Assessing the potential of *Artemisia annua* in the fight against COVID-19

Scientific justification for research

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Abstract

During the course of infection with SARS-CoV-2, which is a highly contagious respiratory disease, 14% of patients will develop severe illness requiring hospitalization and oxygenation and 5% will be transferred to intensive care. The mortality rate varies from country to country and is related to the availability of screening and medical facilities in the country. There is an urgent need for treatments that could be effective from the onset of symptoms in order to avoid transferring patients to intensive care where the mortality rate is very high. SARS-CoV-2 belongs to the betacoronavirus family. The virus enters the body's host cells where the two receptors ACE2 (angiotensin-converting enzyme 2) and serine protease (TMPRSS2) are found together.

After initial viral penetration, via TMPRSS2 which triggers activation of the ACE2 receptor, the virus enters the cell and then, during its maturation and intracytoplasmic penetration, it expresses CyPA (Cyclophillin A). This triggers the extra-membrane ex-

pression of basigin or CD147 or EMMPRIN and its intracytoplasmic activation. CD147 acts intra- and extra-membrane with many proteins. A third molecule, serine/ threonine kinase (PAK1), whose expression is proportional to that of the activation of the ACE2 receptor, participates in the viral pathological process.

The activation of these molecules, which act outside and inside the cell, is at the origin of a cytokine storm and amplification of the body's inflammatory reaction via intense chemotactic activity leading to the activity and circulation of leukocytes and macrophages and finally an induction of metalloproteases (MMP) whose inhibitors are submerged in this pathological state. In addition, there is a sideration of the lymphocyte function.

All clinical signs resulting from this uncontrolled post-invasive inflammatory reaction and activation of metalloproteases appear in the patient after a few days and are responsible for acute respiratory distress and its consequences. At the same time, with the receptors activated, viral replication continues.

Artemisia annua has known antiviral action against human cytomegalovirus, herpes simplex virus type 1, Epstein Barr virus, hepatitis C virus, dengue fever virus, and some strains of HIV-1. Furthermore, it was successfully tested during the SARS-CoV epidemic in 2003 in patients receiving traditional Chinese medicine as a complement to conventional medicine.

In this context, compounds of *Artemisia annua* are likely to induce a decrease in the expression of the ACE2 and TMPRSS2 proteins via alteration of the androgen pathway as well as the CD147 protein. The biomolecules of *Artemisia annua* are also inhibitors of metalloproteases and PAK1. By targeting these 5 membrane or intracytoplasmic proteins that are key to the entry and invasion of SARS-CoV-2 into host cells, *Artemisia annua* may slow the development of infection in the human body and thus slow the worsening of symptoms of COVID-19. We propose to test the efficacy and safety of *Artemisia annua* decoction administered for 14 days to patients infected with mild to moderate forms of SARS-CoV-2 in a phase II randomized controlled trial (standard of care) to be conducted in an open-label setting.

Due to cultural habits, African populations often resort to traditional plant-based pharmacopoeia. This appears to be an essential and pertinent therapeutic approach as and when conditions for monitoring practices validate its efficacy and safety. Validation of the anti-viral, immunomodulating and immunostimulating potential of *Artemisia annua* in the specific case of COVID-19 would make it possible to propose a standardized "improved traditional medicine" in Africa, whose raw material can be cultivated locally and made available at a lower cost, thanks to its capacity to stop the pathological cycle of SARS-CoV-2.

Scientific justification for research

Introduction

Artemisia annua is a medicinal plant that has been included in the Chinese pharmacopoeia for over 200 years. This antiviral medicinal plant has been used extensively in China as treatment for SARSCoV in 2003 and for COVID-19 today. A review of the body of research suggests that *Artemisia annua* is a good candidate to counter the pathological effects of COVID-19. Nevertheless, clinical studies must first be conducted to confirm the virtues of such treatment based on *Artemisia annua*.

