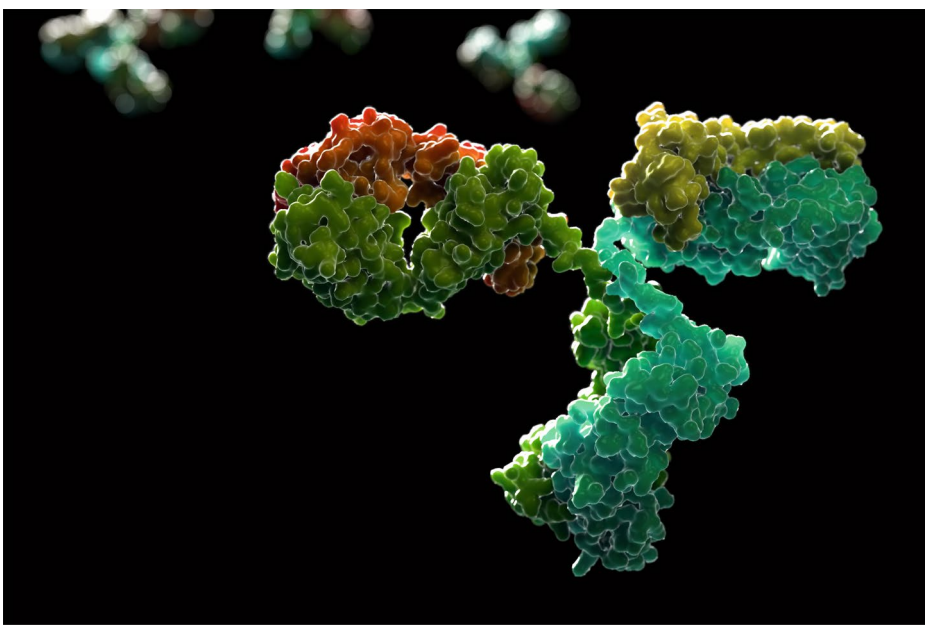
Hunt for improved monoclonals against coronavirus gathers pace

Following mixed results in the clinic against COVID-19, antibody engineers are further optimizing SARS-CoV-2 antibodies with the aim of improving outcomes.

The US government has bought almost a million doses of Eli Lilly's SARS-CoV-2 neutralizing antibody, with the latest purchase announced 2 December. Lilly's monoclonal antibody (mAb) drug, bamlanivimab, was given an Emergency Use Authorization (EUA) on 9 November by the US Food and Drug Administration for use in high-risk COVID-19 patients with mild or moderate disease. Regeneron's two-antibody cocktail (casirivimab and imdevimab) also received an EUA for the treatment of mild-to-moderate COVID-19. Though both drugs represent new options for treating the coronavirus infection, neither is ideal. On the basis of a phase 2 trial report, the overall reduction in viral load between the bamlanivimab and placebo arms was small. The phase 2/3 data on Regeneron's cocktail have yet to be reported in a peer-reviewed venue, but the company claims a similar small reduction in viral load compared with that in placebo-treated patients, although patients with high viral load did better and there were fewer medical visits than in the control arm. In the meantime, several other companies are applying antibody-engineering strategies to enhance antiviral potency, improve mAb safety profile, provide greater convenience of administration and reduce cost.

The use of antibodies as antivirals is gaining momentum. In the 20 years after Synagis (palivizumab) was approved for use against respiratory syncytial virus (RSV), no other mAbs targeting viral pathogens were registered. In 2018, Roche/Genentech received a green light for Trogarzo (ibalizumab) for the treatment of multidrug-resistant HIV; and early last year, the Food and Drug Administration approved Regeneron's Inmazeb antibody cocktail treatment for Ebola. But the pandemic has now put at least a dozen anti-SARS-CoV-2 mAbs into the clinic (Table 1).

