A*STAR RESEARCH

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KEEPING AN EYE ON COVID-19 CLUSTERS

Lessons learnt from Singapore's early cases

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MINIMIZING OUR PLASTIC WASTE FOOTPRINT

How COVID-19 has changed plastic waste production

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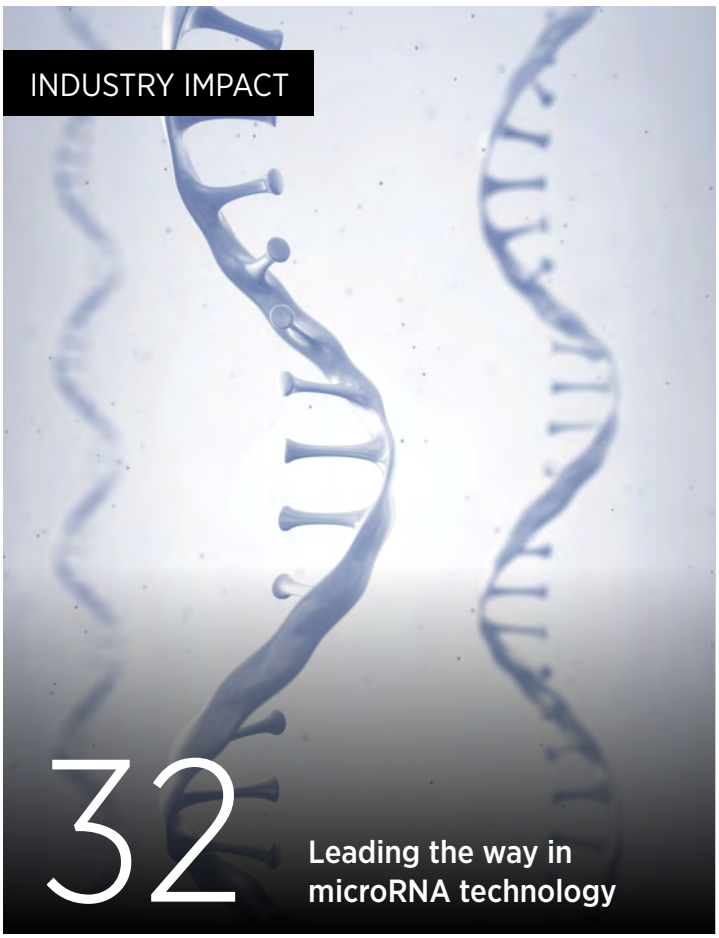

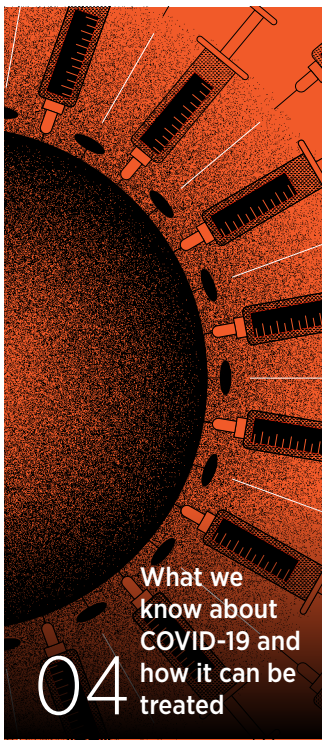

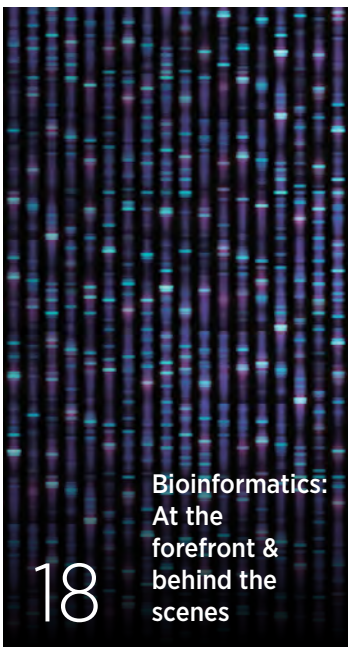

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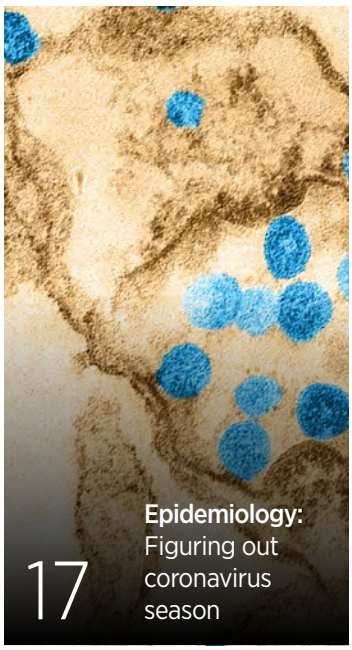
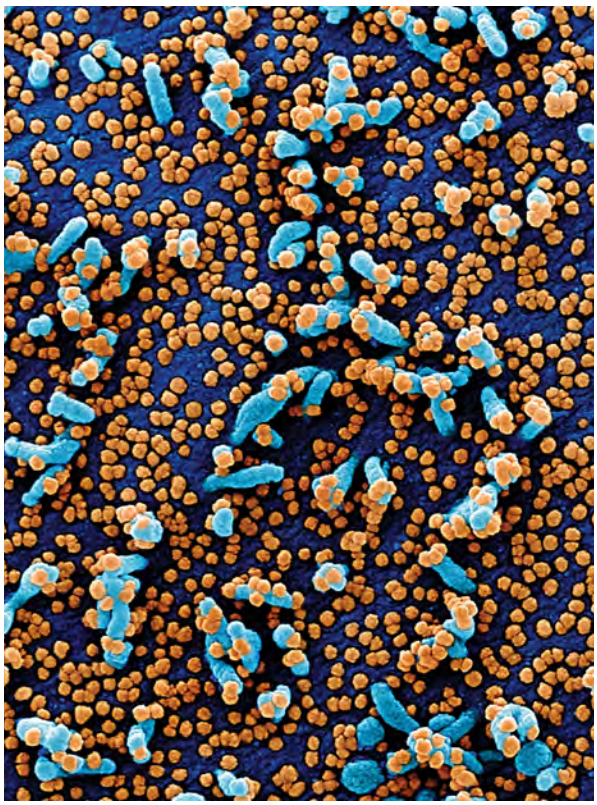


EDITORIAL NOTES



Credit: NIAID

Since it first emerged late last year, COVID-19 has swiftly brought about a remarkable transformation in the way science is performed and disseminated. For instance, the first genomic characterization of the novel coronavirus was released less than two weeks after reports of a mysterious pneumonia emerged from China. Six short months later, over a hundred potential vaccines are in clinical trials—an impressive achievement considering that research is typically a slow, deliberative process.

In this special issue of *A*STAR Research*, we trace how scientists across A*STAR have been involved since the earliest stages of the outbreak and have already made a significant mark on the world stage in these first 200 days.

The story begins with a multidisciplinary team including researchers from the Bioinformatics Institute (BII), who used a combination of epidemiological, clinical and qualitative data to identify ways to curtail the virus' stealthy spread. Read about their findings in our feature story, 'Keeping an eye on COVID-19 clusters, (p. 22).'

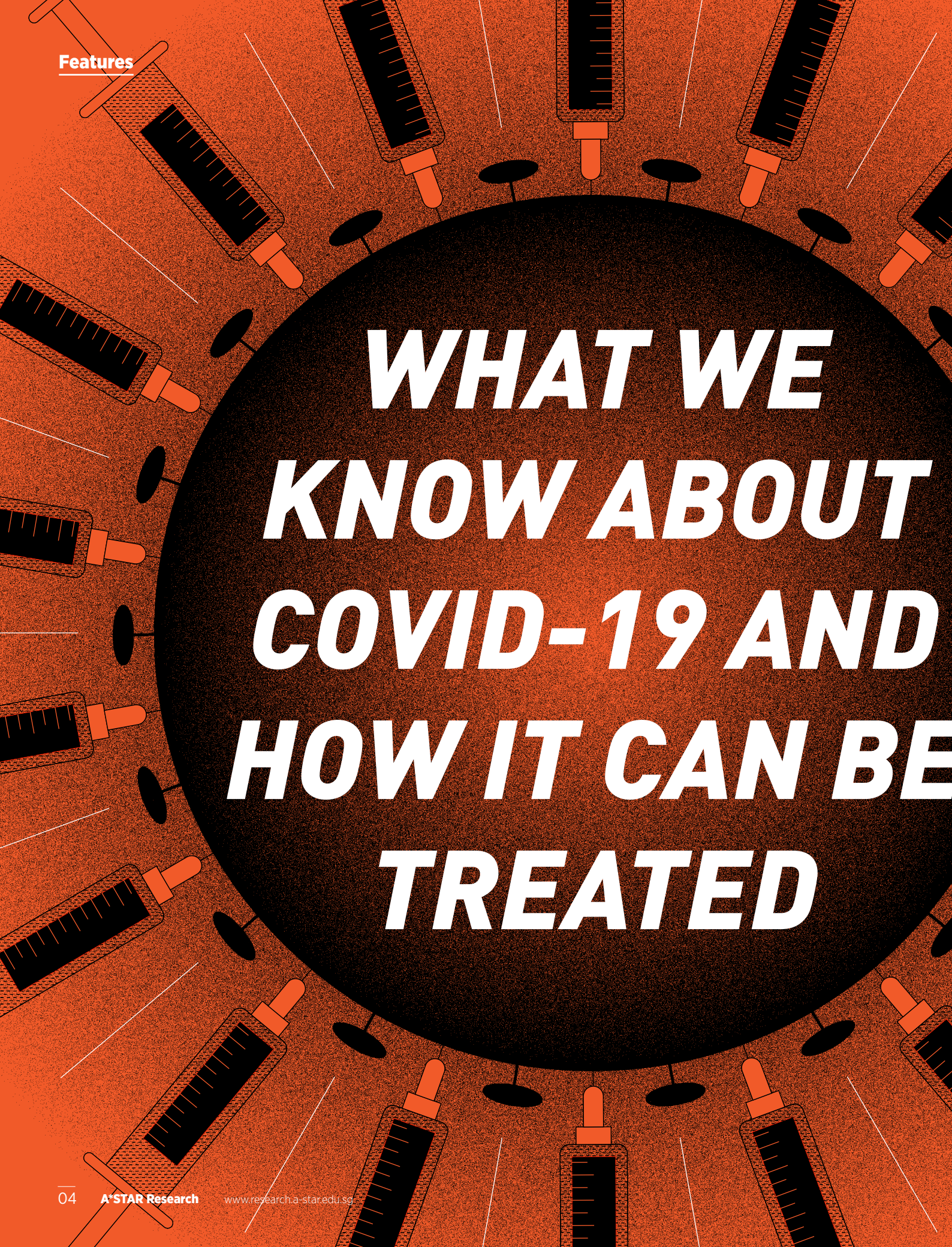
A*STAR scientists have also joined a worldwide effort investigating how genetic variations could influence COVID-19 outcomes. In 'The COVID-19 outliers, (p. 10),' we highlight how the COVID Human Genetic Effort could help us understand why the majority of those infected show no symptoms while other individuals seemingly in the pink of health have succumbed to the disease.

