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Open questions for harnessing autophagy-modulating drugs in the SARS-CoV-2 war: Hope or Hype?

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Abstract

At a time when the world faces an emotional breakdown, crushing our dreams if not taking our lives, we realize that together we must fight the war against the COVID-19 outbreak even if almost the majority of the scientific community finds itself confined to home. Every day, like everyone else, we, scientists, listen to the latest news with its promises and announcements. Across the world, a surge of clinical trials trying to cure or slow down the coronavirus pandemic has been launched to bring hope instead of fear and despair. One first proposed clinical trial has drawn worldwide hype to the benefit of chloroquine (CQ), a well-known and broadly used anti-malarial drug, in the treatment of patients infected by the recently emerged deadly coronavirus (SARS-CoV-2). We should consider this information in the light of the long-standing anti-inflammatory and anti-viral properties of CQ-related drugs. Yet, none of the articles promoting the use of CQ in the current pandemic evoked a possible molecular or cellular mechanism of action that could account for any efficacy. Here, given the interaction of viruses with macroautophagy (hereafter referred to as autophagy), a CQ-sensitive anti-viral safeguard pathway, we would like to discuss the pros, but also the cons concerning the current therapeutic options targeting this process.

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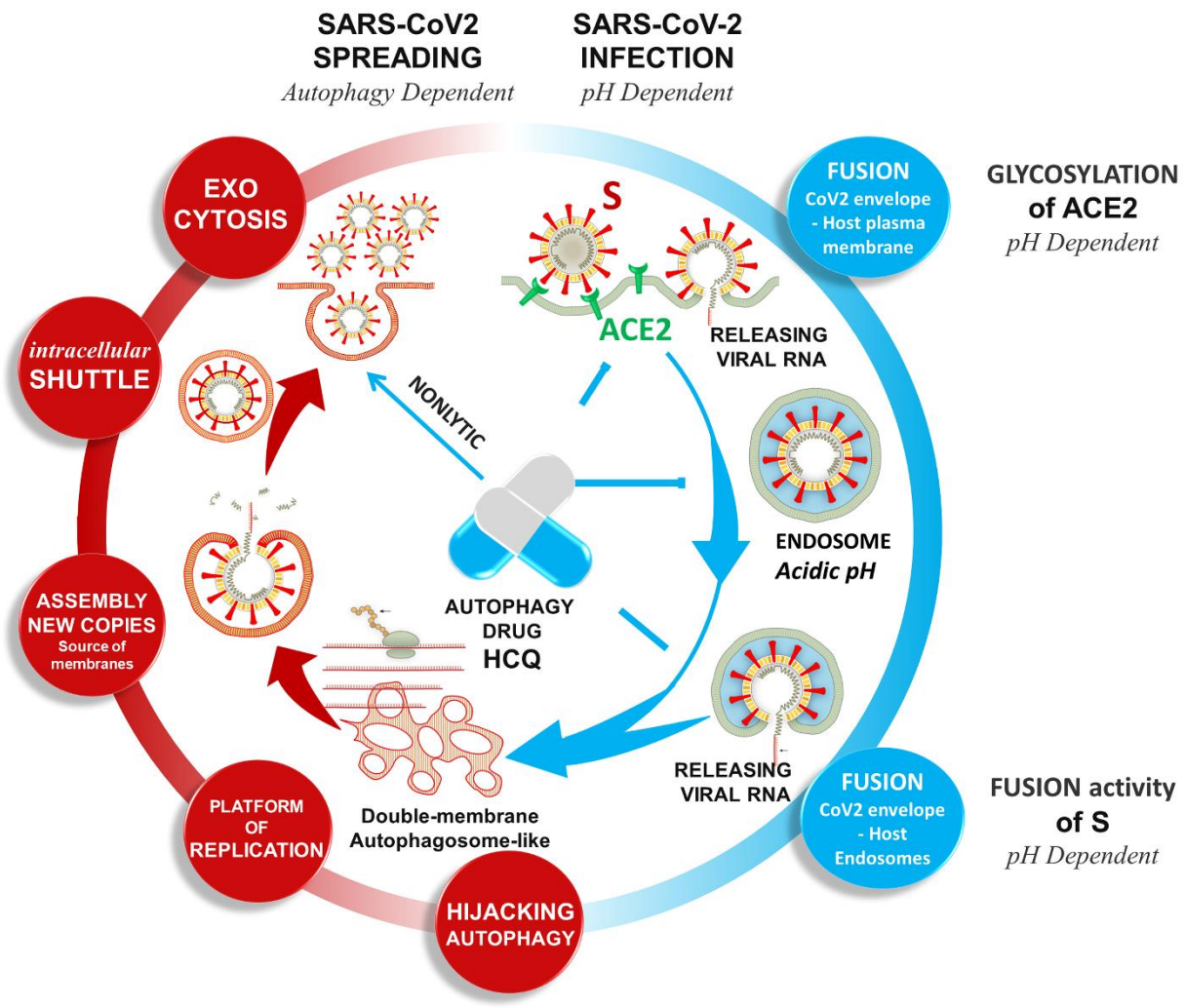

Figure 1. Hypothetical schematic diagram showing the replication cycle of coronavirus and the putative steps controlled by autophagy. The infection of lung epithelial cells starts with the binding of the viral SARS-CoV-2 particle to a cell surface receptor ACE2 and subsequent cell entry either at the plasma membrane or in endosomes upon endocytosis. There, the released genomic RNA highjacks the autophagy pathway (red) for active replication, assembly of newly synthesized particles, and intracellular shuttling to exocytosis. The pH-sensitive steps inhibited by Hydroxychloroquine are hypothetical and indicated in blue.

