Learning from past failures: Challenges with monoclonal antibody therapies for COVID-19

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A B S T R A C T

COVID-19, the disease caused by infection with SARS-CoV-2, requires urgent development of therapeutic interventions. Due to their safety, specificity, and potential for rapid advancement into the clinic, **monoclonal antibodies (mAbs)** represent a highly promising class of antiviral or anti-inflammatory agents. Herein, by analyzing prior efforts to advance antiviral mAbs for other **acute respiratory infections (ARIs)**, we highlight the challenges faced by mAb-based immunotherapies for COVID-19. We present evidence supporting early intervention immediately following a positive diagnosis via inhaled delivery of mAbs with vibrating mesh nebulizers as a promising approach for the treatment of COVID-19.

1. mAbs as a platform for the rapid deployment of highly targeted antivirals

The advantages of mAb therapies are manifold. Currently, most mAb therapeutics against viruses are isolated from B-cells of patients who survived a prior infection, a strategy motivated by the assumption that some of the isolated mAbs may confer a survival benefit. High throughput screening, coupled with microfluidics and single cell sequencing, allows many B-cells to be screened quickly, enabling rapid isolation of mAbs with exceptionally high potency within weeks [1–3], a task that previously required many months of iterative screening and optimization. Unlike small molecule antivirals, the specificity of mAbs for viral antigens contributes to both their efficacy and safety, and likely lowers the regulatory requirements prior to initiating human studies. Concerns of viral escape can be minimized by combining complementary pairs of mAbs [4,5]. The processes of developing, manufacturing, and advancing mAb therapies into the clinic are well understood. These biotechnological advances underpin how companies such as Eli Lilly and Regeneron have been able to advance unique mAb therapies into the clinic within months, and underscore the promise of mAb therapies as an interim therapeutic approach for COVID-19 until effective vaccines can be developed and broadly implemented among the general population.

2. Many promising therapeutic mAbs have failed to treat or prevent ARIs

There are many ARIs for which no vaccine or effective therapies are available, including Respiratory Syncytial Virus (RSV), Metapneumovirus (MPV), Parainfluenza Virus (PIV), adenovirus, seasonal coronavirus (e.g. NL63-CoV), Rhinoviruses (RV), and others. Notably, these ARIs affect millions each year, providing ample financial incentives to develop therapeutic interventions. Indeed, the potential advantages of mAbs as antivirals have attracted many groups to attempt to develop mAbs against these common ARIs over the past two decades.

Nearly all such efforts have been met with disappointing results. Table 1 provides a list of human or humanized mAbs developed as antivirals that have advanced past Phase 1 studies (this list does not include mAbs currently under clinical studies, as their eventual outcome is not known). None of these mAbs were noted to have major safety concerns. Unfortunately, none have shown appreciable efficacy as a therapeutic, either, and only one has received approval for prophylaxis (palivizumab, also known as Synagis®, which offers only modest efficacy and is recommended only for severely premature infants due to limited cost-effectiveness).

The reasons why so many promising antiviral mAb therapies have failed to show clinical efficacy are multifold. For some, clinical development was halted due to actual (e.g. suptavumab for RSV

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NCT02325791 [6]) or anticipated (e.g. CR8020 for Influenza [7]) viral escape, contributing to a failure to meet primary endpoints [8,9]. Motavizumab's biological license application as an immunoprophylaxis against RSV infection was withdrawn due to slightly increased rates of injection site reactions that the FDA concluded did not outweigh the limited improvements in prophylactic efficacy over palivizumab [10,11]. Neither palivizumab [12,13] nor motavizumab [14] showed appreciable clinical benefit as therapies for RSV [15].

It should be noted that many of the mAbs in Table 1 possess lower affinity and neutralizing potency compared to the latest mAbs being developed against SARS-CoV-2. Nevertheless, mAbs are generally administered at very high doses, such that the mAb levels in the systemic circulation should be many orders of magnitude greater than the mAb's actual neutralization potencies (i.e. IC_{50} or IC_{80}) in vitro. This suggests their failure is unlikely to be caused by inadequate dosing of a poorly neutralizing mAb. It is also not clear if binding affinity and neutralization potencies in vitro predict clinical effectiveness. For instance, there did not appear to be an appreciable difference in the prophylactic effectiveness of MEDI-8897 vs. motavizumab in early clinical studies [16,17] despite ~5–10-fold greater affinity [18] and 9-fold better activity in a cotton rat model of RSV infection [19]. Greater neutralization potency in vitro also may not predict effectiveness in vivo, as exemplified by an exceptionally potent mAb against Ebola (in vitro) that provided no efficacy in vivo despite no evidence of neutralization escape [20].