For patients with early or mild disease, mAbs for COVID-19 face



The next generation of antibodies to the new coronavirus will be engineered to produce improved versions. Credit: Science Picture Co / Alamy Stock Photo

Thumbs up for cholesterol-slashing siRNA blockbuster

The European Union has approved a small interfering RNA (siRNA)-based drug for reducing low-density lipoprotein cholesterol (LDL-cholesterol) in patients with abnormally high blood cholesterol. The go-ahead for Leqvio (inclisiran), announced on 11 December, is an important milestone for Novartis, of Basel, Switzerland, as it attempts to recoup some of the $9.7 billion it spent in acquiring the drug's developer, The Medicines Company. Leqvio, an *N*-acetylgalactosamine-conjugated 2′-*O*-methyl, 2′-H-phosphorothioate oligonucleotide, prevents the mRNA encoding the liver enzyme PCSK9 from being translated into protein. PCSK9, short for proprotein convertase subtilisin/kexin type 9, maintains LDL-cholesterol in circulation by preventing LDL receptors from returning to the plasma membrane after they have bound and internalized an LDL-cholesterol molecule. By targeting and reducing PCSK9, Leqvio boosts such recycling, which encourages further cholesterol uptake from circulation. Two antibody PCSK9 inhibitors have already reached the market: Repatha (evolocumab), marketed by Amgen, and Praluent (alirocumab), from Regeneron Pharmaceuticals. Leqvio has a similar effect to both — it cuts LDL-cholesterol by about 50% — but market observers expect it to dominate because of its small-molecule-like manufacturing costs and its more convenient dosing schedule: Leqvio requires a twice-yearly subcutaneous injection, whereas each of the antibodies requires systemic dosing at either two-week or four-week intervals. Neither antibody has, so far, realized the blockbuster ambitions their developers had attached to them, but Novartis CEO Vas Narasimhan has said that Leqvio could become one of the pharma's best-selling products. A US approval has been delayed, however, because the FDA has yet to decide whether it will inspect a manufacturing facility in Italy. And it will take several more years before a fuller understanding of the clinical value of Leqvio emerges. A cardiovascular outcomes study, which will evaluate the effect of the drug in reducing death or cardiovascular events, such as heart attack or stroke, will report in 2024.

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Antibody–drug conjugates for cancer score with ROR1

Boehringer Ingelheim has committed up to $1.45 billion, including milestones, to acquire NBE Therapeutics and its ROR1-targeted antibody–drug conjugate (ADC) NBE-002. The deal was inked just a month after Merck plunked down $2.75 billion in cash to acquire VelosBio and its ROR1 ADC VLS-101.

ROR1 is a receptor tyrosine kinase that is expressed primarily during embryogenesis, rather than in normal adult tissues. But various hematological and solid cancers upregulate ROR1 expression, potentially making it a clean cancer target. To selectively kill cancer cells, NBE Therapeutics and VelosBio loaded ROR1-targeted antibodies with chemotherapeutic agents — an anthracycline-based toxin in the case of NBE-002 and monomethyl auristatin E in the case of VLS-101.

Following their respective bets on these biotechs, both Boehringer Ingelheim and Merck now have to wait for the clinical data to see whether their deals will pay off. NBE advanced its NBE-002 into a phase 1 trial, in solid tumors, in October. VelosBio's VLS-101 was well tolerated and seemingly effective in a phase 1 study in hematological cancers, the company reported at the American Society of Hematology annual meeting in December 2020. VelosBio started a phase 2 trial in solid cancers, including breast and lung cancer, in October.

These deals, paired with Gilead's September acquisition of Immunomedics for $21 billion and Merck's September partnership with Seattle Genetics, highlight continued interest in ADC opportunities.

Other ROR1-targeted agents are also in development. Oncternal Therapeutics' cirmtuzumab, a naked monoclonal antibody, is in phase 1/2 trials, in combination with other agents. Bristol Myers Squibb's ROR1-targeted chimeric antigen receptor (CAR)-T cell therapy JCAR024 — acquired from Juno Therapeutics via Bristol Myers Squibb's 2019 merger with Celgene — is in an ongoing phase 1 trial that started in 2016. And LegoChem Biosciences and CStone Pharmaceuticals are working together on a preclinical ROR1-targeted ADC called LCB71.

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Table 1 | Selected SARS-CoV-2 mAbs in clinical development targeting spike protein epitopes

Sponsor	Agent	Clinical stage
Regeneron	Casirivimab and imdevimab (REGN-COV2)	Phase 3; US EUA 21 November 2020
Eli Lilly, AbCellera	Bamlanivimab, etesevimab	Phase 2; bamlanivimab US EUA 9 November 2020
Vir Biotechnology, GlaxoSmithKline	VIR-7831 (GSK4182136)	Phase 3
AstraZeneca, Vanderbilt Univ.	AZD7442 (AZD8895 and AZD1061)	Phase 3
Tychan	TY027	Phase 3
Celltrion	CT-P59	Phase 2/3
Sinocelltech	SCTA01	Phase 2/3
BeiGene, Singlomics, Peking Univ.	BGB-DXP593	Phase 2
Mabwell (Shanghai) Bioscience	MW33	Phase 2
Brii Biosciences, Tsinghua Univ.	BRII-196, BRII-198	Phase 1
Sorrento Therapeutics	STI-1499, STI-2020	Phase 1
Shanghai Junshi Biosciences	JS016	Phase 1