Research Hypotheses

The world is currently facing a pandemic of COVID-19, a recently discovered highly contagious respiratory disease (Zhu N, 2020) called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (Gorbalenya AE, 2020). Although the majority of people infected with SARS-CoV-2 will remain asymptomatic or only mildly ill, 14% of patients will develop severe illness requiring hospitalization and oxygen support, and 5% will be transferred to an intensive care unit. With the number of deaths rising inexorably, there is **urgent need for new treatments that can be used quickly to avoid the transfer of patients to intensive care and their death**.

The entry of SARS-CoV-2 into host cells is dependent on the expression of two proteins on the surface of host cells, angiotensin-converting enzyme 2 (ACE2), a key molecule of the renin-angiotensin-aldosterone system (RAAS) (Hoffmann M, 2020; Zhou P, 2020) and transmembrane protease serine 2 (TMPRSS2) (Glowacka, 2011; Matsuyama, 2010; Shulla, 2011). The expression of these two proteins is androgen-regulated and it is possible that this difference in regulation contributes to the difference in mortality rates observed between men and women with COVID-19. **The action of androgens on the expression of both ACE2 and TMPRSS2 may be an effective therapeutic strategy in COVID-19 patients.**

Artemisia annua is a non-toxic plant that has long been used for the treatment of fevers and malaria due to its anti-parasitic and antiviral properties. **Two of the main compounds of *Artemisia annua* inhibit the androgen pathway** and are therefore likely to induce a decrease in the expression of ACE2 and TMPRSS2 proteins via alteration of the androgen pathway and thus slow the entry of viruses, including SARS-CoV-2, into human host cells.

We hypothesize that the administration of *Artemisia annua* to patients with mild to moderate forms of COVID-19 can slow down SARS-CoV-2 infection, reduce the rate of patients transferred to intensive care units for mechanical ventilation and ultimately contribute to a decrease in mortality related to this infection.

Description of knowledge relating to the pathology concerned

▪ Epidemiology

In China, the average age of patients reported to be infected with SARS-CoV-2 is 47 years, with 58.1% of cases being male (Guan WJ, 2020). Observations to date suggest a mean incubation period of 5 days (Lauer SA, 2020). Persons infected with SARS-CoV-2 may remain asymptomatic throughout the course of infection. However, the risk of symptomatic infection increases with age and comorbidities such as hypertension, diabetes, cardiovascular disease, chronic respiratory disease and cancer (Wu JT, 2020; Huang C, 2020; Zhou F, 2020; Liang W, 2020; Chen N, 2020). A recent estimate of the mortality rate from the disease was 5.6% (95% CI 5-4-5.8) for China (with a rate as high as 20% in Wuhan, the epicentre of the epidemic) and 15.2% (12 - 5-17 - 9) outside China (Mizumoto K, 2020; Baud D, 2020). The time from symptom onset to death ranges from 2 to 8 weeks (WHO China, 2020).

▪ Clinical signs and progression

In symptomatic patients, the clinical manifestations of COVID-19 are fever, cough, nasal congestion, headache, fatigue and other signs of upper respiratory tract infections. Nausea, vomiting and diarrhoea are less frequently observed (Guan WJ, 2020). Other features of the disease are lymphopenia and abnormal chest images with ground glass opacities (Zhang X, 2020; Li Y, 2020; Xia W, 2020). The infection may progress to severe illness with acute respiratory distress syndrome and thoracic symptoms consistent with pneumonia, which requires mechanical ventilation and management in an intensive care unit (ICU). Severe renal failure is also observed, including severe nephrotic syndrome. Eventually death occurs due to massive alveolar damage and progressive respiratory failure, septic shock and/or multi-organ dysfunction or failure (Wu Z, 2020). A strong correlation of serum viral RNA with disease severity has been reported (Chen W, 2020; Liu Z, 2020).