It is clear that mAb therapies do offer substantial promise for treating systemic infections. Recent examples of successful use of systemic antiviral mAbs against Ebola Virus include Regeneron's 3-antibody cocktail REGN-EB3, [21] and NIH's mAb114 [22]. These mAbs reduced death rates from the overall mortality of 67% for the Ituri EBOV outbreak to ~33.5% and 35.1% of treated patients, respectively [23] and to 4.5 and 9.9% in patients with low viral load. It should be noted that both treatments required very high doses of mAb (150 mg/kg), despite their strong potency in vitro (IC_{50} of ~60 ng/mL for REGN-EB3 [24] and ~ 90 ng/mL for mAb114 [25].

Recent data from advanced trials of anti-SARS-CoV-2 mAbs from Eli Lilly (LY-CoV555) and Regeneron (REGN-COV2) suggest there is a potential clinical benefit when mAbs are administered early in the course of disease, but limited efficacy once patients are hospitalized. Indeed, the emergency use authorizations of REGN-COV2 and LY-CoV555 both exclude usage in hospitalized patients due to poor results from clinical trials, adding to the list of failures of virus-directed mAbs in treating hospitalized infections shown in Table 1. Fortunately, the benefits of administering mAb therapies earlier in the course of infection, in the outpatient setting, were more apparent. With LY-CoV555, treatment was associated with a slight decrease in symptom severity up until day 6 (but not after), as well as a trend toward decreased hospitalization rates. Most surprisingly, however, was that only the 2800 mg group in the LY-CoV555 study resulted in a statistically significant reduction in viral load by day 11 relative to placebo, whereas the higher dose (7000 mg) did not [26]. With REGN-COV2, an interim analysis of results from an ongoing phase 2/3 trial showed a reduction in COVID-19-related medical visits by 57% through day 29 in treated patients, relative to placebo. However, there was no apparent dose-dependent effect; there was no significant difference in virologic or clinical outcomes between the 2400 mg and 8000 mg dose groups for REGN-COV2 [27].

3. An underappreciated pathophysiology of many ARIs

The lung has two distinctive epithelia: a ciliated epithelium that lines the airways and a specialized epithelium that line the alveolus. The differentiated morphology and function of the respiratory tract epithelium exists at the air-liquid interface; epithelial cells grown in submerged culture conditions do not accurately recapitulate the properties and functions of authentic respiratory epithelium in vivo. To recapitulate the actual pulmonary physiology as closely as possible, culture models of human ciliated airway epithelium and alveolar epithelium have been developed. The most rigorous model involves culturing human nasal or tracheobronchial epithelial cells, which have been collected from airway brushings or from cadaver airway tissue, at an air-liquid interface to generate a polarized, well-differentiated, ciliated airway epithelium [28–30]. This method, commonly referred to as **well-differentiated human airway epithelial (WD-HAE)** culture, has been used by numerous investigators over the past two decades to investigate how respiratory viruses infect and propagate within the lungs.

Studies based on WD-HAE cultures have revealed that many viruses responsible for common ARIs, including RSV, rhinovirus, influenza, and PIV, almost exclusively infect via the apical (airway) side of the respiratory tract, with little to no productive infection when viruses are introduced into the basal (serosal) compartment (Fig. 1). More importantly, these viruses appear to predominantly, if not exclusively, shed back into the apical compartment (i.e. into airway mucus secretions), with limited to no shedding of virus into the basal compartment. The shedding of progeny virus into the apical compartment was first described for influenza virus [31–33], and later confirmed for RSV [34–38], parainfluenza virus [39], as well as the betacoronaviruses

Table 1
Prior attempts to advance mAbs for ARIs have faced barriers in clinical studies.