Moving into the realm of translational research, our industry feature showcases how A*STAR's R&D ecosystem has directly contributed to Singapore's response to the pandemic. Our feature story 'The fast track to Fortitude, (p. 26)' details how A*STAR researchers developed and deployed the diagnostic Fortitude Kit within weeks of the virus reaching local shores. In 'Identifying antibodies that work, (p. 28),' we also shine a spotlight on the world's first neutralizing antibody detection kit, proudly developed by the Diagnostic Development (DxD) Hub. Readers interested to know more about the Singapore Immunology Network's (SIgN) efforts to co-develop COVID-19 antibody-based drugs with Chugai Pharmabody Research should delve into 'Placing a 'hit' on the crown, (p. 30).'

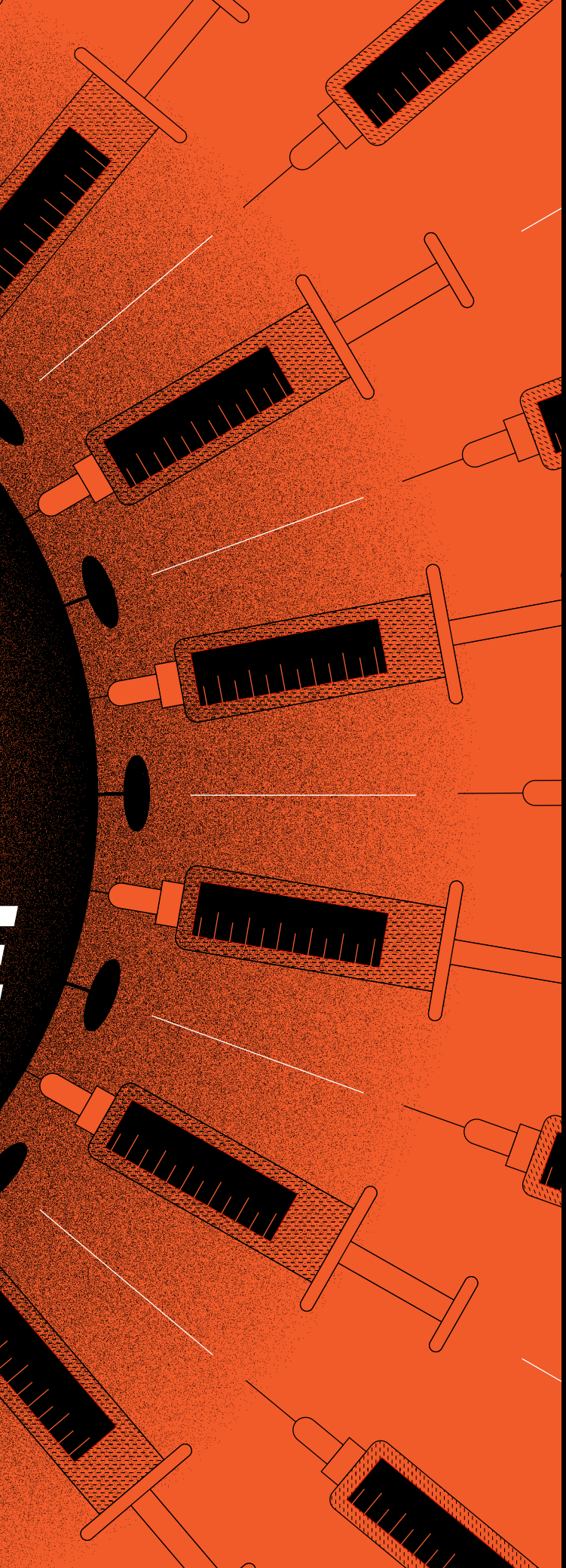
For our latest stories on COVID-19 and other scientific updates from A*STAR, visit our website: **research.a-star.edu.sg** and follow us on Twitter at **@astar_research** as well as on LinkedIn at **A*STAR Research**.

On the cover
A*STAR researchers have been actively involved in battling the COVID-19 crisis since the virus was first detected over 200 days ago.

For the latest on A*STAR's COVID-19 research, please visit: https://research.a-star.edu.sg/tag/covid-19/



WHAT WE KNOW ABOUT COVID-19 AND HOW IT CAN BE TREATED



Lisa F. P. Ng, a leading infectious disease expert on infection and immunity, shares how her team's prior experience has helped them quickly understand the nature of SARS-CoV-2, the virus that causes COVID-19.

When my team and I first learned about the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 2019 (COVID-19), we sprang into action, searching the literature to identify the gaps. In times of crisis, you don't have the luxury of time to go on 'fishing expeditions'—you need to be very targeted and selective about which direction to take.

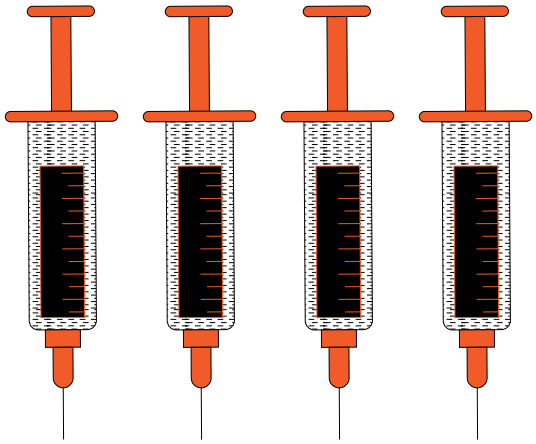
To provide the global scientific community with a roadmap to plan our next steps, we published our findings in *Nature Reviews Immunology*. While the review was addressed to our colleagues on the forefront of COVID-19 research, today, I would like to take a few moments to explain what we know about SARS-CoV-2 to readers who might not necessarily be experts but are nonetheless interested to know where we stand in our understanding of the virus and how it interacts with our immune system.

THE VIRUS: THE SECOND COMING OF SARS

SARS-CoV-2 is a coronavirus, a large family of viruses named after the 'crown' of proteins studded on their surface. Of the hundreds of different coronaviruses known to man, just seven are known to infect humans. Of these, four (229E, NL63, OC43 and HKU1) typically infect only the upper respiratory tract and cause mostly minor symptoms. The other three (SARS-CoV, SARS-CoV-2 and Middle East respiratory syndrome coronavirus, or MERS-CoV) are more deadly, and thus more well known.

As the name suggests, SARS-CoV-2 is closely related to SARS-CoV, a virus that infected approximately 8,000 people and caused almost 800 deaths around the globe between 2002 and 2004. The genomes of SARS-CoV and SARS-CoV-2 are 79 percent similar, but SARS-CoV-2 is most similar to a coronavirus found in bats, RaTG13, sharing 98 percent similarity.

Like SARS-CoV and MERS before it, SARS-CoV-2 spreads primarily through respiratory droplets and contact with contaminated surfaces. It takes about four to five days for symptoms to start showing up in infected patients,



but by 11-12 days most patients will show symptoms like fever and a dry cough. Though less common, patients infected with SARS-CoV-2 might also experience breathing difficulties, muscle or joint pain, dizziness, diarrhea, nausea or the coughing up of blood. Thankfully, most patients experience mild symptoms and recover on their own, but certain patients—particularly the elderly and those with comorbidities—progress on to severe symptoms such as acute respiratory distress syndrome (ARDS).

Once patients develop ARDS, their chances of survival fall dramatically. To improve their low blood oxygen levels, patients with ARDS require assistance from mechanical ventilators to breathe. With their reduced lung function, they are also more likely to develop secondary bacterial or fungal infections. Furthermore, the body's over-exuberant immune response to either SARS-CoV-2 or the secondary infection can result in what is known as a cytokine storm, where the overproduction of immune regulators called cytokines results in uncontrolled inflammation, inflicting damage that leads to organ failure and ultimately death.

This pattern of lung damage and hyperactivation of the immune system matches what we previously observed with SARS-CoV and suggests that the severity of the disease is determined not only by the virus itself but also the body's immune response to it.

THE IMMUNE RESPONSE: RESOLUTION OR OVER-REACTION

The first thing that SARS-CoV-2 does when it enters the body is to look for two key proteins: angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2). ACE2 is recognized by the receptor-binding domain (RBD) of the virus spike protein, while TMPRSS2 is needed for the proper processing of the spike protein required for viral entry into the cell. Once inside, the virus hijacks the cell's machinery and begins to make many copies of itself, eventually causing the death of the cell and the release of new virus particles that can go on to infect neighboring cells.

Apart from virus particles, the death of the host cell also releases molecules that activate the immune response. The first responders are usually cells called macrophages, which secrete signaling molecules called cytokines to recruit other immune cells like T cells and monocytes. In a healthy immune response, these defenders successfully contain and clear the virus without damaging the surrounding tissue, and go on to produce protective antibodies that can neutralize the virus.

However, for reasons that are still not fully understood, the inflammatory process sometimes goes out of control. The overproduction of pro-inflammatory cytokines, called a cytokine storm, can cause serious damage to the lungs and result in ARDS. In severe cases, the cytokines circulate to other organs and could cause multi-organ failure.