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What is this virus?

In December 2019, the etiological agent of an outbreak of pneumonia in Wuhan, China, was identified as **s**evere **a**cute **r**espiratory **s**yndrome **co**rona**v**irus **2** (SARS-CoV-2). On February 11, 2020, WHO named this atypical viral pneumonia '**COVID-19**,' for 'coronavirus disease 2019', and declared it as a global public health emergency situation. By May 19, 2020, only five months later, more than 180 countries worldwide acknowledged the existence of the COVID-19 pandemic [1]. The virus had infected approximately 5 million people worldwide, and the number of deaths had totaled more than 320,000 so far (https://www.worldometers.info/coronavirus/). The most severely affected countries outside of China include the USA, UK, Italy, France, Spain, and Brazil , ruling out a 'Chinese' ethnic susceptibility. This new coronavirus seems to be one of the most hazardous viruses, more than the related SARS-CoV-1 and MERS-CoV, because of its unique features in terms of the clinical severity, high transmissibility, and rapid global spread due to an asymptomatic incubation period and the existence of a high number of positive asymptomatic cases. Interestingly, all of the three coronaviruses that have been transmitted to humans to date (SARS-CoV-1, MERS-CoV, and SARS-CoV-2) are single-stranded, positive-sense RNA, (+)ssRNA, viruses, that generally affect animal species (bats, palm civets, camels, etc.) and accidentally cross over to humans, causing illness ranging from the common cold to sudden and fatal respiratory distress such as for MERS, SARS, and COVID-19.

How can we eradicate it—is it possible to take off-the-shelf anti-microbial drugs already prescribed for Ebola, HIV, or malaria?

With no licensed specific vaccines available to prevent CoV-2 infection, the connection between basic bench research and treatment in humans has never seemed more important. Facing this global emergency, the activity of all approved anti-viral drugs is being re-examined. As first candidates, more than 81 clinical trials are being quickly performed worldwide to repurpose old drugs as a "miracle" cure, including remdesivir that was previously used against Ebola, the lopinavir and ritonavir combination used against HIV [2], and also the anti-malarial drug hydroxychloroquine (HCQ) with broad recognized anti-viral activity against HIV and SARS-COV-1 [3,4].

What is the rationale behind the use of anti-autophagic drugs?

While it is speculative, we presume that HCQ may act on a CoV-2 infection at several levels:

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1) **By reducing the cell surface expression of the host CoV receptor**. The host cell entry constitutes the first step of a viral infection. For CoV-2, similar to the other enveloped viruses, this involves the binding of the coronavirus spike (S) glycoprotein to a single host cell receptor, ACE2 (angiotensin I converting enzyme 2), followed by the fusion of the virus to cellular membranes [5,6].

Of interest, it turns out that the acidic pH of the Golgi lumen is crucial for proper ACE2 terminal glycosylation and sorting to the plasma membrane. Hence raising the Golgi pH with HCQ, a weak base, at doses compatible with patient treatment, is sufficient *in vitro* to induce intracellular retention of ACE2 and thereby abrogate the related SARS-CoV-2-receptor binding and entry at the cell surface [4,7].

2) **By inhibiting the fusion activity of CoV-2**. Alternatively, CoV-2 may take advantage of the endosome to enter deeply into the host cell. Therein the acidic endosomal pH and proteolytic cleavage activate the fusion function of CoV-2 spike protein, controlling in time and space the release of the viral genome into the cytosol. As a result, optimal infection of the host cells by CoV-2 is significantly impaired by drugs that inhibit endosomal acidification [8].

3) **By inhibiting the autophagy flux and thereby virus replication, while promoting nonlytic exocytosis** [9]. As obligate intracellular parasites, viruses during an infection encounter autophagy, an intracellular process by which bulk cytoplasm is enveloped within a double-membrane vesicle, the autophagosome, from where they are shuttled to lysosomes for degradation. Besides its housekeeping role, autophagy emerges to safeguard the cells against infection not only by selectively targeting the viral components or virions for lysosomal degradation in a process termed xenophagy or, more specifically, virophagy but also by playing a pivotal role in antigen processing and the initiation of the adaptive immune response against the virus.