Antibody	Company	Virus	Mode	Last Stage	Status	Route	Dose	IC_{50}	Ref
CR8020	Crucell	Influenza	Tx	Phase 2 (NCT01992276)	Discontinued	IV	30 mg/kg	~9–500 ng/mL	[7]
CT-P27	Celltrion	Influenza	Tx	Phase 2 (NCT03511066)	No new studies announced	IV	10 or 20 mg/kg	~15,000 ng/mL	[87]
Diridavumab (CR6261)	Crucell	Influenza	Tx	Phase 2 (NCT02371668) and (NCT01992276)	Discontinued	IV	30 mg/kg	~18–2200 ng/mL	[88,89]
MEDI8852	MedImmune	Influenza	Tx	Phase 2a (NCT02603952)	Halted following Phase 2a	IV	750 or 3000 mg	~41–4050 ng/mL	[90,91]
MHAA4549A	Genentech	Influenza	Tx	Phase 2 (NCT01980966)	Halted following two Phase 2 studies	IV	3600 or 8400 mg	~195–6765 ng/mL	[57,92]
Motavizumab	MedImmune	RSV	Px, Tx	Px: Phase 3 (NCT00129766) and (NCT00538785) Tx: Phase 2 (NCT00421304)	BLA withdrawn; not effective as Tx [14]	IM	15 mg/kg monthly	~20 ng/mL	[17,93]
Suptavumab (REGN2222)	Regeneron	RSV	Px	Phase 3 (NCT02325791)	Discontinued	IM	30 mg/kg	~2–4 ng/mL	[94]
Synagis (palivizumab)	MedImmune	RSV	Px, Tx	Px: Marketed Tx: Not Marketed	Not Effective (~50% as prophylaxis)	IM	15 mg/kg	~163–360 ng/mL	[93,95,96]
TCN-032	Theraclone	Influenza	Tx	Phase 2 (NCT01719874)	Discontinued	IV	40 mg/kg		[97,98]
VIS-410	Visterra	Influenza	Tx	Phase 2 (NCT03040141)	No clinical activity since 2017	IV	2000 or 4000 mg	30–7000 ng/mL	[99,100]

Px = Prophylaxis; Tx = Treatment.