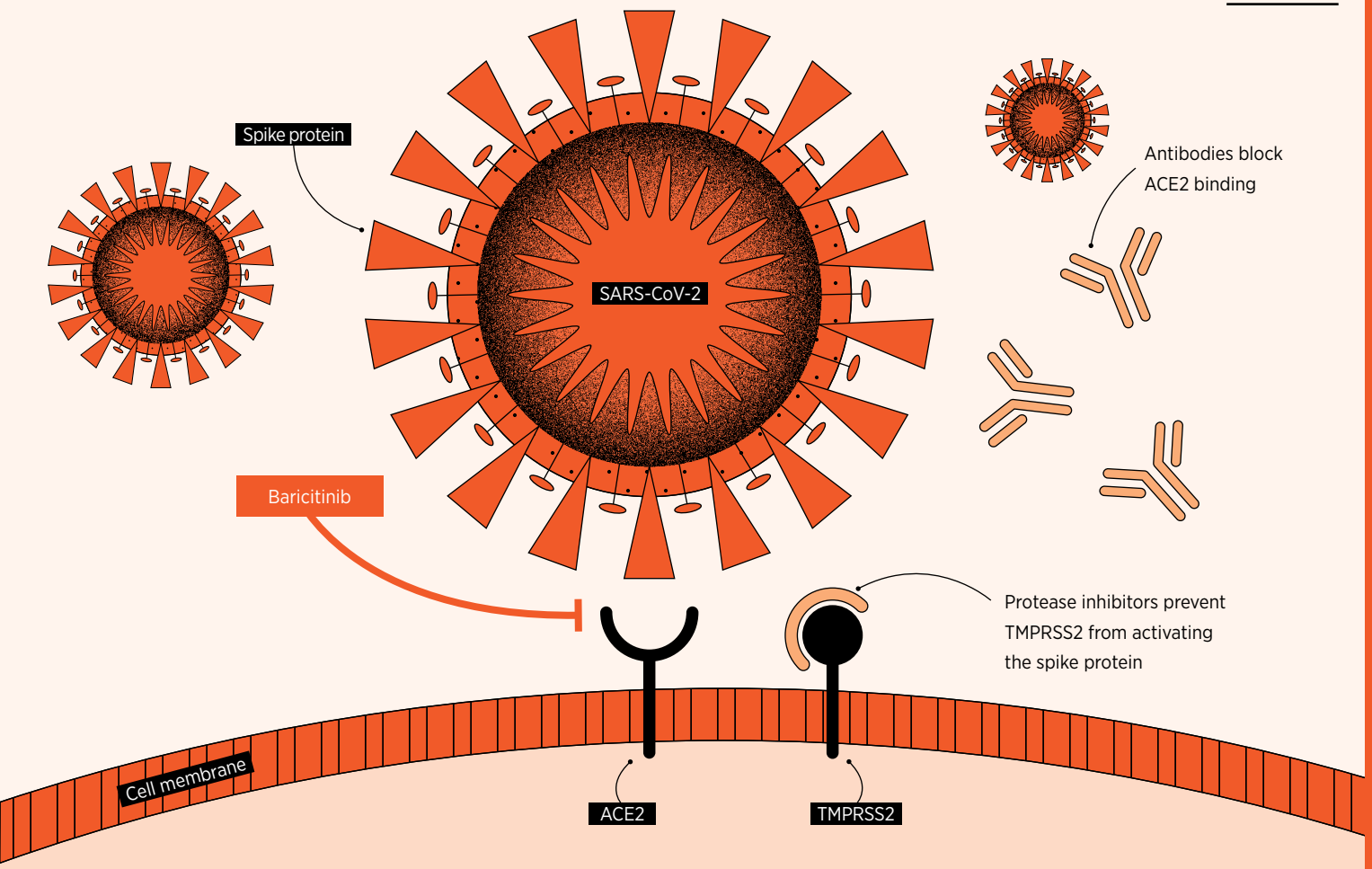
THE WAY FORWARD: TREATMENTS AND VACCINES

Thanks to all the knowledge we have about SARS-CoV-2 across many different studies, we are now in a better position to tackle this new threat. Firstly, because we know that the virus requires both ACE2 and TMPRSS2 to enter cells, one promising approach would be to block or interfere with these two proteins using existing drugs.

A machine-learning study, for example, has predicted that a rheumatoid arthritis drug called baricitinib could inhibit ACE2. Alternatively, patients could be infused with high levels of a soluble form of ACE2 to prevent the virus from binding to cells, a hypothesis being tested in a clinical trial for APEIRON, a recombinant form of ACE2. Drugs like nafamostat mesylate and camostat mesylate, on the other hand, are known to inhibit TMPRSS2 and could be quickly repurposed for treating COVID-19.

Instead of targeting ACE2 on the host cells, scientists are also trying to block the spike protein on the virus using therapeutic monoclonal antibodies, either based on those found in sera from recovered patients or antibodies previously identified against SARS-CoV. Antibodies that can neutralize the virus are also the goal of several vaccine candidates, while others instead seek to provoke a long-lasting memory $CD8^+$ T cell response.

Last but not least, since it appears that an over-active immune response is what contributes to severe disease in COVID-19, researchers are also attempting to find ways to dampen the effect of cytokine storms. For example, several trials are investigating the use of drugs such



as tocilizumab and sarilumab that can block the effect of a key cytokine called IL-6. Similarly, other trials are investigating the blocking of granulocyte–macrophage colony-stimulating factor (GM-CSF), which is believed to be a key driver of lung inflammation in COVID-19 patients. Rather than use drugs, one interesting approach is to filter patients' blood through special columns that can trap excess pro-inflammatory cytokines, thereby reducing their impact on the body.

In addition to my colleague Laurent Renia, Executive Director of A*STAR's Infectious Diseases Horizontal Technology Centre (ID HTC); and collaborator Paul MacAry, Associate Professor at the National University of Singapore; I would like to thank my co-authors Matthew Tay and Chek Meng Poh. Matthew contributed to the writing of this review with his expertise in B cells and antibodies, while Chek Meng brought his wealth of experience from working as an A*STAR International Fellow in one of the best respiratory infection labs in Hong Kong. I would like to encourage younger researchers like Matthew and Chek Meng to continue deepening their knowledge in their chosen fields. As our experience thus far with COVID-19 has shown, whatever expertise you now have in a particular area could one day become useful in another unexpected situation, so keep pursuing knowledge and finding new ways to repurpose it. ★

ABOUT THE AUTHOR:

Lisa F.P. Ng obtained her PhD in molecular virology in coronaviruses from the National University of Singapore (NUS) in 2002. After joining A*STAR's Genome Institute of Singapore (GIS) in 2002 as a postdoctoral fellow, she worked on viral diseases such as hepatitis, severe acute respiratory syndrome and influenza. Ng is currently the Executive Director of A*STAR's Graduate Academy (A*GA) and she holds a concurrent appointment as Senior Principal Investigator at A*STAR's Singapore Immunology Network (SIgN) where she focuses on the immune responses to arthritic arboviruses that are epidemic or highly endemic in the tropical region.

1. Tay, M., Poh, C.M., Rénia, L., MacAry, P.M., Ng, L.F.P. The Trinity of COVID-19: Immunity, Inflammation and Intervention. *Nature Reviews Immunology* **20**, 363–374 (2020).

VIROLOGY

To control outbreaks, go back to basics

Experts suggest that the best way to control an outbreak may be to first understand viral biology and behavior in animals and humans.

First discovered nearly a century ago in animals, coronaviruses have caused serious diseases in animals from mice to chickens and, more recently, humans. SARS-CoV-2 is the third zoonotic coronavirus after SARS-CoV and MERS-CoV, but appears to be the only one so far with pandemic potential.

Lisa F. P. Ng, a Senior Principal Investigator at A*STAR's Singapore Immunology Network (SIgN), says that understanding coronavirus biology and behavior in animals will help us manage the diseases they cause in humans.

This is the basis of an editorial published in *The BMJ* by Ng and Julian Hiscox, Chair in Infection and Global Health at the University of Liverpool, who stem from the same 'lineage' of coronavirus researchers of more than 20 years. They discuss some of the main avenues where prior research on coronaviruses provide context and inform strategies for current and future outbreaks.

For example, the outbreaks of two other deadly coronaviruses, SARS-CoV and MERS-CoV, which jumped into humans from animals, reveal just how difficult it can be to control zoonotic viruses. It took over a year to contain SARS-CoV, which infected fewer people from 2002–03 than SARS-CoV-2 to date. Meanwhile, some MERS-CoV outbreaks have continued to occur since it emerged in 2012.

The authors also note that there are currently no approved vaccines or antiviral drugs against human coronaviruses. Nevertheless, Ng thinks our chances of developing a vaccine are high. "The challenge," she noted, "will be to develop a 'universal' SARS vaccine."

Meanwhile, antivirals are plagued by the possibility of resistant strains. "This happens when the antiviral drugs do not completely inhibit virus replication, but partially control the virus," explained Ng. "This will allow a sub-species of the virus to replicate at low levels that could become totally resistant after several rounds of passages."

To reduce the risk of resistance, the authors suggest targeting proteins that viruses use for replication in host cells, which could also reduce development time and work against multiple coronaviruses because they share similar replication mechanisms.

Given the impact of asymptomatic infections, underlying conditions and coinfections in the current and past outbreaks, the authors note that it will be important to develop accurate serological assays to help us understand the immune response of individuals.

"Understanding infection kinetics will enable the development of novel antiviral compounds to target the virus," said Ng. "Understanding immunity and disease progression will enable the development of novel immune-based therapies against SARS-CoV-2."

As for future outbreaks, Ng expects that the COVID-19 pandemic will equip us with the tools and knowledge needed to shut down transmission chains quickly, ranging from real-time viral genomic sequencing to the development of prognostic biomarkers. ★