▪ Pathogenesis

SARS-CoV-2 belongs to the betacoronavirus subfamily of the Coronaviridae family, which can infect humans and cause severe respiratory illness and death, unlike other subfamilies (Chan JF, 2013). It is closely related to the SARS-CoV virus with an 82% nucleotide identity (Chan JF, 2020). Like other coronaviruses, SARS-CoV-2 is an enveloped, positive, large single-stranded RNA (+ ssRNA) virus with a genome size of approximately 30 kb (Lu R, 2020; Zhou P, 2020). The genome encodes four major structural proteins, including the spike (S), nucleocapsid (N), membrane (M) and envelope (E) necessary to make a complete viral particle. Entry of coronaviruses into cells depends on the binding of viral spike (S) proteins to cellular receptors, facilitating viral attachment to the host cell surface (Li W, 2003). Like SARS-CoV, SARS-CoV-2 binds to the angiotensin-converting enzyme 2 receptor (ACE2), a key molecule of the renin-angiotensin-aldosterone system (RAAS), to enter the host cell (Hoffmann M, 2020; Zhou P, 2020). Given that ACE2 is not only expressed in the lungs, SARS-CoV-2 can spread to other tissues expressing this receptor (Hamming, 2004; Shieh, 2005). Binding of SARS-CoV-2 is followed by ACE2 degradation, inducing an imbalance of RAAS mediated by the angiotensin-converting enzyme ACE. This imbalance has a hypertensive and hypokalemic effect that may contribute to the severe cardiac and pulmonary disorders observed in patients with severe forms of COVID-19 (Kuba, 2005: Ingraham, 2020).

This binding to ACE2 is followed by cleavage of the virus spike proteins by host cell proteases and, in particular, transmembrane protease serine 2 (TMPRSS2) (Glowacka, 2011; Matsuyama, 2010; Shulla, 2011). This cleavage results in fusion of the viral membrane with the host cell membrane, allowing the transmission of coronavirus genetic information into the cytoplasm of the host cell. Protein cleavage by TMPRSS2 has been shown to be critical for the entry into host cells and spread of various viruses such as SARS and Middle East Respiratory Syndrome (MERS) coronavirus, Asian Influenza Virus H7N9 2013, and several influenza viruses of subtype H1N1. TMPRSS2 appears to be one of the keys to the transmissibility of SARS-CoV-2 (Matsuyama S, 2010; Shirato K, 2013; Tarnow C, 2014; Sakai K, 2014; Iwata-Yoshikawa N, 2019; Kawase M, 2012; Zhou Y, 2015). TMPRSS2 is expressed primarily in the prostate but also at lower levels in the lungs, kidneys, colon, liver and pancreas, confirming that the SARS-CoV-2 virus can take advantage of the expression pattern of both ACE2 and TMPRSS2 to infect the lungs and spread to other tissues (Lin B, 1999).

▪ Difference in male/female mortality: gender-dependent regulation of ACE2 and TMPRSS2?

According to the World Health Organization (WHO) COVID-19 weekly report consulted on March 26, 2020, the mortality of male COVID-19 patients is remarkably higher than that of women with 72.8% of deaths (3 times more than women) (WHO, 2020). This observation remains valid for other WHO weekly reports.

ACE2 protein is expressed on the surface of prostate epithelial cells (Song, 2020) and its expression appears to be androgen-regulated (Dalpiaz, 2015). In the myocardium, the expression of ACE and ACE2 is significantly higher in spontaneously hypertensive male mice than in females. After orchiectomy, a significant decrease in ACE and ACE2 expression was observed in these animals, suggesting that androgens stimulate ACE2 expression.

Interestingly, the TMPRSS2 protein is also androgen-dependent.

First, TMPRSS2 is primarily expressed in the prostate, an androgen-regulated organ. Second, several androgen receptors are located upstream of the transcription start site and first intron of the TMPRSS2 gene (Park Y, 2010; Lin B, 1999). Administration of androgens (including testosterone) induces positive regulation of TMPRSS2 gene expression in lung tissue and prostate cancer cells (Lin B, 1999; Mikkonen L, 2010). High levels of TMPRSS2 protein are correlated with prostate cancer progression (Lucas, 2008; Chen YW, 2010) and more recently, TMPRSS2 has been shown to promote the growth, invasion and metastasis of prostate cancer cells (Ko CJ, 2015). Thus, the positive regulation of TMPRSS2 by androgens has a deleterious effect in the body. This may be a contributing factor to the differences between male and female patients infected with SARS-CoV-2.