several limitations. They need to be given intravenously, and outpatient infusions are costly and difficult. Also, the limited supply forces institutions to make decisions about which patients are likely to benefit. "There's always a concern about healthcare equity," says Adam Lauring, an infectious disease doctor at the University of Michigan. mAbs drugs are expensive. Lilly, for example, is charging the government $1,250 a dose, not including the infusion cost.

So far, the clinical results from these mAbs have been less than stellar in moderate COVID-19 and even worse in severe disease. The US National Institutes of Health stopped treating hospitalized patients with Lilly's bamlanivimab in October, on the basis of phase 3 data showing no improvement in clinical outcomes. The same month, Regeneron paused enrollment of sicker patients to one of its hospital trials "based on a potential safety signal and an unfavorable risk/benefit profile," according to the trial's independent data monitoring committee.

But even with vaccines rolling out, finding effective antibody therapies for passive immunization continues to matter. "They're extremely complementary approaches — we need them both," says Erica Ollmann Saphire, a biologist at the La Jolla Institute for Immunology. Mass vaccination will take time and vaccines won't work in everyone, she points out, and many people can't be vaccinated with certain vaccines because they're elderly and immunocompromised.

The Lilly and Regeneron mAbs are standard engineered formats: they are basically wild type, unmodified IgG1 mAbs that target the SARS-CoV-2 spike (S) protein. They were cloned from B cells taken from patients convalescing from COVID-19 or, in the case of one Regeneron antibody, generated in humanized mice. "Everyone just had to make a choice and launch their therapeutic, to get something where it needed to be, rather than wait for the perfect thing," says Saphire, who directs the Coronavirus Immunotherapy Consortium, which is evaluating over 200 SARS-CoV-2 antibodies.

Many next-generation mAbs to the new coronavirus are heavily engineered, specifically in the antibody Fc region. The Fc binds to receptors on immune cells and elicits a broad array of Fc effector functions during viral infections, including macrophage phagocytosis and natural killer (NK) cell–mediated cytotoxicity. Certain Fc mutations extend antibody half-life and increase lung bioavailability, something clearly desirable in respiratory infections. But mutating the Fc can also alter antibody binding to NK cells or macrophages. And, because no one yet knows the clinical benefits and risks of doing so in COVID-19, there remains a lack of consensus as to the best approach. Different companies are placing different bets.

Some are choosing to knock out the antibody's effector cell functions, by introducing the so-called LALA double mutation into the Fc. By interfering with antibody binding to Fc receptors on NK cells and macrophages, such mutations can mitigate activation of these effector cells, which mediate inflammation. "They cause cytokine release, they cause direct attack on the infected tissue, they cause

inflammation," says Jake Glanville, CEO of Centivax, which has an anti-SARS-CoV-2 mAb in preclinical development. For a newly infected patient, that may be okay, he says. "You can put up with a little inflammation in exchange for better viral clearance. But that's a terrible idea to give to someone who has their lungs totally colonized." Centivax therefore knocked out effector function in its lead antibody. Lilly's second antibody, etesevimab, does this too, as do Sorrento Therapeutics' antibodies.

Another safety concern is antibody-dependent enhancement (ADE), whereby the antibody potentiates virus uptake by a macrophage, enabling entry and replication, increasing viral load and worsening disease. But evidence is mounting that the ADE risk may be small. "SARS-CoV-2 doesn't naturally target these cells [macrophages]," says University of North Carolina molecular virologist Tim Sheahan. "It's not known technically if these cells are even able to support the complete life cycle of virus replication." Hundreds of thousands of COVID-19 patients have received convalescent plasma therapy — containing a wide variety of antibodies — without ADE. Enhanced disease has also not been reported in human COVID-19 vaccine trials.

Nevertheless, an antibody with intact effector function could lead to an exaggerated immune response. This is a real threat to patients with advanced COVID-19. "In the very severe patients hospitalized on respirators, it probably is a concern," says Vir Biotechnology CEO George Scangos. "In newly hospitalized patients, I think the jury is still out."