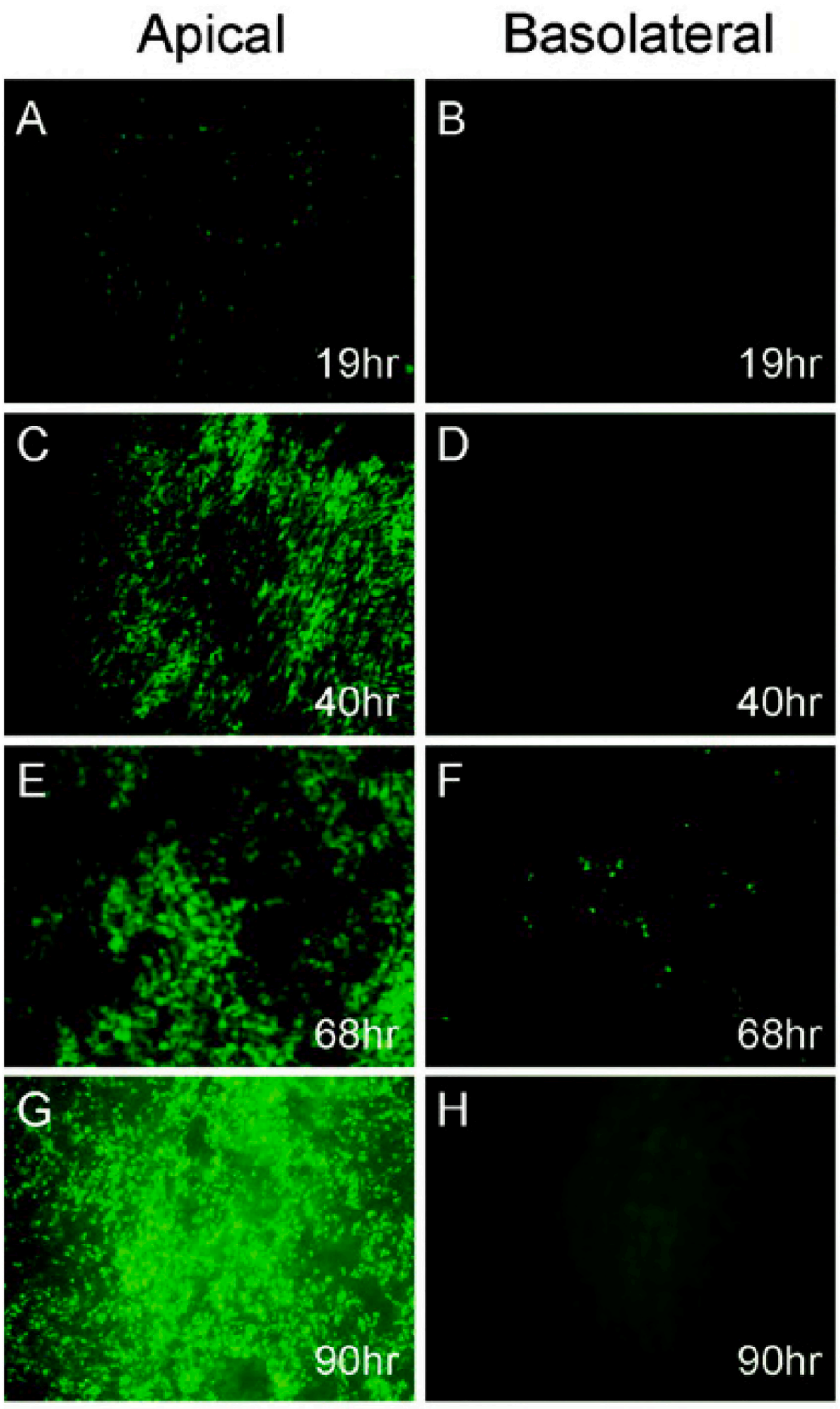

Fig. 1. Infection and spread of SARS-CoV GFP in WD-HAE cultures over time after apical or basolateral inoculation. HAE were inoculated via the apical (left: A, C, E, G) or basolateral (right: B, D, F, and H) compartments with SARS-CoV GFP and GFP-positive cells and assessed over time. Apical inoculation resulted in significant numbers of GFP-positive cells at 40 h post-infection (C), with extensive spread of infection by 90 h post-infection (G). In contrast, basolateral inoculation resulted in a low proportion of cells positive for GFP only at 68 h post-infection (F). These images are representative of duplicate cultures from at least three different patient sets. Original magnification, 10×. Image reproduced from [41].

HKU1 [40], SARS-CoV-1, and now SARS-CoV-2 [41]. Thus, apical shedding of virus and subsequent reinfection appears to be the primary mode responsible for the spread of these viruses from the **upper respiratory tract (URT)** to the **lower respiratory tract (LRT)** before eventually infecting the deep lung (alveolar epithelium). This mechanism of apical shedding and propagation is consistent with analysis of blood from infected patients that typically showed low to no systemic viremia, including those infected by influenza virus [42], RSV [43] and MPV [44], and explains why nasal or upper airway rather than blood-sampling represents the most accurate means of diagnosing ARIs during the early stages of infection. It is likely that substantial titers of infectious viruses will only begin accessing the systemic circulation when the infection has reached the deep lung and infection and inflammation have led to sufficient tissue damage and injury to compromise epithelial barrier function [45,46].

Similar to ARIs caused by commonly circulating viruses, both SARS-

CoV-1 and SARS-CoV-2 appear to spread infection through the respiratory fluids at the apical airway surface (Fig. 2). Apical infection and shedding is consistent with the apical localization of their common receptor, ACE2, to the apical membrane of human airway epithelium in vivo and in WD-HAE cultures [41,47]. It also agrees with clinical reports to date that suggest relatively limited viremia of SARS-CoV-2 (i.e. infectious viruses in the blood) *before* the disease has progressed to more severe infection, hyper-inflammation, and lung injury in the more fragile alveolus [48]. This gradient of progression of virus infection and extent of disease also agrees with the lag between the first symptoms of virus infection in the URT to when these patients begin to experience dyspnea (5–7 days after symptoms [49,50]).

4. Systemic vs. inhaled delivery of antiviral mAb therapies using vibrating mesh nebulizers

The therapeutic mAbs listed in Table 1 were all administered systemically to patients by either intramuscular (IM) or intravenous (IV) injection. Whether these administration routes are optimally suited for neutralizing viruses in the apical side of the respiratory tract is highly questionable. We believe the administration route, together with the timing of initiating treatment relative to the stage of the infection, are two factors that substantially impact the efficacy of mAb therapies.

