# Prevent COVID-19 severity by repurposing mTOR inhibitors

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## Abstract

COVID-19 has become a severe global public health concern. The critical illness has a mortality rate of 61.5%, and thus, reducing the severity and mortality is top priority.

Currently, inflammatory storms are considered as the cause of critical illness and death due to COVID-19. However, After systematical review of the literature, we proposed that cross-reactive antibodies-associated antibody-dependent enhancement (ADE) may actually be the cause of cytokine storms.

If the activation of memory B cells can be selectively inhibited in high-risk patients at an early stage of COVID-19 to reduce the production of cross-reactive antibodies of the virus, we speculate that the ADE can be avoided and severe symptoms can be prevented. The mammalian target of rapamycin (mTOR) inhibitors satisfy such conditions.

We recommend that pharmaceutical companies conduct clinical trials urgently.

## Key words:

COVID-19; coronavirus; cytokine storm; immunity; ADE; cross-reactive antibody; rapamycin; mTOR inhibotors

## Introduction

COVID-19 has become a severe global public health concern. It has infected more than one million individuals as of April 3, 2020. About 20% of the patients with COVID-19 have developed severe illness, and 5% have further developed critical illness. The mortality rate of critical case illness is 61.5%[1]. Thus, reducing the severity and mortality of the disease is a top priority.

According to the general understanding of diseases, high antibody levels indicate that pathogens are easily controlled and infections can be alleviated. Conversely, Zhang and colleagues found that COVID-19 severity is associated with increased IgG response[2].

What does this phenomenon suggest?

COVID-19 has many striking similarities to severe acute respiratory ryndrome (SARS) which outbroke 17 years ago. A previous study demonstrated that the peripheral blood CD4+ and CD8+ T cells in SARS-infected survivors showed a reversible decline. The decline and duration of T-cells and the severity of the disease are closely related, while the irreversible decline leads to mortality. T cell decline coexists with the increase in IL-6, TNFα, and other proinflammatory cytokines[3]. The recent data collected from COVID-19 patients also confirmed that T cell counts were negatively correlated with the changes in IL-6, TNFα, and other proinflammatory cytokines[4].

Currently, the inflammatory storms are considered as the cause of critical illness and death. After systematical review of the literature, we proposed that cross-reactive antibodies-associated antibody-dependent enhancement (ADE) may actually be the cause of cytokine storms in highly pathogenic human coronavirus infection, including SARS and COVID-19. ADE is also the underlying pathology in elderly people, and those with comorbidity would be into a more severe situation.

## Antibody-dependent enhancement characteristics of SARS and COVID-19

Patients with SARS who have developed antibodies earlier in the serum and have high antibody levels experienced severe infection[5]. The median time that SARS-CoV antibodies were detected in the serum was 16 days. It is remarkable that IgG

antibodies were first detected in some patients as early as day 4 of the disease. The early occurrence of serum IgG antibodies is associated with a high incidence of entering the intensive care unit (ICU)[6]. This phenomenon has also been reported in patients with COVID-19[2]. Among the 80 patients who received convalescent plasma therapy, 33 showed good and 47 showed poor results. Among the 33 patients with good results, the majority (n=28, 58.3% of 48) received plasma therapy within 14 days of disease onset[7]. We speculate in here that patient's early high-level antibodies are not the same as those in the convalescent plasma. These premature antibodies may be cross-reactive antibodies related to memory immunity.

Therefore, we hypothesize that SARS and COVID-19 have the characteristic of ADE. Briefly, when virus A enters the body, it activates memory B cells and inhibits the activation of naive B cells. The memory B cells produce antibodies capable of binding to virus A. However, these are cross-reactive antibodies, based on the immune memory of the previous infection by virus A1 which has one or more similar epitopes to that virus A. These cross-reactive antibodies are capable of delivering the virus to monocytes-macrophages via the Fc receptor, following which, a large number of viruses are replicated and released outside the cells after the immune escape. This is the process of ADE.

**ADE can explain numerous laboratory and clinical characteristics of COVID-19**

Pathological observations revealed that the exuding cells in the alveolar cavity of the patients died due to COVID-19 were mainly monocytes-macrophages. Virus inclusions were visible in the macrophages. Immunohistochemical staining showed that the macrophages were SARS-CoV-2 positive, while nucleic acid was tested positive by PCR. The spleen and other immune organs exhibit macrophage hyperplasia, phagocytosis, and a decrease in the number of lymphocytes and necrosis[8].

Proinflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNFα) are mainly derived from macrophages. The activity of macrophages and the massive release of these cytokines mutually confirm that the cytokine storm is the secondary event of macrophage activation. A recent study demonstrated significantly enhanced production of cytokines and chemokines by macrophages in those treated with sera from deceased SARS patients and virus as

compared to cells treated with virus alone. Sera treatment alone did not induce cytokines or chemokines. Blockade of FcγRs reduced the production of proinflammatory cytokines from macrophages[9].

Typically, after the body is infected with a virus, virus-specific T cells are required to kill the virus by killing the infected cells. However, cytokine IL-6 can inhibit the differentiation of T cells and impair cell immunity[10]. Therefore, the observed decrease in the number of T cells can be a secondary event of a cytokine storm.

