

FOUND IN TRANSLATION

SARS-CoV-2 spike therapeutic antibodies in the age of variants

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Christos Kyratsous, Vice President of Research, Infectious Diseases, and Viral Vector Technologies at Regeneron Pharmaceuticals, and Alina Baum, Associate Director, Infectious Diseases Associate at Regeneron Pharmaceuticals, discuss the development of antibody therapeutics targeting the spike protein of SARS-CoV-2.

In the fight against the COVID-19 pandemic, anti-spike antibody therapeutics have been at the forefront of discovery of novel antivirals. By targeting the spike protein on the surface of SARS-CoV-2, these antibodies block the ability of the virus to enter host cells and limit viral replication in both prophylactic and therapeutic settings in animal models (Baum et al., 2020a; Hansen et al., 2020). Recent clinical data have demonstrated the ability of these mAb therapeutics to lead to faster viral clearance and reduce incidence of hospitalizations and deaths when administered early in infection and prevent infection in the setting of high-risk exposure, and early data indicate that they may help reduce virus load and stop progression to mechanical ventilation and death in the hospitalization setting (Weinreich et al., 2021; Chen et al., 2021; unpublished data). At the time of the writing of this article, three mAb therapeutics have been authorized under Emergency Use Authorization (EUA) for treatment of COVID-19 in a nonhospitalized setting: monotherapy (bamlanivimab) and combination (bamlanivimab and etesevimab) from Eli Lilly, and combination therapy (casirivimab and imdevimab) from Regeneron, with other anti-spike mAbs in late-stage clinical trials. Clinical trials are ongoing in the hospitalized population, but it is clear that similar to other direct antivirals, treatment earlier in infection provides the greatest benefit,

highlighting the need to treat patients as early as possible after diagnosis (Mulangu et al., 2019).

The recent increase of emerging SARS-CoV-2 variants has prompted a debate on whether antibody therapeutics can provide protection against an increasingly diverse viral population, as studies have now shown that many highly potent neutralizing antibodies lose activity against some of these variants (Wang et al., 2021). The seriousness of this concern is highlighted by the recent US government recommendation against continued use of Eli Lilly monotherapy bamlanivimab due to a complete loss of neutralization against variants commonly found in the US (Department of Health and Human Services, 2021; Food and Drug Administration, 2021).

As with any antiviral therapy, there are two facets of viral resistance that are critical to consider for an effective therapeutic: (1) the ability of the therapeutic to safeguard against emergence of treatment-induced resistance and (2) the breadth of therapeutic coverage against viral variants circulating in the population. These two distinct but related properties of anti-spike mAbs are key to assessing the overall risk of treatment failure with any individual therapeutic. And while the risk of viral resistance cannot be completely eliminated, it can be significantly reduced through rational design of antibody therapies.

In vitro escape studies with anti-spike antibodies have been instrumental in assessing the relative risk of treatment-induced resistance, clearly demonstrating that viral resistance rapidly emerges under single antibody pressure (Baum et al., 2020b; Copin et al., 2021 Preprint; Weisblum et al., 2020). The relevance of these in vitro systems for predicting the likelihood of rapid virus escape has been confirmed in animal models and in the clinic; in the hamster model of SARS-CoV-2, antibody-resistant viruses were rapidly selected during monotherapy treatment, and in the clinic, a high prevalence of treatment-associated variants was detected in patients treated with bamlanivimab monotherapy (Copin et al., 2021 Preprint; Food and Drug Administration, 2021). Several strategies to minimize the risk of such resistance have been proposed; these include using combinations of antibodies targeting separate nonoverlapping epitopes or distinct but partially overlapping epitopes, as well as using single antibodies targeting more conserved epitopes on the spike protein (Baum et al., 2020b; Pinto et al., 2020). The relative risk of treatment-induced virus escape associated with these strategies has now been assessed in multiple studies. The results clearly demonstrate that rapid resistance arises with any monotherapy treatment independent of epitope conservation, and

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that a combination of antibodies with partially overlapping epitopes behaves similarly to monotherapy, resulting in rapid selection of single amino acid mutants that significantly impact both antibodies simultaneously (Copin et al., 2021 Preprint; Baum et al., 2020b). Therefore, clinical use of either monotherapy or combination therapy with antibodies that have overlapping epitopes has the potential to drive selection of mAb-resistant variants, potentially increasing the risk of treatment failure and/or seeding resistant variants into the population. The second possibility is especially concerning since mAb escape variants are often located in immunodominant epitopes within the spike protein (e.g., E484K), mutations which have been shown to not only be associated with reduced potency of several mAbs, but also with impaired natural infection and vaccine-induced neutralization titers and possible reinfections (Wang et al., 2021). Recent characterization of intra-host virus populations in humans illuminates the full spike protein sequence diversity present within an infected individual, with a multitude of minor spike protein variants readily available for selection under the right pressure (Copin et al., 2021 Preprint). Contrary to monotherapy or mAb combinations with overlapping epitopes, use of combinations of neutralizing antibodies targeting distinct noncompeting epitopes greatly reduces the risk of viral escape both *in vitro* and *in vivo*, and most importantly in clinical studies (Copin et al., 2021 Preprint). This finding is in line with many years of antiviral therapy experience, which has clearly shown the utility of combination therapy in minimizing the risk of resistance (Mendoza et al., 2018).

In addition to treatment-emergent mutants, monitoring the breadth of neutralization coverage against circulating virus variants has become critically important with the recent emergence of variants that appear to be under strong selection pressure in the human population. The Center for Disease Control and Prevention has recently classified these variants into risk categories based on potential impact on transmissibility, association with more severe disease, significant reduction in neutralization titers from infection or vaccination, or decreased efficacy of vaccines (Center for Disease Control and Prevention, 2021).