The pharmacokinetics of systemically administered mAbs have been reviewed in great detail in excellent prior publications [51,52]. Notably, antibodies are large (~150 kDa for IgGs), hydrophilic molecules with a correspondingly low volume of distribution and slow kinetics of distribution out of the plasma, leading to limited passive transport of IgG from the circulation to mucosal surfaces. Although IgM and secretory IgA can be directly secreted into the airway fluids through a mechanism relying upon transcytosis across epithelial cells [53,54], IgG does not benefit from the same active mechanism in the lung. This makes the distribution of IgG antibodies into the lung in sufficient quantities for efficacy exceedingly challenging and necessitates a very systemic high dose, relying on a small fraction of the dose to make it to the site of infection. Detailed pharmacokinetic studies in primates suggest the concentration of systemically administered mAb is roughly ~500–2000 fold lower in broncheoalveolar lavage fluid (BALF) compared to plasma [55,56]; our recent, unpublished studies in neonatal lambs also yielded a comparable magnitude difference in BALF vs. plasma mAb concentration following IM delivery.

The preferential shedding of viruses into the airway mucus as infection spreads from the URT to the LRT implies that adequate therapeutic concentrations of mAbs must be achieved in the airway mucus secretions to effectively inactivate viruses and limit the continued spread of the infection. Greater levels of anti-flu mAb in the nasal mucosa appears to correlate with more rapid elimination of the virus in humans [57]. Among the handful of studies that compared inhaled delivery of mAbs vs. systemic delivery, inhaled delivery consistently afforded greater efficacy. For instance, intransal dosing of anti-influenza mAb provided ~3-fold improved survival over IV administration of the same mAb [58]. In cotton rats, 160-fold more mAb is required when dosed systemically (4 g/kg) in order to match the efficacy of the same mAb dosed intranasally (0.025 g/kg) [59]. These preclinical studies would suggest that inhaled delivery of anti-SARS-CoV-2 mAbs currently under clinical testing will likely achieve comparable efficacy even when dosed at a substantially lower dose compared to IV delivery. Given the limited mAb supply relative to number of surging cases (e.g. Regeneron recently estimated the maximum production capacity for REGN-COV2 to be ~250,000 doses per month through early 2021, based on current IV dosing, whereas 200,000 new cases are being diagnosed *every day* in the United States as of late November 2020), the lower dosage requirement for direct inhaled delivery of mAb should be further investigated.

For prophylaxis against RSV infection, we believe the modest clinical efficacy observed with palivizumab, motavizumab, and MEDI-8897 is likely attributed in part to the low titers of incoming virions during a transmission event. However, once an infection is already established in the respiratory tract leading to high local viral load, much higher levels of mAb dosed systemically is required compared to prophylaxis. Another potential shortcoming of systemically delivered therapeutics is the relatively slow diffusion of mAbs into the respiratory tract, leading to substantial delays before reaching C_{max} in the lung. For example, it takes *3 days* of twice-daily dosing for osteltamivir to achieve steady-state drug concentrations in the lung [60]. The distribution of mAbs into the lung after systemic administration may similarly take a few days before reaching Cmax; depending on how quickly mAbs can reach inhibitory levels in the airways following systemic dosing, this could mean that SARS-CoV-2 is afforded an additional period for exponential increase in viral titers and further inflammation. We suspect the frequent failures of mAbs as treatment of ARIs is at least partly due to the limited and/or delayed distribution of antiviral mAbs into the airway mucus secretions.

In contrast to systemic delivery, administering antiviral mAbs directly into the airways offers several important advantages. First,