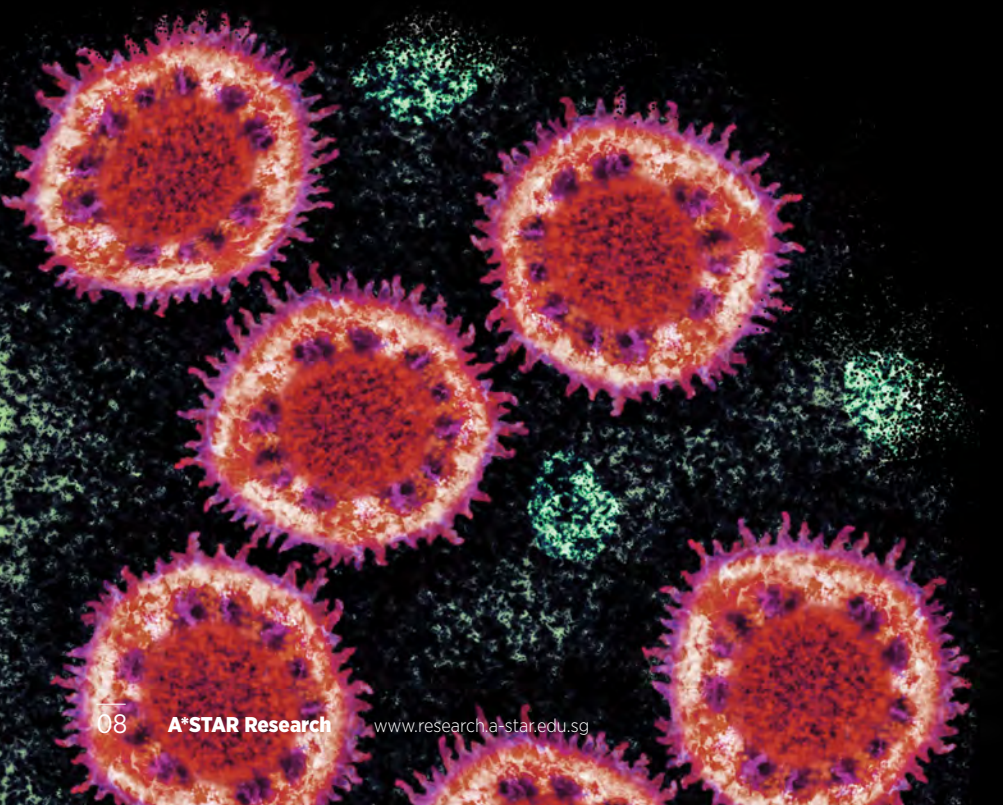


Photo credit: Nixx Photography / Shutterstock

BACKGROUND

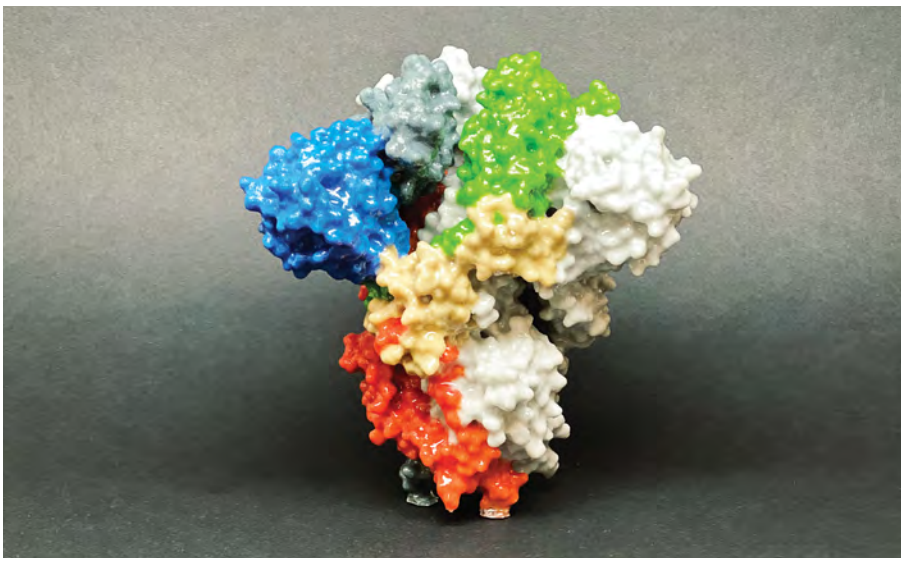
The SARS-CoV-2 pandemic and previous coronavirus outbreaks are caused by zoonotic viruses, which jumped into humans from animals.

1. Ng, L. F. P., Hiscox, J. A. Coronaviruses in animals and humans. *BMJ* **368**:m634 (2020).

IMMUNOLOGY

Getting the SARS-CoV-2 antibody response straight

Two linear peptides on the SARS-CoV-2 spike protein may improve the specificity of serological assays and show potential as vaccine candidates.



Antibodies that recognize linear epitopes could form the basis of more accurate COVID-19 tests. 3D print of a spike protein on the surface of SARS-CoV-2, the virus that causes COVID-19.

Photo credit: © US National Institutes of Health

As researchers work around the clock to find ways to stop SARS-CoV-2, one feature—the 'crown' of spike proteins after which the family of viruses is named—has received a lot of attention. SARS-CoV-2, like its deadly cousins SARS-CoV and MERS-CoV, uses its spike protein to attach to and invade cells. Understanding how the human immune system recognizes the spike protein is thus essential for designing more accurate diagnostics and vaccines against COVID-19.

When a person is infected with SARS-CoV-2, the antibodies they produce detect either small fragments of the virus in its 3D form (conformational epitopes) or a sequence of amino acids with no secondary structure (linear epitopes). However, one possible reason that early diagnostic tests targeting conformational epitopes were not as accurate as hoped is that other viruses may have similar 3D motifs. Furthermore, SARS-CoV-2 conformational epitopes might also be recognized by non-specific antibodies, potentially giving rise to false-positive results.

"Linear epitopes are a lot less likely to be recognized by non-specific antibodies due to their short sequences," said Lisa F. P. Ng, a Senor Principal Investigator at A*STAR's Singapore Immunology Network (SIgN). In a study published in *Nature Communications*, Ng and her team identified two linear epitopes of SARS-CoV-2 that were strongly and specifically recognized by antibodies from COVID-19 patients.

The researchers, led by study co-first authors Chek Meng Poh and Guillaume Carissimo, Research Fellows in Ng's lab, exposed pools of five overlapping peptides covering the length of the SARS-CoV-2 spike protein to antibodies from patients who had developed COVID-19, healthy controls and those who had recovered from SARS, which struck in 2003.

Through multiple rounds of screening, the researchers found two peptides, S14P5 and S21P2, that were recognized by antibodies from COVID-19 patients but not SARS patients or healthy controls. Ng expects that these peptides will be useful for designing highly specific serological assays to determine the extent of exposure to SARS-CoV-2 in the population, as well as for measuring immune responses in clinical trials for vaccine candidates.

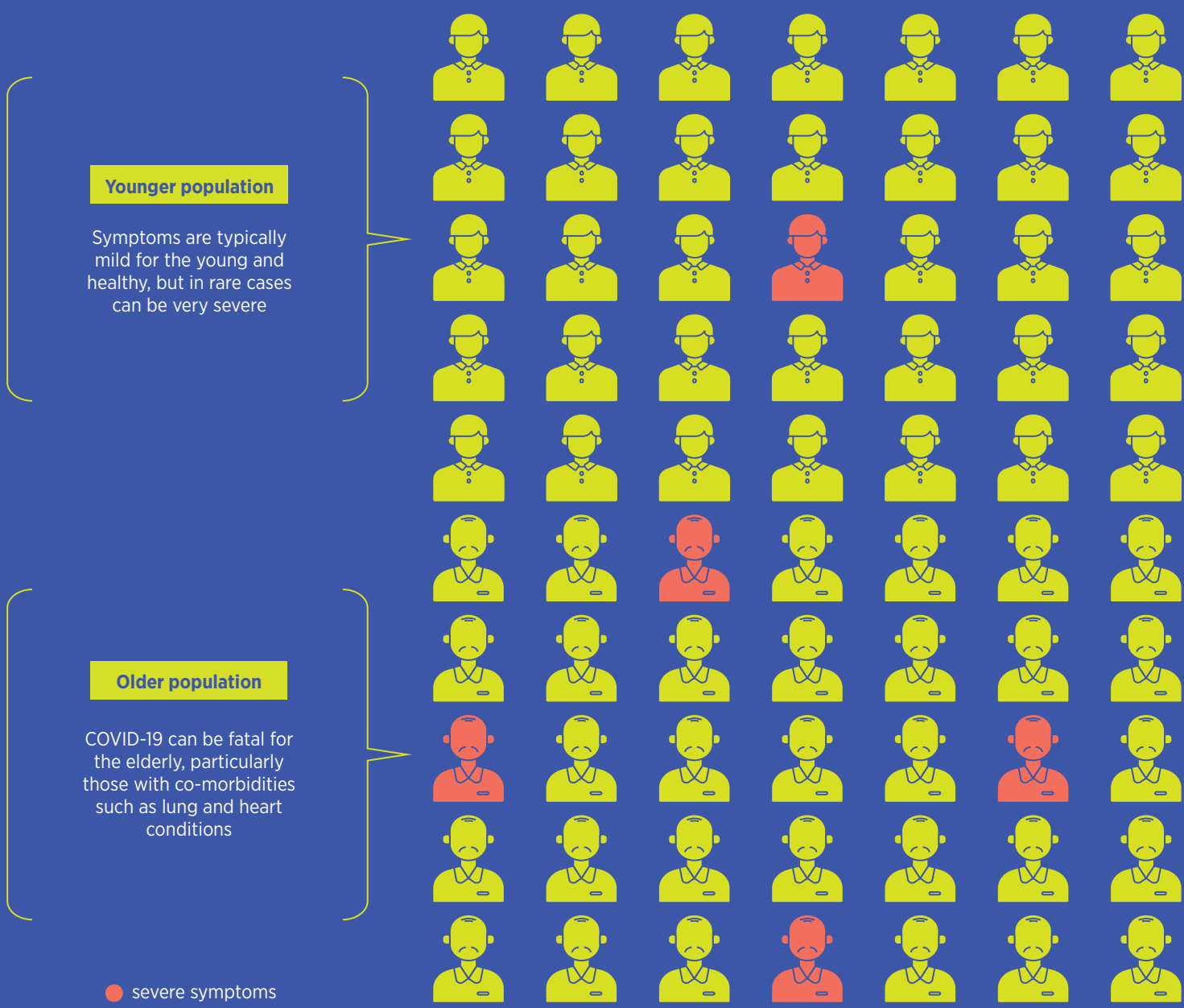
The researchers also showed that antibodies targeting S14P5 and S21P2 can neutralize or block SARS-CoV-2 infection. "Further studies will be needed to determine if these epitopes can be used as vaccine candidates to generate sufficient levels of antibodies in humans and protect against COVID-19," said Ng. "Developing specific monoclonal antibodies against these epitopes as a potential treatment for COVID-19 would be the next step." ★

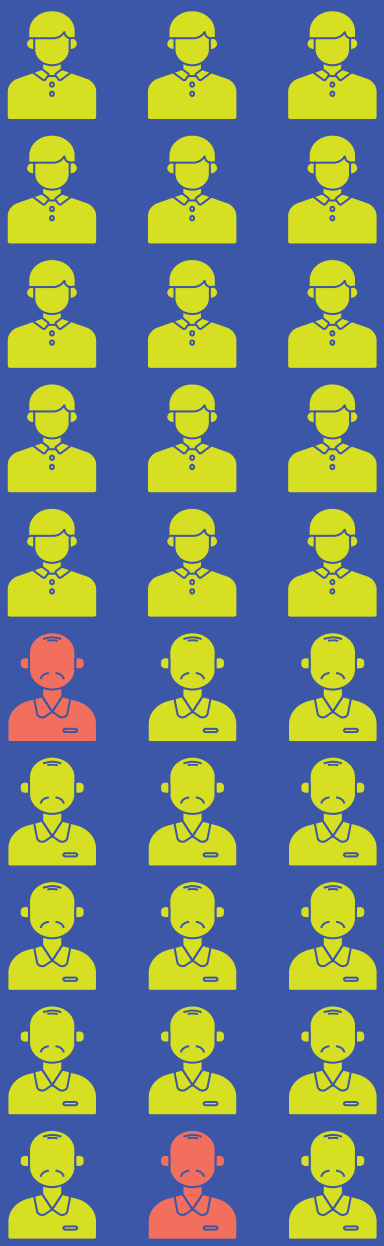
1. Poh, C. M., Carissimo, G., Wang, B., Amrun, S. N., Lee, C. Y. *et al*. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. *Nature Communications* **11**, 2806 (2020).

GENETICS

The COVID-19 outliers

An international genetics consortium seeks to unravel the mystery of why COVID-19 infection is silent in some cases and lethal in others.





