Inhalable antibodies for the treatment of COVID-19

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By the end of July 2022, the SARS-CoV-2 pandemic had caused more than 6 million deaths worldwide. This viral infection results in a series of atypical respiratory diseases termed COVID-19, from asymptomatic infection to severe symptoms such as acute respiratory distress syndrome and pulmonary fibrosis. Development of vaccines and therapeutic measures that mitigate the sufferings caused by the pandemic has been achieved in a short period of time. Only 1 year after the emergence of the pandemic, COVID-19 vaccines have been approved in several countries. The current four types of approved vaccines, i.e., mRNA vaccines, inactive virus vaccines, virus-vectored vaccines, and protein subunit vaccines, are designed based on viral surface spike protein and induce
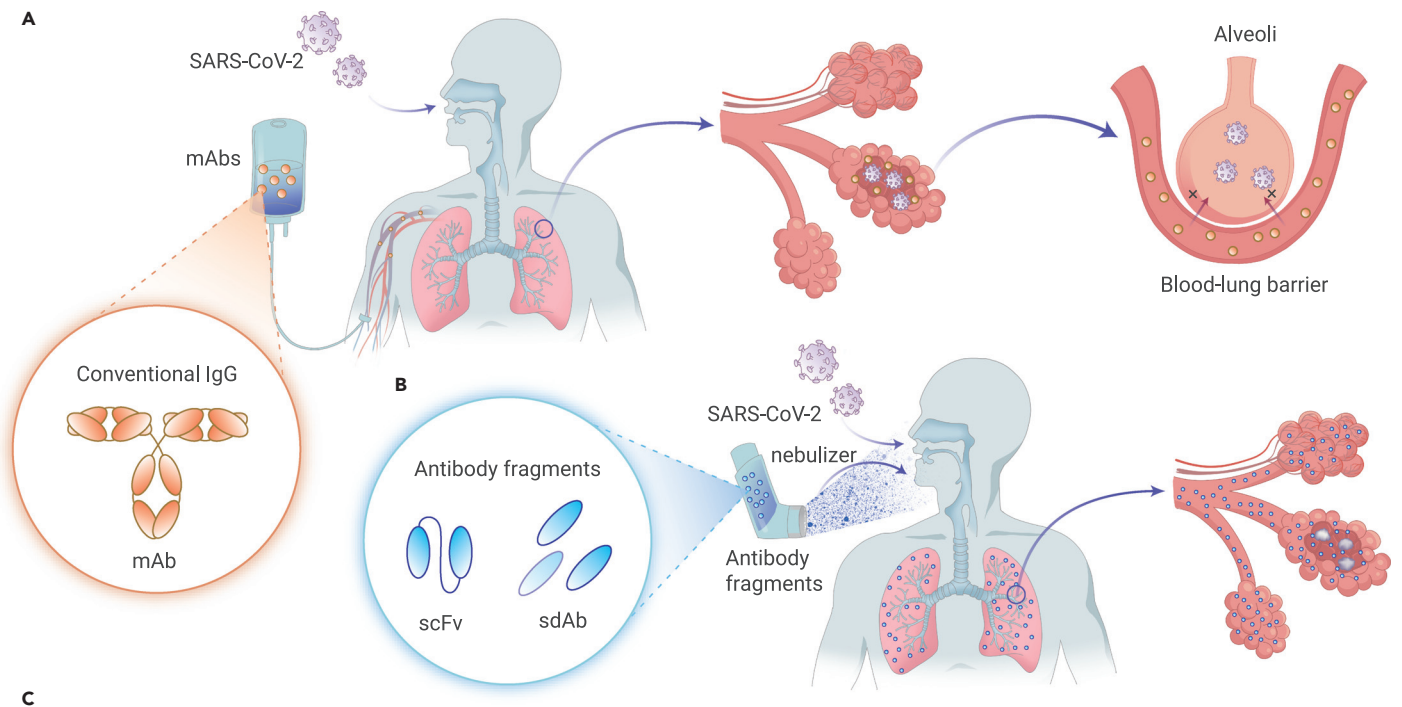
INN	Target	Antibody	Company	Status	Omicron BA.1/BA.2	Omicron BA.4/BA.5
Bamlanivimab	SARS-CoV-2 Spike	LY-CoV555	Eli Lilly/Shanghai Junshi Biosciences	EUA granted by the US FDA, suspended	Inactive	Inactive
Etesevimab	SARS-CoV-2 Spike	LY-CoV016/CB6			Inactive	Inactive
Casirivimab	SARS-CoV-2 Spike	REGN10933	Regeneron Pharmaceuticals	EUA granted by the US FDA, suspended	Inactive	Inactive
Imdevimab	SARS-CoV-2 Spike	REGN10987			Inactive	Inactive
Sotrovimab	SARS-CoV-2 Spike	S309	GSK/Vir Biotechnology	EUA granted by the US FDA, suspended	Marked reduction	Marked reduction
Regdanvimab	SARS-CoV-2 Spike	CT-P59	Celltrion Group	Approved in South Korea (MFDS) and EC	Inactive	Inactive
Amubarvimab	SARS-CoV-2 Spike	Brii-196	Brii Biosciences Ltd.	Approved by NMPA (China National Medical Products	Marked reduction	Marked reduction
Romlusevimab	SARS-CoV-2 Spike	Brii-198			Marked reduction	Inactive
Bebtelovimab	SARS-CoV-2 Spike	LY-CoV1404	AbCellera/Eli Lilly	EUA granted by the US FDA	Active	Active
Tixagevimab	SARS-CoV-2 Spike	AZD8895/COV2-2196	AstraZeneca	EUA granted by the US FDA, for Pre-exposure prophylaxis (PrEP)	Marked reduction	Inavtive
Cilgavimab	SARS-CoV-2 Spike	AZD1061/COV2-2130			Acive	Mild reduction
Tocilizumab	IL-6R		Roche	EUA granted by the US FDA		
Itolizumab	CD6		Biocon Biologics India Ltd.	EUA granted by the Indian drug regulatory agency		