Countering the action of androgens on ACE2 and TMPRSS2 expression may therefore be an effective therapeutic strategy in COVID-19 patients. Currently, there are no therapies based on this hypothesis being used in COVID-19 patients.

▪ Current COVID-19 treatments

There is currently no vaccine to prevent COVID-19 or SARS-CoV-2 infection, nor are there therapeutic agents to treat COVID-19. Current therapeutic strategies are only symptomatic or compassionate. In France, on 25 March 2020, the Ministry of Health authorized the prescription of hydroxychloroquine, an anti-malarial agent that has been used for decades, for the treatment of COVID-19 patients (JORF, 2020) for its antiretroviral effect (Keyaerts E, 2004; Vincent MJ, 2005; Biot C, 2006; Sperber K, 1995, 1997 Yao X, 2020). Ongoing clinical trials are mainly testing drugs with antivi-

ral or anti-inflammatory properties, such as chloroquine, hydroxychloroquine, azithromycin, lopinavir-ritonavir, favipiravir, remdesivir, ribavirin, interferon, convalescent plasma steroids and IL-6 inhibitors (clinicaltrials.gov). However, these therapies are unlikely to be sufficient to slow COVID-19 pandemics: no treatment currently used in COVID-19 patients targets the actual entry of the virus into the host cell prior to replication.

Although the majority of people infected with SARS-CoV-2 will remain asymptomatic or only mildly ill, 14% will develop severe illness requiring hospitalization and oxygen support, and 5% will be transferred to an intensive care unit (ECDC, 2020). With the number of deaths rising inexorably, there is urgent need for new treatments that can be used quickly to avoid the transfer of patients to intensive care units and their death.

▪ African health strategy for the COVID-19 pandemic

In Africa, as of April 13, there were about 14,000 confirmed cases compared to 160,000 in Italy and more than 560,000 in the United States (Africa Centres for Disease Control and Prevention, 2020; Center for Systems Science and Engineering (CSSE), 2020). Despite the slow arrival of the COVID-19 pandemic, the 1.2 billion people living in Africa are at enormous risk. Prevention strategies are difficult to put in place on the African continent as social distancing cannot be respected in densely populated urban areas and access to clean running water is scarce. Countries have only a few ventilators for millions of people. In urban communities in Africa, health facilities are generally overcrowded and understaffed, while in rural areas, poor roads and unreliable transport make access to care difficult. Advanced health care is sorely lacking in almost all countries. Finally, winter is coming to the southern hemisphere, where most of Africa is located, and some experts fear that drier and colder weather may increase viral activity.

With the number of deaths rising inexorably worldwide, there is urgent need for new treatments that can be used quickly to prevent COVID-19 patients from deteriorating, being transferred to intensive care units and dying.

▪ Traditional pharmacopoeia as an alternative for poor populations

A large proportion of the African population is confronted with three major difficulties which are obstacles to a beneficial and efficient health strategy:

- the possibility of drug shortages and supply disruptions in rural areas

- the cost of conventional medicines, and the exclusion for economic reasons of a significant part of the population from this type of treatment

- the significant proportion of counterfeit medicines in circulation that poses a major risk to those who buy them.

In this context, and because of cultural habits, populations often resort to traditional pharmacopoeia. Indeed, this appears to be an essential and pertinent therapeutic approach as and when conditions for monitoring practices validate its efficacy and safety.

When a traditional herbal medicinal product has proven its efficacy and safety in terms of methods of preparation and dosage to be followed, this type of medicinal product has the advantage that it can be grown and produced locally, that it is available and accessible at an affordable cost to the whole population and that it is difficult to falsify (i.e. its taste, smell and appearance can be recognised).