When describing an 'outlier,' we may think of someone who is exceptionally gifted at sports, or someone with a particularly high IQ. It turns out that there are also outliers among COVID-19 patients—they exhibit massive variability in outcomes ranging from silent infection to lethal disease.

So why do some people remain seronegative despite heavy or repeated exposures to the virus, while young, previously healthy individuals end up getting admitted to intensive care for a life-threatening disease? Here, the role of human genetics in determining clinical response to the SARS-CoV-2 virus remains to be defined.

To study the immense clinical variability observed among infected individuals, the COVID Human Genetic Effort consortium was established in February 2020 by Jean-Laurent Casanova, a professor at the Rockefeller University, to recruit patients from multiple centers and countries, targeting patients below the age of 50 years with a life-threatening disease and no pre-existing medical conditions.

Not only will this consortium facilitate the detection of individuals naturally resistant to SARS-CoV-2 infection, it will also open up new avenues for controlling COVID-19 in the general population by providing potential pharmacological targets for development.

"This global consortium aims to study COVID-19, which is a completely new disease, and the human genetics that could define disease resistance or severity," said Lisa F. P. Ng, a consortium member and Senior Principal Investigator at A*STAR's Singapore Immunology Network (SIgN). Ng is joined on the consortium by Laurent Renia, Executive Director of A*STAR's Infectious Diseases Horizontal Technology Centre (ID HTC).

In a commentary in the journal *Cell*, the authors reveal that the consortium has embarked on genome-wide association studies of COVID-19 patients based on their exome and genome data. Data analysis will take place both locally at the hubs and centrally by the consortium, followed by clinical and immunological studies.

Establishing the consortium in the early stages of the pandemic means that there remains low signal interference from confounding variables such as vaccines, previous related infections and herd immunity, the authors explained in the commentary.

Based on the data collected, the consortium researchers hope to find out whether inborn errors of inflammation, or mutation of key genes involved in the immune response, play a role in infection or immunity to COVID-19. Their effort will also determine any links between such genes and ethnicity, should they exist.

"Not only will this consortium facilitate the detection of individuals naturally resistant to SARS-CoV-2 infection, it will also open up new avenues for controlling COVID-19 in the general population by providing potential pharmacological targets for development," Ng said. ★

1. Casanova, J-L., Su, H. C., and the COVID Human Genetic Effort. A Global Effort to Define the Human Genetics of Protective Immunity to SARS-CoV-2 Infection. *Cell* **181**(6), 1194-1199. (2020).

STAYING AHEAD OF THE

Serology testing may be fast and easy to perform, but there are still some limitations that need to be overcome, suggests Laurent Renia.

Because of its high sensitivity and accuracy, quantitative RT-PCR is currently the gold standard for diagnosing COVID-19 infection in the acute phase of the illness. However, there are certain limitations: apart from being costly and time-consuming, false negative results may arise due to insufficient viral genetic material, biological variation or improper handling of nucleic acid samples.

This has motivated industry and academic scientists to consider immunoassays, a rapid test format that may deliver results in a shorter time window and at lower cost. To date, tests are commercially available and can be used to detect SARS-CoV-2 antibodies from patient serum or plasma samples.

In this review, my colleagues and I at A*STAR's Singapore Immunology Network (SIgN) and the National University of Singapore provide an overview

of the immunoassays currently available for SARS-CoV-2 detection. We also discuss the strengths, limitations and applications of this diagnostic technique, and what to expect next.

We wrote this review to help clinicians understand serology assays better, building on our experience working on serology for infectious diseases such as Zika and chikungunya. We also clear up any misconceptions about serology testing and clarify the technical parts of it without making it too complicated for non-immunologists.

WHICH ANTIBODIES ARISE AND WHEN?

To draw reliable conclusions from immunoassays, it is important to note that the timing of testing matters greatly, since it provides a snapshot of an individual's antibody profile at that point in time.

According to recent studies, both anti-SARS-CoV-2 IgM and IgG antibody levels increase gradually along with infection phases. IgM was detected as early as three days post-illness onset, peaking between two to three weeks post-illness onset, while IgG was detected as early as four days post-illness onset, peaking after 17 days post-illness onset.

Similar to SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV), both IgM and IgG levels in SARS-CoV-2 patients appear to be correlated with disease severity, with a higher level of both antibodies present in patients with more severe infections.

However, one study showed no specific chronological order in terms of IgM and IgG seroconversion. In addition, there also seemed to be no correlation between seroconversion rates and age, gender or time of hospitalization. Thus, these findings suggest that it may be important to test for both IgM and IgG antibodies to confirm a positive infection.

IDENTIFYING ANTIBODY TARGETS

Current serology assays mainly target two immunogenic coronavirus proteins: S protein, which is the most exposed viral protein; and N protein, which is abundantly expressed during infection. The receptor-binding domain (RBD) of the S protein is also a target of interest.

To reduce the time needed to develop novel neutralizing antibodies, it may be possible to use computational prediction to identify existing SARS-CoV antibodies that may recognize SARS-CoV-2 epitopes, the specific part of the target protein that binds to the antibody. For example, 49 out of 298 linear SARS-CoV-derived B-cell epitopes have an identical match with SARS-CoV-2 protein sequences, with the majority of matches located at the

S and N proteins. Further mapping revealed several regions on the S2 subunit that may allow cross-neutralization of both SARS-CoV and SARS-CoV-2.

Beyond the development of new immunoassays, studying SARS-CoV-2 epitopes where SARS-CoV-2-specific antibodies bind to may also help guide the design of a vaccine.

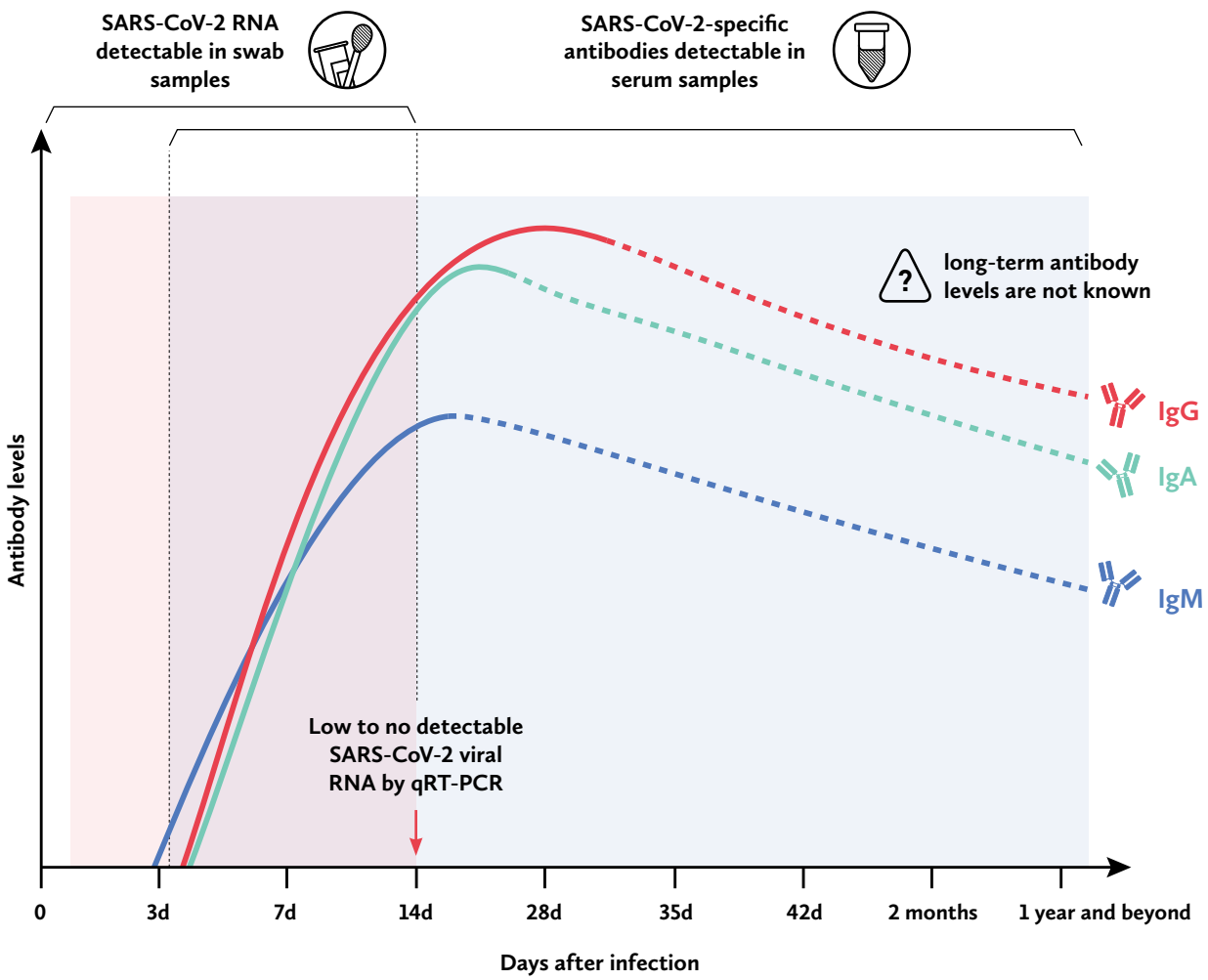
ENSURING TESTS ARE SPECIFIC TO SARS-CoV-2

As the majority of human populations have had prior exposure to endemic human coronavirus infections, it is necessary to validate the specificity and sensitivity of current immunoassays against SARS-CoV-2 to avoid false positive outcomes.

The high level of cross-reactivity between SARS-CoV and SARS-CoV-2 can be attributed to the high degree of genetic homology. Detailed analysis reveals a highly conserved S2 subunit domain across the coronaviruses. Thus, using an S1 subunit-based immunoassay may be more specific than the entire S antigen for diagnosing SARS-CoV-2 infections.

Since respiratory diseases are the hallmark of coronavirus infections, several studies have exploited the detection of IgA antibodies to diagnose SARS-CoV-2 infections. However, IgA-based immunoassays have been hypothesized to be less specific than IgG-based ELISA assays due to cross-reactivity with serum samples from patients infected by other coronaviruses.

Given the availability of immunoassays against various coronavirus structural proteins, it may be prudent to



Antibodies against SARS-CoV-2 (IgG, IgA and IgM) develop by three or four days after infection but only reach peak levels within two or three weeks. The long-term persistence of antibodies in the blood is still not known, as indicated by the dotted line.

We hope to consolidate what we have learnt about COVID-19 to help us prepare for future outbreaks.

use more than one antigen-based serological approach to establish a true positive SARS-CoV-2 infection. In addition, the use of saliva samples and other bodily fluid swabs could also be explored as a less invasive alternative for sample collection.