Cross-reactive antibodies effectuate ADE. Hitherto, seven coronaviruses have been found infecting humans, of which, three are SARS-CoV, SARS-CoV2, and MERS-CoV that cause severe acute respiratory syndrome as well as severe illness and mortality. The remaining four (HCoV-229E, -OC43, -NL63, and -HKU1) only cause cold-like symptoms. The SARS virus has multiple cross-reactive epitopes with these low-pathogenic coronaviruses[11]. The antibodies of these four low-pathogenic coronaviruses have being investigated objectively in healthy individuals with >70% positive rate of serum IgG antibodies[12], i.e., >70% individuals have been infected with coronavirus. Interestingly, 75.75% (359/499), 75.95% (379/499) and 71.54% (357/499) individuals had remarkably low total T cell counts, CD4+ and CD8+ T cell counts in patients with COVID-19[4].

Most of the severe illnesses occur in adults, elderly, and those with comorbidity diseases that could transform into a critical situation. Since adolescents are less likely to develop severe illnesses, we focused on the correlation between cross-reactive antibodies and age, comorbidity diseases, and other high-risk factors. Among all the serum anti-HCoV IgG antibodies that had been collected from 105 adults, aged ≥50 (mean, 67.9) years from the seven sites in the USA, the detection rate of four low pathogenicity HCoV antibodies was 91‒100%[13]. HCoV-229E and -OC43 antibody levels continuously tracked in 44 members of 10 families in Seattle have shown that mean titers increased directly with age[14]. In addition to causing common colds in man, human coronaviruses may also be involved in the etiology of severe diseases in all age groups[15]. Compared to the positive detection rate of 2/5 in children, that of serum antibody in healthcare workers is 100%[16]. This fact might explain why some young healthcare workers <50 years of age have progressed to severe illness and some were

deceased due to COVID-19.

Collectively, the ADE pathogenic mechanism of COVID-19 is that once the human body is infected by SARS-CoV-2, it activates the immune memory left by the previous infection with low pathogenicity coronavirus, i.e., infection by HCoV-229E or OC43 or NL63 or HKU1. The memory B cell rapidly produces cross-reactive antibodies. Fc receptors mediate the viral antibody complex enter monocytes-macrophages. The virus replicates after immune escape and causes a rapid rise in the number of viruses leading to the release of multiple proinflammatory cytokines, lymphocyte reduction, and immune dysregulation. As a result, severe and critical COVID-19 occurred.

**mTOR inhibitor would be the solution for ADE**

If the activation of memory B cells can be selectively inhibited in these high-risk patients at an early stage to reduce the production of cross-reactive antibodies of the virus, we speculate that the ADE process can be avoided and severe symptoms can be prevented. mTOR inhibitors can suppress early B-cell production in germinal centers[17]. Therefore, we are expected to reduce early cross-reactive antibody production and further prevent ADE.

mTOR inhibitors enhance the magnitude and quality of viral-specific CD8+ T cell responses to vaccination in macaques[18]. In consideration of the pivotal role of mTOR in controlling the metabolism, dendritic cells and macrophage function, pathogens have evolved strategies to target this pathway to manipulate these cells and manipulate mTOR and cellular metabolism to promote immune escape[19]. These studies elucidated new mechanistic characteristics of mTOR inhibitor and suggested immune applications extending beyond its role as an immunosuppressant.

On the other hand, mTOR inhibits the replication of MERS coronavirus in vitro up to 61%[20]. mTOR inhibitors are superimposed on corticosteroids and can significantly improve the outcome in ICU patients infected with the HINI influenza virus[21], which provided additional evidence supporting their clinical use for similar diseases.

There is a variety of mTOR inhibitor on the market used for immunosuppressants during organ transplantation, which is clinically safe. Therefore,

in order to prevent ADE and reduce the severity and mortality, early use of mTOR inhibitors in patients with high-risk factors of severe illness is a direction for investigation. The latest published research also supports the use of mTOR inhibitors for COVID-19[22, 23].

### Clinical trial proposal

It is recommended to conduct a randomized, double-blind, placebo-controlled, multicenter clinical trial to urgently confirm the safety and effectiveness of mTOR inhibitors in the prevention of COVID-19 severity.

The NLR ratio in patients with COVID-19 is an early warning indicator of the development of the severe disease. Among the patients >50 years of age, 50% of patients with NLR >3.13 will develop severe illness[24]. Pivotal clinical trials may enroll this subset of individuals at an early stage, within 2 weeks after onset, and evaluate the difference in the incidence of severity between the mTOR inhibitor intervention group and the control group.

The intervention of the clinical trial should choose one of marketing-approved mTOR inhibitors, i.e., sirolimus, everolimus or temsirolimus.

The primary endpoint should be the incidence of severe and critical symptoms. The secondary endpoints should include 28-day recovery and mortality, changes in lymphocyte subpopulation count, viral load, cytokine levels, and incidence of lung injury and respiratory distress.

### Significance

It would be more difficult to develop vaccines for highly pathogenic human coronavirus if we consider their ADE characteristics. Therefore, it is emergency to find an effective way to prevent the occurrence of severe illness before the development of SARS-CoV-2 specific drugs or vaccines is completed. It will greatly ease the tension of medical resources and save countless lives worldwide.

### Contribution

YZ proposed the concept, searched the literature, and wrote the first draft.

SL confirmed the theoretical basis of immunology and improved the final

manuscript.

## Disclosure

YZ is serving as the CEO of Beijing Aldans Biotech Company which has a patent pending of rapamycin repurpose with application No. CN202010129707.8.

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