In addition, the procedure for the development of "improved traditional medicinal products" makes it possible to offer standardised herbal medicinal products that have been validated according to scientific standards. The alliance between traditional and contemporary knowledge can thus enable the creation and recognition of a modern pharmacopoeia underpinned by ancestral knowledge and experience.

Potential actions of *Artemisia annua* in the treatment of COVID-19

▪ Antiviral action of artemisinin

Artemisia annua (*A. annua*) is a non-toxic medicinal plant of the Asteraceae family native to China. Its aerial parts have been used for centuries in the traditional Chinese pharmacopoeia to treat fevers and especially malaria (Hsu, 2006; Tu, 2016). *A. annua* contains more than 600 compounds, including artemisinin, which was discovered in the 1970s for its powerful antimalarial properties (Klayman, 1985). Currently, combination therapies based on artemisinin and its derivatives, such as artesunate and artemether, are recommended by WHO for the treatment of uncomplicated malaria caused by Plasmodium falciparum (P. falciparum) (World Health Organization, 2017).

Several *A. annua* compounds have antiviral activity against several types of viruses. For example, artesunate inhibits the in vitro replication of several strains of human cytomegalovirus (HCMV), herpes simplex virus type 1 (HSV-1) and Epstein-Barr virus (Kaptein, 2006; Efferth, 2002). Its antiviral activity has also been demonstrated in humans with high efficacy and tolerability in the treatment of HCMV in a patient

who developed a drug-resistant infection after stem cell transplantation (Shapira, 2008). Artemisinin also inhibits in vitro replication of certain viruses such as hepatitis C (HCV) (Paeshuyse, 2006; Obeid, 2013) and certain strains of HIV (Oguariri, 2010; Lubbe, 2012).

After the outbreak of SARS-CoV Severe Acute Respiratory Syndrome Coronavirus in 2003, the antiviral effects of over 200 Chinese medicinal plants were studied in vitro by the Shi You Li team. The alcohol extract of *Artemisia annua* with those of *Lycoris radiata*, *Pyrrosia lingua* and *Lindera aggregata* presented a significant inhibition of SARS-CoV host cell lysis (Shi You Li, 2005). In a study comparing the efficacy of several types of treatments against SARS-Cov, the administration of medicinal decoctions including *A. annua* in addition to conventional treatments was found to effectively reduce the symptoms of the infection (World Health Organization, 2007). **This inhibitory effect of *A. annua* compounds on the replication of different viruses could be considered in the treatment of coronavirus infection to prevent it from multiplying in human host cells.**

▪ Anti-androgen action of *Artemisia annua*: blocking ACE2 and TMPRSS2

We have seen that the expression of ACE2 and TMPRSS2 proteins, both necessary for the entry of SARS-CoV-2 into host cells, is stimulated by androgens. Recently, artemisinin has been shown to induce androgen receptor degradation and disrupt the androgen response in several prostate cancer cell lines (Steely, 2017). Artemisinin induces AR degradation by the 26S proteasome, particularly by activating the ubiquitination of the AR protein in LNCaP and PC-3 prostate cancer cell lines. This disruption of the androgen pathway by artemisinin inhibits the proliferation of androgen-sensitive prostate cancer cell lines. Artesunate also alters the androgen pathway in vitro in the same manner as artemisinin (Nunes, 2017; Wang, 2017). Finally, artemisinin derivatives such as dihydroxyartemisinin and artemisinin dimers inhibit the proliferation of different cell lines of prostate cancer tumor, a hormone-dependent cancer (Morrissey, 2010). It has been found in vitro that resistance of prostate cancer cells to artemisinin does not induce resistance to another of the biomolecules contained in the *Artemisia annua* plant such as artemisitene and arteannuin B (Efferth, 2011).