LIMITATIONS OF SEROLOGY TESTING

While it may be fast, robust and easy to perform, one of the major setbacks of serology testing is its inability to detect the presence of infection during the early stages of disease, as antibodies take several days to weeks to be generated after exposure to foreign material. As such, serological testing may provide false negative results for an early SARS-CoV-2 infection and the use of quantitative RT-PCR may be more suitable in acute cases.

Furthermore, due to the unique genetic makeup of each individual, an inherent variability in antibody response could explain the variety of antibody profiles elicited among COVID-19 patients.

Cross-reactivity could be another limitation of immunoassays as it severely impacts the specificity and sensitivity of the test. Although the phylogenetically closest coronavirus, SARS-CoV, has not been reported to be circulating in the human population since 2004, other endemic human coronaviruses may still pose a problem to the accurate diagnosis of a SARS-CoV-2 infection.

POTENTIAL FOR SURVEILLANCE, DETECTION AND INTERVENTION

Given the rapid rise in the number of confirmed COVID-19 cases coupled with the worldwide demand for test kits, decentralized point-of-care tests may provide a cost-effective and straightforward option for SARS-CoV-2 diagnosis. The lateral flow assay, for example, is a paper-based platform used to detect and quantify analytes in complex mixtures.

Ultimately, the goal of serology testing for SARS-CoV-2 is to achieve an axis of surveillance, detection and intervention. Surveillance is how can you anticipate or recognize signs of an upcoming outbreak, while detection is about developing tools to identify the new pathogen. Last but not least, intervention covers measures to prevent the virus from spreading, such as by using face masks and social distancing, and ultimately vaccines.

It is also for these reasons that A*STAR's Infectious Diseases Horizontal Technology Centre (ID HTC), where I serve as Executive Director, was established. We will always have infectious disease because Singapore is based on an open economy requiring goods exchange and travel. Once country borders are re-opened, and as soon as you facilitate the movement of people, goods and animals, you facilitate the movement of pathogens.

The idea of the Horizontal Technology Centre is to bring together different pockets of expertise on a regular basis. We hope to consolidate what we have learnt about COVID-19 to help us prepare for future outbreaks. ★

ABOUT THE AUTHOR:

Laurent Renia is Executive Director of the A*STAR Infectious Diseases Horizontal Technology Centre (ID HTC), which was established on July 1, 2020. He received his PhD degree in 1991 from the University Pierre et Marie Curie in Paris, France, where he studied the immune response against malaria. Prior to joining A*STAR, Renia was a Research Director at the French National Institute of Health and Medical Research (INSERM), and also Director of the Department of Immunology at the Institut Cochin. After joining the Singapore Immunology Network (SIgN) in 2007, he served as its Executive Director from 2014 to 2020. He has published more than 260 articles and book chapters on the research topics of infection, immunity and oncoimmunology.

1. Lee, C. Y-P., Lin, R. T. P., Renia, L. and Ng, L. F. P. Serological Approaches for COVID-19: Epidemiologic Perspective on Surveillance and Control. *Frontiers in Immunology*. **11:**879 (2020)

ABOVE
Viral-directed therapies such as remdesivir target the coronavirus replication cycle.

DRUG DISCOVERY

A two-pronged attack on COVID-19

Antiviral strategies against COVID-19 broadly fall into two groups: those that target the virus and those that target the host factors that are essential for virus replication.

Among the coronaviruses known to infect humans, the clear winner at human transmission is the novel coronavirus, SARS-CoV-2, despite a lower mortality rate when compared to its cousins, SARS-CoV and MERS.

Since its discovery in the Chinese city of Wuhan in December 2019, numerous antiviral drugs have been tested for efficacy against SARS-CoV-2. At the time of publication, more than 400 clinical trials have been registered in ClinicalTrials.gov.

Providing a bird's eye view of the current landscape, Justin Jang Hann Chu, a Joint Senior Principal Investigator at A*STAR's Institute of Molecular and Cell Biology (IMCB), and colleagues review the two main antiviral strategies currently in play—those that directly target the virus and those that indirectly target the cellular factors required for virus replication.

"Both antiviral strategies are promising," said Chu. "Ideally, having a combination of an antiviral that targets both the virus and cellular factors required for virus replication could result in shorter treatment times, reduced emergence of drug resistance and fewer side effects," he explained.

The first strategy—viral-directed therapies—targets the main viral replication processes in the coronavirus replication cycle. Drugs that fall into this group hinder viral entry into the host cell, or stop the virus from replicating itself. For example, viral genome replication inhibitors under testing include ribavirin, remdesivir and favipiravir.

"Drugs that target viral proteins or the viral replication process have been shown to exert good efficacy and be highly translational for clinical application," said Chu. "Targeting the virus is straightforward and leads to fewer side effects. However, as SARS-CoV-2 is an RNA virus with a higher mutation rate, the emergence of drug-resistant virus mutants remains a serious concern."

In contrast, host-directed therapies indirectly target the virus by managing clinical symptoms of the disease. Through a myriad of ways, it is possible to enhance the innate immune response or reduce inflammation to fight the effects of the virus on the host. Candidate treatments include resveratrol, indomethacin and HIV-1 protease inhibitors such as nelfinavir and lopinavir.

Describing the COVID-19 pandemic as one that will require "intense and sustained effort to control," the authors are nonetheless optimistic that at least one of the 400 antiviral strategies in clinical trials will lead to its successful resolution, given the collaboration across academia, industry, governments and international organizations. ★

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EPIDEMIOLOGY

Figuring out coronavirus season

Understanding how closely related coronaviruses circulate seasonally could impact the timing of vaccination against SARS-CoV-2.

As scientists around the world race to develop a vaccine against COVID-19, one hurdle that stands in their way is the possibility that antibodies against SARS-CoV-2 end up worsening the disease instead of eradicating its target. Called antibody-dependent enhancement (ADE), this phenomenon has been observed for other viral diseases such as dengue.

The issue with SARS-CoV-2 is that its similarity to other human coronaviruses circulating in the population means that pre-existing antibodies to these 'common cold' viruses could drive ADE. For this reason, ADE has been hypothesized as one possible explanation of why some COVID-19 cases are more severe than others.

"If and when the SARS-CoV-2 vaccines are licensed, these may initially protect against SARS-CoV-2 infections, but we don't know if they may predispose people to more severe infections via ADE with any of the seasonal coronavirus infections," said study co-author Hong Kai Lee of A*STAR's Singapore Immunology Network (SIgN).

Driven by this concern, Lee and a team of international collaborators compared the seasonal peaks of common human coronaviruses with that of influenza A and B, using data from diagnostic laboratories from various countries. "Setting influenza incidence as the baseline for comparison will help guide vaccination strategies once the yearly mutation rates of the novel coronavirus are established," he said.

The study found that in temperate countries such as Canada or the UK, coronavirus infections typically peak either slightly before or simultaneously with the influenza A and B peaks. In contrast, there was no consistent relationship between the influenza A, influenza B and coronavirus peaks in Singapore.

"If there is a predictable seasonality for coronaviruses which differs from the SARS-CoV-2 seasonality, you could try to vaccinate against SARS-CoV-2 when seasonal coronaviruses are not circulating," Lee explained. In the case of Singapore, where coronaviruses are found to be circulating all year round, the vaccines also need to be available all year round. Policymakers thus may consider the significant challenges for future SARS-CoV-2 vaccine procurement, stock maintenance and administration, he added.

"Ideally, we want to first develop accurate and reliable serology assays for each of the four seasonal coronaviruses, OC43, 229E, NL63 and HKU1," Lee said. Patients with COVID-19 can then be screened to determine if there is any correlation between antibodies against other coronaviruses and disease severity, and ultimately answer the question of whether ADE occurs in COVID-19 as it does for diseases like dengue and respiratory syncytial virus. ★

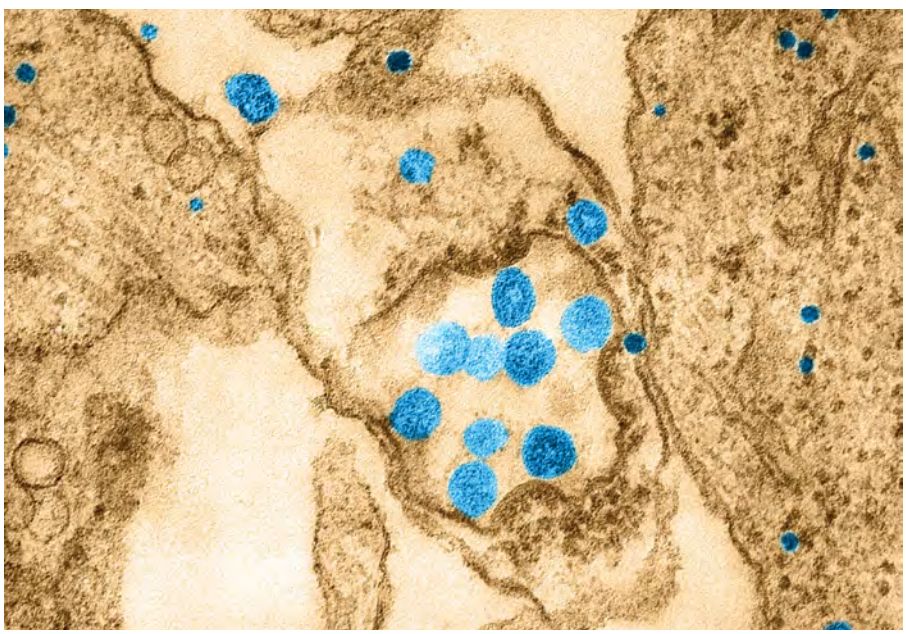

Photo credit: Science Source / Science Photo Library

LEFT
Scientists are investigating the best time to vaccinate people against SARS-CoV-2 to minimize interactions with other viruses.

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BIOINFORMATICS: AT THE FOREFRONT & BEHIND THE SCENES

Sebastian Maurer-Stroh shares how bioinformatics has enabled Singapore to respond quickly to COVID-19 and will continue to play an important role in ongoing surveillance.

Bioinformatics is an inherently interdisciplinary effort, combining the molecular biology revolution heralded by pioneers like Watson and Crick with advances in computer science that have placed previously unimaginable abilities at our fingertips. Reflecting the collaborative nature of bioinformatics, my colleagues and I at A*STAR's Bioinformatics Institute (BII) study everything from sequence analysis to image processing, working with academic, clinical and industry partners alike.

Nowhere has the synergistic power of bioinformatics been made more clear, however, than during the ongoing COVID-19 pandemic, where more than 15 million people have been infected and over half a million have sadly lost their lives in the first six months of the pandemic. Amid the uncertainty and upheaval, bioinformaticians have been at the forefront of supporting the development of urgently needed diagnostics and drug repurposing, as well as behind the scenes carefully monitoring the virus genome for potentially dangerous mutations and tracing the virus evolution to study and help curb transmission.