Undoubtedly, being a highly pathogenic virus, we propose that the interaction between CoV-2 and the autophagy pathway is complex: A) upon the destabilization of the lysosome (lysophagy) and the release of the viral genome, the alarm sirens sound, LGALS8 (galectin 8) within the GALTOR complex would inhibit MTOR, leading to the induction of autophagy [10]; B) however, given the severity of the disease, it might be expected that CoV-2 encodes virulence factors that block the host autophagy machinery to escape lysis and immune surveillance [11], the nature of which remains to be determined. C) Importantly, all known members of the coronavirus family have evolved to control the autophagy pathway to foster their own growth

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[12]. One feature of coronavirus-infected cells is indeed the dramatic accumulation of double-membrane vesicles resembling autophagosomes. This provides the virus with a platform for active viral RNA replication, a source of membrane for their envelope, and an intracellular shuttle for their exocytosis.

Can we, therefore, target autophagy to treat COVID-19?

Although there is no experimental evidence to suggest that targeting autophagy will benefit people infected with COVID-19, we highlight the following observations from the literature: pharmacologically activating autophagy with rapamycin could enhance coronavirus production, whereas inhibiting it with HCQ could counteract it, explaining in part the reported efficacy. 'Optimistically', HCQ has the benefit of being low cost, largely available throughout the world, and possessing a strong background safety history. Given early ‘promising’ results, Didier Raoult and co-workers, together with other academic and pharmaceutical laboratories, announced HCQ and azithromycin as a miraculous end to the COVID-19 pandemic [13–15]. Ridding this first wave of hype, on March 25, 2020, the US Food and Drug Administration (FDA) approved HCQ use for COVID-19 treatment (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-daily-roundup-march-25-2020). Since then, this treatment has been highly debated worldwide by different experts and political leaders. Lately, three studies found that HCQ (without azithromycin) led to neither a higher nor lower chance of patients ending up with intubation or death, but underlined possible toxic effects [16–18]. Finally, the USA FDA cautioned in late April against the use of hydroxychloroquine or chloroquine for COVID-19 patients not being treated in a hospital because of the risk of heart arrhythmias (https://www.fda.gov/safety/medical-product-safety-information/hydroxychloroquine-or-chloroquine-covid-19-drug-safety-communication-fda-cautions-against-use).

What is next for the future—can we define a population at risk for COVID-19 carrying autophagy-related gene polymorphisms?

Once an effective treatment that mitigates the symptoms of COVID-19 is identified, it would be important to understand why a SARS-CoV-2 infection is associated with a broad range of symptoms starting from complete asymptomatic cases to deadly acute respiratory distress syndrome and subsequently death, independent of ethnicity. At present, no one knows why some people — and not others — develop this deadly response; but there are likely host risk factors, outside of aging, including genetic mutations that may predispose to the severe form of this disease.

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The first epidemiological data point to a clear sex difference, with males being more frequently affected. Interestingly, *ACE2*, the receptor of SARS-CoV-2, is present on the X chromosome, underlying a possible gender sensitivity. However, similar to other complex diseases, we propose that the risk of COVID-19 may be influenced by multiple host genetic components (i.e., among others, glycosidases, or proteases affecting the ACE2-S interaction, and the inflammatory cytokines) along with the virus. We and others have provided evidence that *ATG16L1* and *IRGM* SNPs that impair the efficiency of autophagy to clear invasive pathogens predispose to the inflammatory storm in Crohn disease [19], a threat relevant for the COVID-19 infection [20]. Importantly, we should keep in mind that these autophagy polymorphisms are frequent in the general population, from 10 to 50%, and could explain in part the extreme variability of the COVID-19 disease. This observation points out the importance of precision medicine.

CQ translation to COVID-19 treatment: Hope or hype?

As a global public health emergency, public fear along with that of political leaders has created an urgency for a vaccine, prioritizing not only people but also the associated profits. Under this increasing pressure, the race for an effective treatment has never been so fast: in just four months, at least 1600 clinical trials have already been launched worldwide (https://clinicaltrials.gov/ct2/results?cond=COVID-19). However, we have little, if any, time to design their clinical management. More than ever, we are in the rush of the early phases of development, and do not have the time required for the classical three-step validation—from basic bench research, to animals, then to the patients—to rigorously confirm their relevance, safety, and efficacy. As the most urgent issue is to treat patients, the fact remains that we must not lose our critical spirit [21,22]. Looking back on the latest clinical trials of HCQ that failed expectations, many potential culprits can be identified, such as poor planning, small sample size, or a misunderstanding of the key underlying biological principles. Offering too much hope based on therapies that have not been validated places a huge burden on society, and is the result of the dilemma related to the health urgency of treating patients who may be in very severe circumstances, tempered by the extreme importance of practicing evidence-based medicine, the significance of which has been clearly demonstrated [22]. The danger of this hype is multiple: believing in the effectiveness of a particular treatment, people may no longer protect themselves or could be exposed to the harmful side effects of an overdose (cardiac arrhythmias, blindness, deafness, and even death) [18,23], and subsequently no longer agree to participate in controlled therapeutic trials.

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That said, we should still consider autophagy as a potential pharmaceutical target, as autophagy is a critical host process that controls all steps harnessed by SARS-CoV-2. This short non-exhaustive list of questions aims to very rapidly stimulate a collective reflection on how the manipulation of autophagy could be used to fight SARS-CoV-2 and future viral threats that will continue to emerge.

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