To treat non-intubated patients more effectively, Vir selects its antibodies to recruit immune effector cells and mobilize them against the infection. "In all our preclinical models, effector function matters," Scangos says. Neutralizing antibodies fight infection by blocking viral entry into cells. But if these antibodies also mobilize effector cells — triggering antibody-dependent cellular cytotoxicity (by NK cells) and antibody-dependent cellular phagocytosis (by macrophages) — they also indirectly kill cells already infected by the virus. "Two ways to eliminate the infection," says Scangos. Vir expects to report phase 3 data for its lead fully human mAb, VIR-7831, by the end of January.

Vir is engineering additional effector function into its next antibody, making three Fc-region amino acid changes first described by Rockefeller University immunologist Jeff Ravetch. These mutations tighten binding to stimulatory Fc receptors on immune cells while reducing binding to inhibitory receptors. "The result is a dramatic increase in potency, in the short term," says Scangos. In animal models, Vir found that the engineered antibody boosts not just NK cell and macrophage cell killing, but also the T-cell response, because the antibody Fc engages Fc receptors on antigen-presenting dendritic cells, says Scangos. A clinical trial is planned for early 2021.

But there's limited published evidence that anti-COVID-19 antibodies require effector function to be fully protective. A mAb therapy might not need to kill infected cells with effectors: just blocking viral entry might be enough. On the other hand, mopping up infected cells could prove necessary because not all antibodies that strongly neutralize viruses in culture are protective in vivo. Sheahan's group recently reported that, in a mouse model, immune effector function does, for some antibodies, help protect against the virus. "There is a difference in efficacy, yes," says Davide Robbiani, director of the Institute for Research in Biomedicine in Bellinzona, Switzerland, and a coauthor on the paper. But "regarding efficacy and safety of antibody effector functions, it's early to be conclusive."

Besides effector function, another big divide in the field is cocktails. In addition to Regeneron, Lilly is also testing an antibody cocktail, as is AstraZeneca, which is developing two antibodies discovered at Vanderbilt University. An antibody pair can bind the virus spike protein at two distinct epitopes to overcome drug resistance if one mutates. "The virus may evolve; the virus may find ways to escape the treatment with monoclonal antibodies, especially if a single monoclonal antibody is being used," says Robbiani.

But Vir is testing single-antibody therapy, in part because its antibody binds to a viral epitope that rarely mutates, says Scangos. It will be "more difficult for the virus to escape this antibody than a cocktail of two antibodies that Regeneron or Lilly or others are bringing forward," he says. "Two isn't better than one, necessarily. Quality matters."

Centivax is also going with a single antibody. Glanville points out that resistance mutations to the anti-RSV mAb, Synagis, are rare. For SARS-CoV-2, as based on an analysis of viral sequences in public databases, "I think the amount of escape variants is going to be pretty low, and in the low cases that happens, there are these other antibodies people can take," says Glanville. "My goal is to make a medicine that's less expensive, [so] more people can take it."

Furthermore, at lower doses, intravenous infusions could be replaced by intramuscular (IM) or subcutaneous injections. Regeneron is also teaming up with gene therapy pioneer

AstraZeneca joins Russia to boost coronavirus vaccine

AstraZeneca will begin clinical trials in December on a combination regimen that includes their own COVID-19 vaccine candidate and part of Russia's Sputnik V. The UK-based pharma announced it would explore combinations of different types of vaccines following phase 3 data published in *The Lancet* showing its own vaccine yielded lower efficacy than that achieved by mRNA vaccines from Moderna and Pfizer/BioNTech. The US Food and Drug Administration granted Emergency Use Authorization for the Pfizer/BioNTech vaccine on 11 December and for the Moderna vaccine on 18 December.

Russia's Sputnik V vaccine was developed by the Gamaleya Research Institute and backed by the sovereign Russian Direct Investment Fund. It received a controversial approval from the Russian government in August before phase 3 efficacy trials had begun. The vaccine, which follows a prime-and-boost approach, combines two slightly different human adenovirus-based vectors: a recombinant adenovirus serotype 26 (Ad26) and serotype 5 (Ad5). Each bears genetic fragments that encode the SARS-CoV-2 spike glycoprotein. Interim results announced on 14 December show Sputnik V with an efficacy of 91.4% in phase 3 trials, but data have not yet been published.

Also in December, AstraZeneca and University of Oxford reported preliminary data from a phase 3 trial of their vaccine candidate, AZD1222. The vaccine showed 70.4% efficacy overall after two doses. But the company also revealed a puzzling finding: a subpopulation receiving a lower first dose, due to an error in dosing, experienced 90% efficacy.