Figure 1. The deposition of antibodies administrated by injection or inhalation and mAbs marketed for COVID-19 (A) Intravenously injection administrated mAbs circulate mainly in plasma, with little distribution in alveoli due to the air-blood barrier. (B) Inhalation route delivers the antibody fragments to the lung efficiently. scFv, single-chain fragment variable; sdAb, single-domain antibody. (C) Worldwide approved or EUA granted monoclonal antibodies are listed. INN, International Nonproprietary Names.

neutralizing antibodies against SARS-CoV-2. Notably, vaccinations are protective against severe disease and death, but they are less effective in preventing infections and transmissions. Moreover, poor immune responses may be induced by vaccinations in immunocompromised individuals, resulting in ineffective protection from COVID-19. Therefore, there is still an imperative need for efficacious drugs to treat COVID-19.

During the past 2 years, a dozen antibodies have been approved or granted for emergency use authorizations (EUA) for the treatment of COVID-19. These antibodies significantly ameliorate the hyper-inflammation or eradicate the virus. Earlier intervention in hospitalized patients with mild to moderate COVID-19 using approved antibodies also slowed down disease progression. Except for some re-purposed inflammation-alleviating antibodies, such as itolizumab, levilimab and leronlimab, the SARS-CoV-2 neutralizing antibodies target essentially the glycoprotein spike to prevent viral interaction with the host receptor angiotensin-converting enzyme 2 (ACE2), thus inhibiting the entry of virus into host cells.

In contrast to the approval of antibodies against SARS-CoV-2 at a phenomenal pace, the development of antibodies against other pathogens that induce respiratory tract infection (RTI) has culminated in disappointing results of only three approvals, including palivizumab against respiratory syncytial virus (RSV), as well as obiltoxaximab and raxibacumab against *Bacillus anthracis*. The reason for the limited success is multifold, but the unsatisfied curative effect and high cost may constitute the most dominant factors. Compared with small molecules, therapeutic antibodies bind more specifically to the target antigens and are associated with limited development of drug resistance. However, due to air-blood barrier, the subcutaneously (s.c.) or intravenously (i.v.) administered antibodies diffuse slowly and barely distribute in the lung where the pathogens concentrate, limiting their therapeutic efficiency (Figure 1A). Pharmacokinetic studies in primates and neonatal lambs indicated an approximate 500- to 2,000-fold lower concentration of monoclonal antibodies (mAbs) in bronchoalveolar lavage fluid (BALF) than that in plasma.[1] To increase the efficacy, the necessary antibody dosage is up to grams, which will increase their production cost. The limited cost-effectiveness further restricts the use of mAbs in treating RTIs.

Inhalation (i.h.) of drugs for the treatment of asthma occurred in the 1950s and is now widely used in treating respiratory diseases. Drug aerosols are sprayed or nebulized by instruments and inhaled by patients to shorten the time for drugs to reach maximal concentration (Cmax) in the lung. In animal models, i.h. delivery route was much more efficient than i.v. or intraperitoneal (i.p.) delivery route in terms of depositing the anti-influenza and anti-RSV mAbs into the lung. Consistently, researchers found that 1.7% of the inhaled mAb 1212C2, a potent neutralizing antibody against SARS-CoV-2, was deposited in the BALF of hamsters at 30 min after inhalation.[2] In contrast, less than 0.1% of the i.p. administered 1212C2 was deposited in the lung.