FROM SEQUENCE TO TEST KIT

We first heard reports of an unusual viral pneumonia spreading in Wuhan in late December 2019. In the field of infectious diseases, this is usually not a cause for concern; experts are always on the lookout for 'the next big one' but most of the time, it turns out to be nothing. This one, however, did progress further and by the second week of January, the world was informed by authorities in China that it was caused by a coronavirus, the same family of viruses that caused severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). We immediately sat up and took notice, and were soon called up by our colleagues from the Global Initiative on Sharing All Influenza Data (GISAID) to take action.

Originally designed to rapidly disseminate information about influenza viruses, GISAID was called upon by affected countries to make available its platform known for its unique sharing mechanism, so countries could share their virus sequences with unprecedented speed. It all started with five genomes from three Chinese labs.

The realization that we were dealing with something bigger than we had ever faced before didn't happen the very first moment the sequences of what later became known as SARS-CoV-2 were made publicly available. Instead, the realization grew gradually as the cases climbed to the hundreds and then thousands, spreading from the city of Wuhan to other Chinese cities.

From an average of three or four new sequences per day, the number of submissions quickly escalated to hundreds per day and we soon had to build a more robust database to store all 30 kilobase pairs of each sequence, along with important metadata like where the virus was isolated and when the sample was taken. GISAID could rely on programmers and scientists in different parts of the world working literally day and night for weeks and months.

To deal with the influx, we also had to adapt our procedures for handling incoming genomes, and quickly develop computational tools to screen for mistakes and quality so my colleagues at A*STAR's BII and Genome Institute of Singapore (GIS) developed software tools that allow us to automatically flag genomes for issues such as illegal characters, as well as sort them into categories based on the quality of coverage. Colleagues in France from Institut Pasteur improved checking of metadata and colleagues in Brazil and Argentina also joined our team of up to 50 people in this huge curation effort to be able to respond at every hour of the day by covering all time zones. As of July 2020, the GISAID database has over 60,000 sequences and that number is growing by the day.

While tracking the difference in SARS-CoV-2 sequences can give us important insight into where the virus could possibly have come from and how it is evolving over time, the first point of action everyone had to do to prepare for the virus was to develop accurate and reliable diagnostic kits. My A*STAR colleague Dr Masafumi Inoue at the Experimental Drug Development Centre (EDDC) and Dr Timothy Barkham at Tan Tock Seng Hospital immediately got down to work. Aided by Dr Sidney Yee, CEO of the Diagnostics Development (DxD) Hub, and the entire A*STAR ecosystem, we were able to launch the Fortitude quantitative reverse transcription polymerase chain reaction (qRT-PCR) diagnostic kits by the first week of February.

By following its genomic evolution, we hope to catch up with it or sometimes even be one step ahead.

Photo credit: ktsdesign / Shutterstock

To develop Fortitude, we needed access not just to one genome but reference multiple genomes—from this outbreak relative to previous viruses—to identify a region that is not only unique to the new virus but also relatively stable, so that it is common among all the current outbreak strains. This is where bioinformatics had a profound impact: not only did it enable us to make comparisons rapidly across the thousands of bases in the genome, but it also continues to help us make sense of the constant flow of sequences so that we can ensure that the diagnostic kits will continue to work even as the virus mutates.

Beyond diagnostics, once you have the genome sequenced, you can also use it to predict drug targets and start screening existing drugs in silico, greatly speeding up the search for much needed therapies.

Beyond diagnostics, once you have the genome sequenced, you can also use it to predict drug targets and start screening existing drugs in silico, greatly speeding up the search for much-needed therapies.

THE MEANING OF MUTATIONS

Apart from addressing the urgent need for diagnostics and drugs and triggering vaccine development on the frontlines, bioinformatics also plays a crucial role in helping us understand how the virus is mutating. First of all, I would like to stress that mutations are normal, particularly for RNA viruses like SARS-CoV-2 which naturally make mistakes when replicating, making imperfect copies of themselves. But just because the virus is mutating does not necessarily mean that it is more dangerous.

Secondly, most mutations are small and are either bad for the virus or have no impact at all. To give you an analogy, if the entire virus genome is like a car, the mutated form of the virus would be the same car in the same color with only a tiny difference such as a single letter difference in the license plate. Just as this change in the license plate doesn't affect the performance of the car or make it more fuel-efficient, these mutations do not mean that the virus has become more or less virulent.

However, this 'changed license plate' can tell us where the car came from and when it was registered. Similarly, mutations can give us a sense of how the different viral 'cars' are related to each other, a piece of information that we can then use in contact tracing.

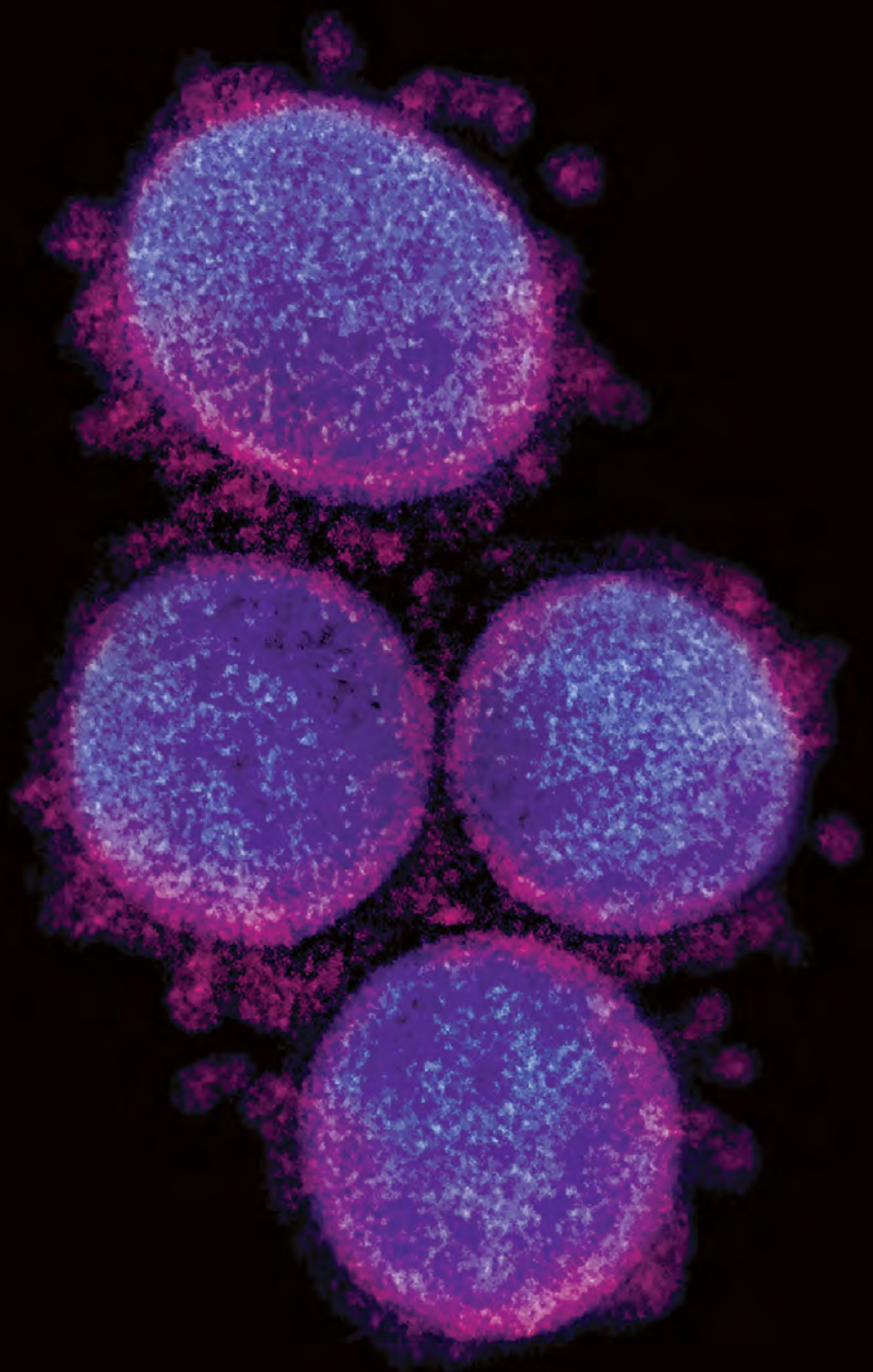
On very rare occasions, it is possible that there are mutations that do actually change the performance and fitness of the virus and it often requires multiple steps. For example, one such set of mutations in the evolution of SARS-CoV-2 is thought to have given it the ability to jump from animals like bats or pangolins into human hosts.

With real-time genomic surveillance enabled by platforms such as GISAID in combination with modern tools of bioinformatics, we can quickly detect these rare changes when they occur and judge if they affect diagnostics, treatments or increase virulence. For example, the virus that caused the outbreak in Europe had evolved to be slightly different from the original strain, such that it was not so well detected by the RT-PCR kit initially developed by colleagues in China based on the first outbreak genomes. As soon as we saw that change, we notified our colleagues and, armed with that information, they were able to quickly change their protocols and subsequently were able to fully detect the new European strains as well.

As the battle against SARS-CoV-2 continues to be waged across the globe, bioinformaticians around the world are racing against the continually evolving virus. By following its genomic evolution, we hope to catch up with it or sometimes even be one step ahead. ★

ABOUT THE AUTHOR:

Originally from Austria, Sebastian Maurer-Stroh leads a group of experts in computational sequence and structure analysis at A*STAR's Bioinformatics Institute (BII), where he has been the Deputy Executive Director (Research) since 2019. Maurer-Stroh has led industry collaborations ranging from applying AI in pharma manufacturing to precision medicine, food safety and consumer care. He is currently leading bioinformatics efforts in the virus outbreak response and surveillance and contributing globally via GISAID and WHO surveillance networks.



The Global Initiative on Sharing All Influenza Data (GISAID) database began with five SARS-CoV-2 genomes from three Chinese labs. As of July 2020, it has over 60,000 sequences.

Credit: NIAID

EPIDEMIOLOGY

Keeping an eye on COVID-19 clusters

Surveillance and contact tracing are key to limiting the widespread transmission of COVID-19 in the community, experts find.

As the COVID-19 pandemic began to sweep the globe in early 2020, Singapore was immediately on the alert, having learned from previous experiences with SARS, H1N1 and Zika. The Lion City is a major Southeast Asian travel hub, and with a local population of over 5.7 million people in a high-density urban setting, it was mindful of the impact of a potential pandemic.

