingly, BIO101 treatment also improved the deep airway structure and the mechanical properties of the lungs (resistance, compliance and elastance) in these mice [71]. Together, these observations strongly suggest that BIO101 could have a protective effect against the ARDS observed in humans suffering from severe forms of COVID-19.

There is, as yet, no direct evidence for beneficial effects of BIO101 in a preclinical animal model of Sars-CoV-2 infection. However, the potential anti-inflammatory, antifibrotic and antithrombotic effects of BIO101 administration, achieved by activating the 'protective' arm of RAS via MasR, allow us to believe that BIO101 could be beneficial in patients with mild and severe forms of COVID-19 (Fig. 1). BIO101 might also benefit patients who are being treated in the ICU by preserving muscle function. A Phase 2/3 clinical trial (COVA, ClinicalTrials #NCT04472728) is ongoing in the US, France, Belgium, the UK and Brazil to test the efficacy of BIO101 in COVID-19 patients who do not require mechanical ventilation.

Conclusions

Because most of the deleterious effects of SARS-CoV-2, including inflammation, fibrosis, thrombosis and pulmonary damage, point towards an imbalance in the RAS, we strongly believe that inhibiting the 'harmful' arm of the RAS (with ARBs or ACEIs) and/or activating the 'protective' arm (through activation of AT2R or MasR downstream of ACE2) could have a beneficial effect in COVID-19-infected patients and could improve their ARDS outcome. Among these strategies, BIO101 could offer a new therapeutic option in COVID-19 patients by improving their respiratory and muscular functions, and might ultimately promote the survival of patients who are at high risk of developing very severe forms of this devastating disease.

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Conflicts of interest

ML, SC, RL, SV, and PD are employees of Biophytis. They declare, however, that their potential commercial interests had no impact on the rational scientific conduct of this review.

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