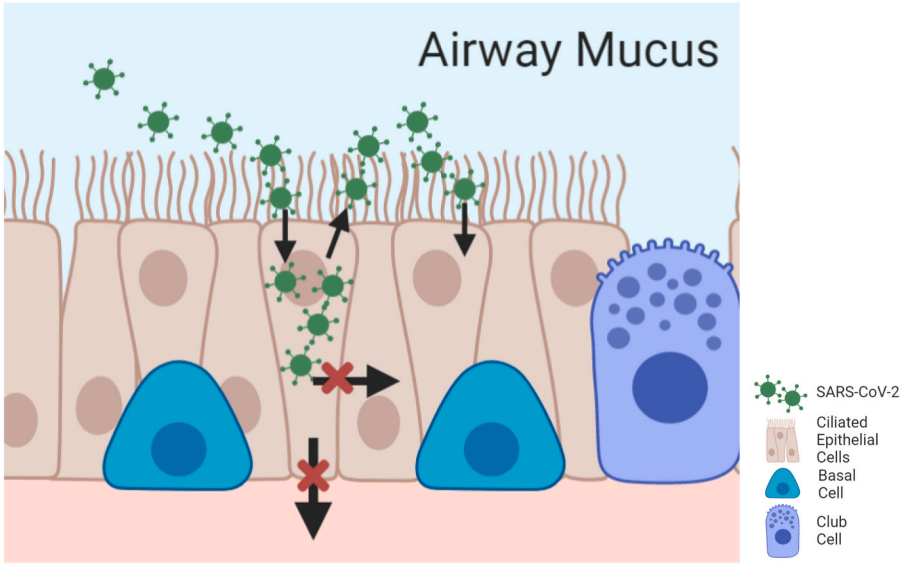

Fig. 2. Preferential apical infection and shedding of progeny viruses in the respiratory tract. SARS-CoV-2 deposited in the upper respiratory tract can diffuse through airway mucus and internalize into airway epithelial cells by binding to ACE2. The red X's indicate that SARS-CoV-2 does not typically spread from an infected cell laterally to a neighboring cell or through shedding into the basal compartment. Instead, SARS-CoV-2 is preferentially shed from infected cells from the apical side, back into the airway fluids, in which it diffuses to the apical face of neighboring cells, interacting with ACE2 and initiating the process of cellular entry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inhaled mAbs are immediately available to exert antiviral activity as they deposit into airway mucus secretions, the site of virus infection and spread. This approach effectively enables earlier intervention during the exponential growth phase of viral infection. Relative to systemic administration, inhalation either greatly reduces the amount of mAb needed to achieve the same inhibitory concentrations in the lung [58], and/or achieves much greater local mAb concentrations in airway mucus secretions. Given the large quantities of SARS-CoV-2 that are shed into airway mucus secretions, pulmonary delivery of mAb appears particularly well suited to address the spread of the infection within the lung. Given the large quantities of endogenous IgG that are already present in airway mucus secretions, inhaled mAb therapies are also likely to be well tolerated. Finally, by harnessing Fc-mucin interactions [61–64], inhaled mAbs may also facilitate rapid elimination of viruses from infected airways through mucus clearance mechanisms including muco-ciliary mucus transport and/or cough clearance [65], thereby physical eliminating the viral antigens that drive pulmonary hyper-inflammation. Consistent with the aforementioned advantages of direct delivery into the lung as well as the apical route of infection and spread of these viruses, prior work has shown that human mAbs delivered directly into the lung are highly efficacious [58,59,66,67], and more effective than the same mAbs introduced systemically [58,59].

Inhaled delivery of mAbs requires a delivery device that is effective and efficient. Vibrating mesh nebulizers (VMNs) represent an attractive approach for the pulmonary delivery of proteins and antibodies as VMNs can: 1) deliver a high dose of mAb to the airways while keeping the total volume relatively low [68]; and, 2) achieve uniform dispersion throughout the airways [68–71]. Further, by generating aerosols using a vibrating mesh, protein degradation is kept to a minimum, unlike jet or ultrasonic nebulizers, which rely on heating elements. Whereas traditional jet nebulizers possess only a ~ 10% delivery efficiency, the latest VMNs exceed 60% and directly avoid problems associated with hygroscopic growth and agglomeration of proteins - common challenges for dry powder formulations of proteins [72,73]. VMNs also directly avoid the coordinated breath inhalation frequently required for dry powder or metered dose inhalers, which can be difficult for geriatric and pediatric patients. VMNs are already routinely used at home and in outpatient settings.

5. The best time to treat SARS-CoV-2?

COVID-19 is predominantly a respiratory disease, with early infection in the upper airways and progression to lower airway disease over time. In severe cases of COVID-19, infections in the deep lung result in severe inflammation, leading to Acute Respiratory Disease Syndrome (ARDS). The hyperinflammatory response and associated cytokine storm represents the primary driver of mortality [74]. Indeed, respiratory failure alone accounts for 53% of the mortality, and respiratory failure coupled with heart failure accounts for another 33%; thus, 86% of COVID-19 deaths are directly associated with respiratory failure [27]. At later stages of infection, COVID-19 patients also often face a myriad of systemic complications including cardiac arrest, brain inflammation [75–78], and require ICU care and ventilator support [79]. By then, even when the viral load can be quickly controlled, patients still face inflammation-associated morbidities and diverse organ damage, as shown in some early results from convalescent serum studies [80]. Finally, pulmonary fibrosis developed in ~33% of patients who survived MERS [81] and SARS [82]; this permanent disability appears to also be common among hospitalized patients who survived COVID-19 [83]. We believe these realities motivate exploring interventions that can be administered soon after an outpatient diagnosis, prior to hospitalization, to halt SARS-CoV-2 infection from spreading past the lower respiratory tract, inducing hyperinflammation within the lung, and infecting other organs.