The deposition of inhaled particles in the respiratory tract is principally dependent on the force that dominates their motion. The particles with large size principally sediment in the upper airways following high inertia. Particles with smaller size gravitate downward to the middle airways and move to the deep alveolar region by Brownian diffusion. In other words, it is the particle size and molecular mass that determine the deposition location. The molecular size dependency of inhalation efficiency was also proven by the aerosol delivery of a trimeric nanobody PiN-21 and its albumin-fused version.[3] The amounts of recovered antibodies and antibodies deposited in the respiratory system, especially the lower respiratory tract, were inversely correlated with the size of molecules. The recovery rates for nanobodies with molecular weights of approximately 30, 45, and 60 kDa were 70%, 40%, and 20%, respectively. Therefore, it is not surprising in our recent findings that the majority of conventional IgG retained in the vibrating mesh nebulizer.[4] This also explains why the clinical oral inhalation is limited to small molecules except for one macromolecule insulin.

Stimulated by the urgent need for treating COVID-19, a number of inhalable biologics are reported, and some of them are under clinical evaluation. For example, the inhaled HH-120 aerosol, a decoy receptor constituted by the soluble extracellular domain of ACE2 (sACE2), neutralizes SARS-CoV-2 potently *in vitro* and is now under clinical phase I stage (NCT05116865). Encouraging results were also reported in anti-SARS-CoV-2 single-chain variable fragment (scFv) antibodies. Intranasal administration of scFv76, a molecule of about 28 kDa, completely inhibited the SARS-CoV-2 infection at dose of 74 µg/mouse

(~3 mg/kg dose).[5] Immunohistochemistry assay showed that nebulized scFv76 deposited efficiently down to the alveolar space. Among the biologics, nanobodies, owing to their small molecular weight (around 15 kDa) and superior biophysical properties, serve as promising candidates for nebulization, exampled by the nanobody ALX-0171 that has been developed as an inhalable anti-RSV drug at clinical phase II stage. Aerosol administration of the anti-SARS-CoV-2 nanobody PiN21 at a minimal dose of 0.2 mg/kg was proven to be effective in animal models.[3] The low-dose administration by inhalation also ameliorates the side effect of drugs. However, the camelid origin of nanobodies limits their application as therapeutics in humans. Therefore, we recently developed a technology platform for the development of fully human single-domain antibody (UdAb), which may have better safety profile and clinical efficacy compared with camelid-derived nanobodies.[4] By implying the next-generation impactor, we found that the nebulized bispecific UdAb bn03 (~28 kDa) has a cutoff diameter within the range of 3−5 µm, suitable for respiratory tract deposition. Via intranasal inhalation, bn03 exhibited robust protection against SARS-CoV-2 infection in both mild and severe mouse models. The above findings indicate the promising therapeutic effects of inhalable antibodies and the superiority of small-sized antibody fragments in the treatment of COVID-19 (Figure 1B).

The prevalence of SARS-CoV-2 variants, especially the recent Omicron strains that contain over 30 mutations in spike, diminishes the efficacy of vaccines and several marketed antibodies (Figure 1C). It is likely that SARS-CoV-2 will keep evolving to escape the humoral immunity, promoting the development of therapeutics with broad neutralization against all emerging variants. Therefore, identifying the highly conserved epitopes, such as regions in S2 and cryptic site inside the trimeric interface of spike,[4] and developing conserved-sites-targeting antibodies may be a promising strategy to deal with the challenges. Beyond the conserved-sites-occupying antibodies, the aforementioned sACE2 molecules are incomparably effective against all the spike mutations. However, the therapeutic usage of sACE2 is still controversial because of its potential off-target toxicity, since ACE2 is a protease that participates in the regulation of the renin-angiotensin-aldosterone system. Nevertheless, modified sACE2 with inactivated enzyme activity may surpass the limitation and be marketed for the treatment of COVID-19 in the future.

The rapid evolution and fast spread of SARS-CoV-2 has led to an unprecedented need for innovative, potent, and cost-effective therapeutics. Inhalable small-sized antibody fragments with broad neutralization, cheap manufacturing, and easy storage and transportation may meet the needs during long-term epidemics. It can also lead to greater convenience for patients and improved treatment compliance compared with the invasive routes. On the other hand, inhalable biologics may also be accompanied by certain risks, like burdening the respiratory clearance system. Other issues, such as the devices for the nebulization and formulations of the products, need to be well addressed before the clinical usage of inhalable antibodies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.