Close monitoring of circulating viral sequences since the beginning of the pandemic

has allowed an unprecedented understanding of dynamics of viral evolution as a novel pathogen is introduced into a naive population and immunity begins to mount. For the first few months of the pandemic, the viral spike protein appeared relatively conserved with little variation in viral sequence. The emergence of the D614G variant, initially detected in March 2020, for the first time demonstrated that a fitness advantage, presumably associated with greater transmissibility of the virus, can lead to a remarkably rapid expansion of that variant, resulting in the vast majority of globally circulating viruses encoding that mutation by May of 2020 (Korber et al., 2020). A second example of a rapidly expanding lineage, B.1.1.7 (UK) encoding the N501Y mutation, similarly demonstrated that a variant can rapidly take over the viral population, with dominance of this variant now observed in multiple countries in Europe and rapid increase occurring in the US (Kemp et al., 2021 Preprint). The expanding dominance of the B.1.1.7 lineage has raised well-founded concerns regarding the efficacy of vaccines and mAb therapeutics against this lineage. Multiple studies have demonstrated no or minimal loss of neutralization potency of clinical stage antibodies and vaccine-induced polyclonal antibody responses against the B.1.1.7 virus (Wang et al., 2021). The impact of the B.1.1.7 variant on infection and vaccine-induced polyclonal antibody responses, albeit small, is likely associated with the deletion deltaH69/V70 in the N-terminal domain (NTD) of the receptor-binding domain (RBD), as the NTD has been shown to be a common target of neutralizing antibodies. Indeed, the majority of mAbs targeting the NTD have been shown to completely lose neutralization potency against the B.1.1.7 lineage, thus making the NTD less attractive as a potential target for mAb therapy (Wang et al., 2021).

Perhaps the greatest concern when it comes to emerging SARS-CoV-2 variants stems from identification of rapidly expanding lineages with mutations in the RBD that have significant impact on neutralization potency of many RBD-targeting antibodies, including clinical stage antibody therapeutics, as well as natural infection and vaccine-induced antibody responses. In addition to the N501Y mutation, the B.1.351 and P.1 lineages, originating in South Africa and Brazil respectively, also carry mutations

E484K and K417N/T (Tegally et al., 2020 Preprint; Martins et al., 2021). Other lineages containing the E484K mutation but not N501Y, such as P.2 (Brazil) and B.1526 (New York), have also been expanding, suggesting that the E484K mutation independent of N501Y may confer sufficient advantages to the virus (Voloch et al., 2020 Preprint; West et al., 2021 Preprint). The E484K mutation is especially worrisome as it is now clear that this particular residue is commonly found within epitopes of highly potent neutralizing antibodies, and lineages containing this mutation are less susceptible to neutralization by natural infection and vaccine-induced polyclonal sera (Wang et al., 2021). Indeed, the E484K mutation was initially identified by several studies as an escape variant to potent RBD-targeting mAbs and convalescent sera from SARS-CoV-2-infected donors (Baum et al., 2020b; Weisblum et al., 2020). Recent data emerging from vaccine trials demonstrated that the overall efficacy of multiple vaccines was significantly lower in South Africa (at the time that the B.1.351 variant was widely circulating) than in the US or UK, providing the most concerning real-world evidence that some virus variants may have a dramatic impact on the overall global burden of SARS-CoV-2 even with increasing immunity in the human population (Madhi et al., 2021). Although the prevalence of the B.1.351, P.1, and P.2 lineages has remained low in the US, the B.1.526 lineage rapidly expanding in the New York region (30–40% prevalence at the time of the writing of this article) also carries the E484K mutation, and as such represents the most imminent threat to vaccine and therapeutic efficacy in the US.

Assessment of clinical stage and EUA-authorized antibody therapeutics has made it clear that the same approaches that protect against treatment-induced resistance also provide benefit when it comes to coverage of emerging variants. Multiple studies have now shown that an EUA-approved antibody monotherapy bamlanivimab completely loses activity against the B.1.351 (South Africa), P.1 (Brazil), B.1.427/B.1.429 (California), and B.1.526 (New York) variants, and while a combination of mAbs with overlapping epitopes, bamlanivimab and etesevimab, performs better than monotherapy, it is also significantly impacted by single amino acid mutations

(Copin et al., 2021 Preprint; Food and Drug Administration, 2021). On the contrary, antibody combinations with nonoverlapping epitopes (casirivimab and imdevimab, COV-2130, and COV2196) have so far shown no loss of neutralization potency against any of the variants of concern, as full neutralization potency is retained by the combination even when one of the antibodies is impacted. Although the broad coronavirus antibody, VIR-7831, also provides coverage against these circulating variants, its inherent susceptibility to drug-induced resistance when used as monotherapy counters this advantage (Copin et al., 2021 Preprint; Wang et al., 2021).

It is clear that SARS-CoV-2 will continue to mutate as ever-greater immunity is reached in the human population. In vitro escape studies with mAbs and convalescent serum have clearly shown that many mutations in addition to E484K impact neutralization potency of multiple mAbs, and most of these mutations have been detected in circulating viruses, albeit at relatively low frequencies (Baum et al., 2020b; Weisblum et al., 2020). As immune pressure continues to mount, it is likely that some of these variants will expand and may represent new

threats to therapeutic and vaccine efficacy. The need for potent antivirals that can prevent severe illness and death will remain, even with increasing use of highly efficacious vaccines, as global elimination of SARS-CoV-2 appears to be unlikely. Future drug and vaccine development will need to be guided by widespread sequence surveillance and will require collaboration with regulatory authorities to ensure creative strategies are used to keep up with the ever-evolving virus.

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