Currently, to minimize the burden on the healthcare system, the clinical practice in the U.S. is to send most patients who receive a positive diagnosis of COVID-19 home, and only hospitalize those who experience dyspnea and require supportive care. Unfortunately, by the time patients present to the hospital with severe symptoms, the window of opportunity to avoid pulmonary inflammation and systemic spread of the infection may have already lapsed. Furthermore, the average duration of hospitalization for COVID-19 patients ranges from 15 to 20 days. Even if a mAb therapy for hospitalized patients turns out to be highly effective in reducing deaths and shortening the hospitalization stay, such therapies will only modestly reduce the burden on the healthcare system.

An alternative approach for mAb-based intervention is to passively immunize all high-risk individuals to prevent initial infections and/or limit the spread of SARS-CoV-2 infection to the more vulnerable lower airways and alveolus. Unfortunately, the manufacturing capacity to produce sufficient mAb to passively immunize large populations is simply not available. Assuming the same 15 mg/kg dose used to passively immunize infants with MEDI-8897 for RSV and the use of a highly potent mAb with an IC_{50} comparable to the most potent mAbs currently being advanced for COVID-19, even passively immunizing just 1000 subjects would require more than 1.2 kg of mAb. Passively immunizing six million people (i.e., ~2% of the USA) would likely exhaust the entire manufacturing capability of a typical large pharmaceutical company.

Based on the apical pattern of infection and spread of SARS-CoV-2 and the possibility to directly delivery mAb to the lung airways using VMNs, we propose an alternative strategy for early intervention that focuses on administering nebulized mAb therapies to high-risk patients as soon as they receive a positive RT-PCR-based SARS-CoV-2 diagnosis. The median time from first symptoms to hospitalization and ARDS have been estimated to be in the range of 5–7 and 8 days, respectively [75,84–86]. Given the accelerating deployment of rapid diagnostics, we believe it is increasingly likely that patients will be diagnosed when infections are still largely restricted to the URT, with limited LRT involvement. We believe this represents a golden window of opportunity for intervening prior to the development of significant lower airway and systemic morbidities. Initiating a mAb therapy immediately following outpatient diagnosis may effectively reduce spread of virus infection into the distal airways and alveolus, thus reducing the likelihood of subsequent pulmonary complications that lead to hospitalization. As noted above, nebulization may also substantially reduce the overall dose of mAb needed per patient, which would increase the scalability of such an approach to a much larger patient population. By potentially preventing hospitalization (rather than simply shortening duration of hospitalization) early nebulized mAb therapy against SARS-CoV-2 may greatly reduce the burden on hospital systems should the number of COVID-19 patients continue to climb.

6. Conclusions

Technological advances have allowed pharma and biotech companies to identify lead mAb candidates and advance them into Phase 1 studies on the order of months, an impressive feat in advancing life-saving therapies for the millions of patients infected with SARS-CoV-2. Coupling these ultrapotent therapeutic mAb candidates with advances in rapid diagnostics potentially enables an early intervention against COVID-19 that is distinct from classical passive immunization and systemic therapy. We believe early inhaled mAb therapy represents an additional modality for mAb-based therapies that should be assessed in parallel with the systemic mAb-based therapies that have shown early signs of clinical benefit, offering the potential for more effective treatments that minimize the progression to severe pulmonary disease and hospitalization, while minimizing the dose of mAb needed and thus enabling treatment of more patients. Beyond addressing the current COVID-19 pandemic outbreak, early intervention via direct nebulized delivery of mAb may also be a promising strategy to treat ARIs caused by commonly circulating pathogens or newly emerging pathogens in future

pandemics.

Declaration of Competing Interest

S.K.L is founder of Mucommune, LLC and currently serves as its interim CEO. S.K.L is also founder of Inhalon Biopharma, Inc., and currently serves as its CSO, Board of Director, and Scientific Advisory Board. S.K.L has equity interests in both Mucommune and Inhalon Biopharma; S.K.L's relationships with Mucommune and Inhalon are subject to certain restrictions under University policy. The terms of these arrangements are managed by UNC-CH in accordance with its conflict of interest policies. M.M. has equity interests in Inhalon Biopharma.

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