AZD1222 uses the chimpanzee ChAdOx1 adenovirus vector to express the spike protein. AstraZeneca will test its own vaccine with Gamaleya's Ad26 vector. Some experts have warned that the other vector used by Gamaleya, Ad5, is derived from a human cold virus, which means some people may have a pre-existing immunity that could hamper response to the COVID-19 spike protein.

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Jim Wilson of the Perelman School of Medicine at the University of Pennsylvania to develop an AAV-based intranasal therapy that will express its COVID-19 antibodies in nasal epithelial cells. "Infusions are just not practical for mass release," says Glanville, who is working on injectables. Scangos agrees. "The key is going to be an IM or subcu injection," he says. "You need a lower dose to do that; you can't possibly do an IM injection with the 2-mg dose." Regeneron's infusion dose is 2.4 grams — 1.2 gram per antibody — while Vir is dosing at only 500 milligrams. "We're on our way to getting an IM formulation," says Scangos. Single antibodies would also cost less. "The right choice is to make an inexpensive monoclonal that will be mass used," says Glanville. Whether the newer antibodies can accomplish that goal is a question for 2021. ❐

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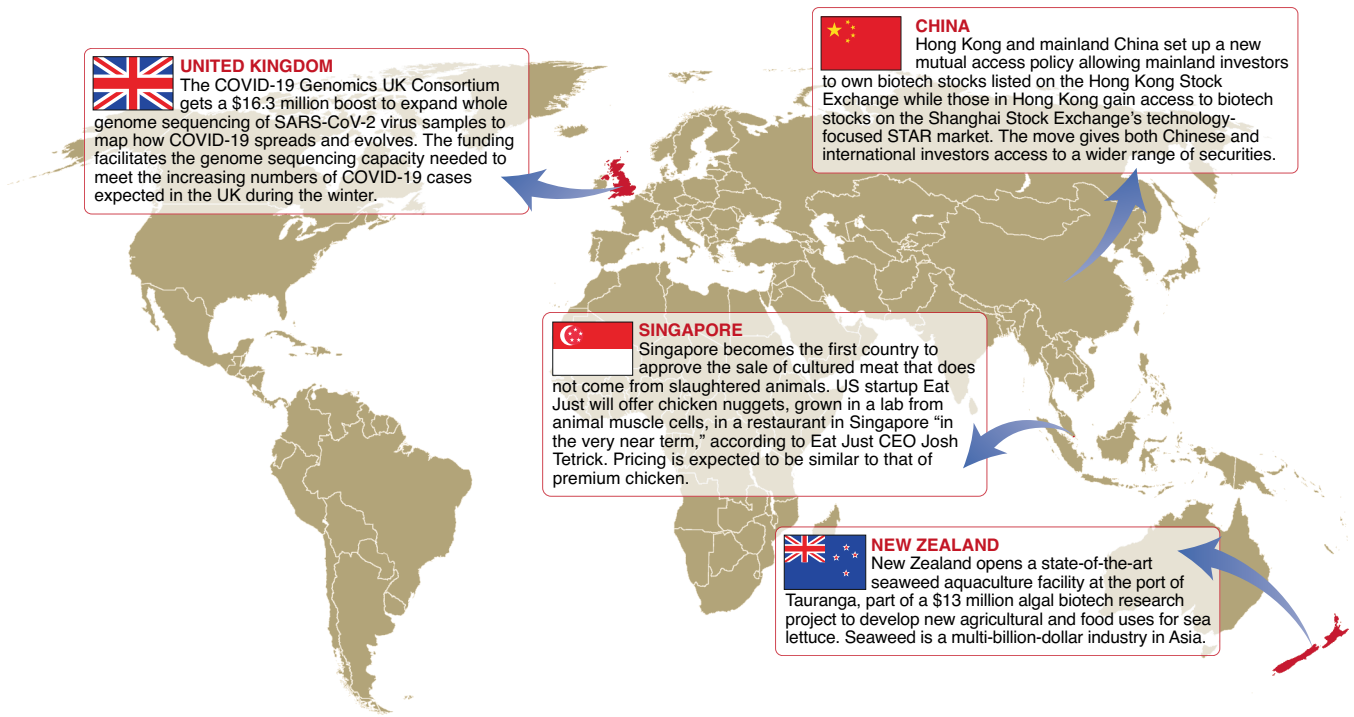
Around the world in a month



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