In humans, *Artemisia annua* has been used as a dried plant capsule at 5x50 mg/d for long-term use in a patient with metastatic prostate cancer who had previously been treated with bicalutamide 50 mg/d for 14 days. This treatment (bicalutamide followed by *Artemisia annua*) was followed by a very significant regression of metastases of prostatic carcinoma but without remission (Michaelsen, 2015). In terms of toxicity, *Artemisia annua* and artemisinin do not present severe toxicity. They are generally

well tolerated and do not cause serious adverse events as demonstrated in the meta-analysis of Ribeiro et al. including 108 studies (Ribeiro, 1998).

▪ Anti-metalloprotease action of *Artemisia annua*: inhibition of CD147

The transmembrane protein CD147 (also known as basigin or EMMPRIN, extracellular matrix metalloproteinase inducer) is known to facilitate the invasion of host cells by various viruses, in particular SARS-CoV (Chen Z, 2005), HIV-1 (Pushkarsky, 2001), MHV-4 (Joseph, 1993), herpes (Qin, 2010), measles (Watanabe, 2010), human cytomegalovirus (HCMV) (Nguyen, 2018) and Chikungunya virus (De Caluwé, 2019). CD147 is widely expressed in various cell types, including epithelial and glandular cells, cells of the seminiferous ducts and renal tubules, vascular endothelium in the brain and cardiac myocytes, allowing viral invasion into many tissues of the body. CD147 is a key receptor in erythrocyte invasion for most strains of the malaria parasite Plasmodium falciparum (Crosnier, 2011). Finally, the SARS-CoV-2 S protein has been shown to bind to CD147 to enter the host cell (Wang K, 2020). Following this last study, a Phase II clinical trial entitled "Clinical Study of Anti-CD147 Humanized Meplazumab for Injection to Treat With 2019-nCoV Pneumonia" (ClinicalTrials.gov Identifier: NCT04275245) is currently underway in China to block the CD147 protein by meplazumab, a monoclonal antibody that prevents the binding of the SARS-CoV-2 S protein to CD147 and thus subsequent infection. This drug is a highly specific molecule directed against CD147, but such specificity does not exclude that other drugs affecting CD147 expression may also have beneficial effects on COVID-19 treatment. Interestingly, artemisinin (20-80 µg / ml) significantly inhibited in vitro the induction of EMMPRIN and the expression of MMP-9 metalloprotease at the transcriptional and translational level in a dose-dependent manner in PMA-induced macrophages. In addition, artemisinin strongly blocked the activity of EMMPRIN and MMP-9 by suppressing the PKCδ / ERK / p38 cascade (Wang Y, 2011).

CD147 is mainly known as an inducer of extracellular matrix metalloproteinase but it has also been shown to regulate lymphocyte reactivity. Expression levels of CD147 and MMP, including MMP-9 and MMP-2, are often increased in inflammatory processes and tumors (Iacono, 2007; Sun, 2001). Thus, inhibition of CD147 and the MMPs it induces may inhibit the inflammatory processes that lead to cytokine storm in COVID-19 (Hsu, 2015). However, various *A. annua* compounds including artemisinin and artesunate have demonstrated in vitro and in vivo inhibition activities of metalloproteases, mainly MMP-2 and MMP-9, but also MMP-8 (Buommino, 2009; Ma, 2019). The polyphenols of this plant also have inhibitory activity on MMP-9 (Ko, 2020).

In this context, compounds of *Artemisia annua* are likely to induce a decrease in the expression of the ACE2 and TMPRSS2 proteins via the alteration of the androgen pathway as well as the CD147 protein. By targeting three key membrane proteins involved in the entry of SARS-CoV-2 into host cells, *Artemisia annua* may slow the development of infection in the human body and thus slow the worsening of COVID-19 symptoms. We propose to test the efficacy and safety of *Artemisia annua* decoction administered for 14 days to patients infected with mild to moderate forms of SARS-CoV-2 in a Phase II randomized controlled trial (standard of care) to be conducted in an open-label setting.

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