Decision-makers had to deploy countermeasures to quickly contain the spread of the coronavirus. However, at the time, COVID-19 was an invisible enemy; relatively little was known about the nature of viral transmission and how best to protect the wider community from a potentially devastating outbreak.

In a bid to fill in the blanks, the Singapore 2019 Novel Coronavirus Outbreak Research Team set to work, assessing the settings and activities linked to spikes in COVID-19 transmission. Their study, published in the medical journal *The Lancet*, describes the team's observations on three outbreak clusters that originated from a tour group from China, a corporate event and a church.

Sebastian Maurer-Stroh, Deputy Executive Director (Research) at A*STAR's Bioinformatics Institute (BII), was among a multidisciplinary team of scientists, clinicians, bioinformaticians and epidemiologists involved in the study.

Using a combination of epidemiological and clinical data as well as field interviews, the work provided much-needed answers for how surveillance and contract tracing are vital in curtailing the spread of the virus.

The dynamics of COVID-19 transmission within the spotlighted clusters revealed some interesting findings: of the 36 individuals who tested positive for COVID-19, the transmission of the virus was, in most cases, linked to close contact with an infected individual, such as people living in the same household. Prolonged contact with international travelers, at events such as conferences, also put individuals at a higher risk of infection.

Indirect transmission, through sharing food or touching common surfaces was found to also be a possible route. To

It is also important for countries to do active case-finding among close contacts of affected individuals, including contacts with mild symptoms, to contain clusters and stop them from spreading.

Photo credit: Oleg / Shutterstock

mitigate the virus from spreading this way, the authors call for public health messages that stress the importance of proper hand hygiene and social distancing.

Lessons learned from the local clusters indicate that transmission tracking is key. "It is also important for countries to do active case-finding among close contacts of affected individuals, including contacts with mild symptoms, to contain clusters and stop them from spreading," the authors write.

They also called for further studies to address how the virus is spread by individuals who display no symptoms, which remains a grey zone for epidemiologists. "Although there is interest in asymptomatic transmission, we are unable to address this point in our study, and further studies should be done to better understand disease transmissibility of asymptomatic cases," the authors write. ★

IMPACT

This study highlighted the importance of active case-finding among close contacts of affected individuals to contain COVID-19 clusters and stop them from spreading.

LEFT
The researchers used a combination of epidemiological and clinical data as well as field interviews to unravel the dynamics of COVID-19 transmission within clusters.

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GENETICS

The day COVID-19 landed in Thailand

Two early cases of COVID-19 in Thailand represent the first confirmed exported cases from China, a Thai-Singapore team reports.

In late December 2019, an outbreak of unidentified pneumonia was reported in the bustling Huanan Seafood Market in China's Wuhan city. Responding quickly to the escalating situation, several countries established thermal screening for travelers from Wuhan and stepped up surveillance at hospitals.

What happened next played out like a classic movie plot about a pandemic: two individuals—the first confirmed exported cases of COVID-19 from China—boarded separate flights from Wuhan to Thailand's Bangkok Suvarnabhumi Airport, where they were discovered on January 8 and January 13.

Racing against time, Thailand's Ministry of Public Health confirmed both cases to be COVID-19 positive. By studying case histories, clinical characteristics and genomic profiles together with Sebastian Maurer-Stroh, Deputy Executive Director (Research) at A*STAR's Bioinformatics Institute (BII), a Thai-Singapore team was able to piece together the early transmission patterns of the outbreak.

Although the two imported COVID-19 cases were not directly linked, their viral genomes were identical with each other and with four other viral sequences collected from patients in Wuhan. Since both individuals had not visited the Huanan Seafood Market, nor did they contact persons with COVID-19, it indicates potentially wider distribution beyond Wuhan before January 23, when travel restrictions were enforced in the city.

"Taking together the history and onset of symptoms of these two COVID-19 cases, it suggests that transmission within Wuhan beyond the Huanan Seafood Market likely occurred in the first week of January or earlier," Maurer-Stroh explained.

Comparing the two genome sequences with known coronavirus families, the SARS-CoV-2 genome showed 80 and 88 percent identity with SARS-CoV and SARS-like bat CoV genomes from China, respectively. On a structural level, the ACE2 surface protein of SARS-CoV-2, which is responsible for viral attachment to human cells, had only 76 percent identity with SARS-CoV.

"Given several mutations in the binding interface, SARS-CoV-2 may differ in host-cell binding efficiency as compared with SARS-CoV. This could result in differences in virulence and transmission potential," said Maurer-Stroh.

While asymptomatic cases in their incubation period would have been missed, Thailand's rapid response early in the outbreak proved successful in these two cases, the authors noted. Another important lesson is that the genome sequences were shared in real-time via the Global Initiative on Sharing All Influenza Data (GISAID) online portal, which gave researchers everywhere immediate access to study the new virus and early outbreak patterns. ★

BACKGROUND

Thailand was the first country outside of China to report confirmed cases of COVID-19. A*STAR researchers were involved in the genome analysis of those first few cases.

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Photo credit: Viroj Changyencham / Shutterstock

INDUSTRY IMPACT:

SCIENCE ON THE MOVE

A*STAR scientists and their industry partners are racing against time to take crucial COVID-19 technologies from bench to bedside.

THE FAST TRACK TO FORTITUDE

In a display of sheer scientific willpower, A*STAR researchers developed and deployed the COVID-19 diagnostic Fortitude Kit within weeks of the virus reaching Singapore's shores.

It may seem like a lifetime ago, but it was only in late January 2020 that Singapore confirmed its first case of the then-novel coronavirus causing COVID-19. Amid the frenzied atmosphere of the Chinese New Year holidays, few would have suspected that the strange pneumonia would snowball into the global pandemic that it is today.

But a couple of scientists at Singapore's Agency for Science, Technology and Research (A*STAR) had their ears pricked up. "We already heard through different channels in December that there was some sort of pneumonia in Wuhan," recalled Dr Sebastian Maurer-Stroh, Deputy Executive Director (Research) of A*STAR's Bioinformatics Institute (BII).

Haunted by the memory of previous outbreaks—including SARS in 2003, swine flu in 2009 and Zika in 2016—local researchers jumped into action even before the virus had reached local shores. With the advancement of sequencing technologies over the years, the draft genome of the SARS-CoV-2 virus was released by Chinese researchers as early as mid-January, allowing scientists worldwide to quickly create their diagnostic kits.

By the first week of February, A*STAR researchers, along with their collaborators at Tan Tock Seng Hospital (TTSH), had already unveiled a locally developed COVID-19 diagnostic, called the Fortitude Kit, and sent it to various local hospitals, both local and overseas. In stark contrast, during the 2003 SARS outbreak, diagnostic kits were made available in Singapore only months into the outbreak. Here's a look into the Fortitude kit's fast track journey from bench to bedside.

SPOTTING A STEALTHY KILLER

The Fortitude Kit is based on a technique known as the real-time reverse transcription polymerase chain reaction (RT-PCR). The testing process begins by collecting nasal or nasopharyngeal swabs from individuals suspected to have COVID-19. Once transported to the laboratory, viral RNA is extracted from these swabs. That is where the Fortitude Kit itself comes into the equation.

Each kit comprises a one-step RT-PCR test, which comes ready-made with all the reagents needed in the correct amounts. These reagents include reverse transcriptase, an enzyme that converts viral RNA to DNA, as well as short DNA sequences called primers that can detect SARS-CoV-2's unique genetic footprint. During RT-PCR, primers recognize and bind to the viral DNA sequences converted from RNA. Repeated cycles of heating and cooling then trigger the *Taq* polymerase to exponentially create identical copies of viral DNA until they are detected by the machine.

Running the Fortitude Kit only takes around 90 minutes, but the preparation steps add on a few hours and it can take a day or longer for results to be released, depending on the resources of the healthcare providers.

Still, RT-PCR is widely considered to be the gold standard for the detection of viruses such as SARS-CoV-2. Unlike other diagnostics on the market, RT-PCR-based tests have specificity rates of over 99 percent—meaning that individuals who test positive truly have the disease. The sensitivity of RT-PCR tests depends on the stage of illness, being close to 100 percent in the first week of illness but then becoming less sensitive as time goes by. So far, Fortitude has become one of the most widely adopted diagnostic kits on the market.

ALL HANDS ON DECK

The Fortitude Kit was made possible by a powerhouse team of Singapore's leading scientific minds, namely A*STAR scientists, Dr Maurer-Stroh and Dr Masafumi Inoue; as well as CEO of the Diagnostics Development (DxD) Hub Dr Sidney Yee, and TTSH's Dr Timothy Barkham. Each expert brought their own specialized set of skills to the table in the fight against COVID-19.

The A*STAR Fortitude Kit is used routinely in 13 Singapore hospitals and laboratories. To date, the kit has been deployed in more than 20 countries.

With his expertise in computational biology, Maurer-Stroh examined the virus' genetic sequences and its evolution over time, sharing his insights on the Global Initiative on Sharing All Influenza Data (GISAID) public database. Armed with this data, Inoue, Head of the Diagnostics Group at A*STAR's Experimental Drug Development Centre (EDDC), then started developing the prototype of Fortitude Kit. The first order of business? Designing primers that would target SARS-CoV-2.

"Ensuring quality primer designs is always our focus in developing diagnostic kits," said Inoue. To do so, his team first had to identify a distinct sequence to target that was also shared by the current outbreak strains. This required Inoue to compare and contrast multiple coronavirus genomes, enlisting help from colleagues like Maurer-Stroh to narrow down the choice of regions to target using bioinformatics. In the end, they went for a region which codes for an enzyme that catalyzes the replication of RNA from an RNA template.

"We chose that region as a primer target since we knew it had the least number of mutations in the 2003 SARS virus," explained Inoue. By designing primers based on the unique portions of the virus that are less prone to mutations, more circulating viral strains can be readily detected by the kit.

Once the primers and the prototype were in place, TTSH's Barkham stepped in to fine-tune the kit's parameters. His team tweaked the RT-PCR process at various points, carrying it out at different concentrations or temperatures to identify the ideal conditions. They also evaluated the kit on real patient samples, which could contain molecules that may confound the test and result in inaccurate readings.

Fortitude's finishing touches were then added by the Yee's team at DxD Hub, a national platform led by A*ccelerate, A*STAR's commercialization arm. "We optimized the assay so that we are sure that it works every single time," she shared. "We also developed the whole production process, because it's more than just putting things together in a kit. It's also a lot of manufacturing instructions, quality control and quality assurance protocols."

Given the massive effort required to develop a diagnostic, it's remarkable that A*STAR and TTSH managed to successfully do so in less than a month. Their long, sleepless nights were powered by the sheer willpower to help Singapore face its biggest crisis yet. "This is only a small snapshot of the team involved in rolling out just one product, Fortitude Kit," commented Yee. "It really takes the whole Singapore R&D ecosystem and we're very fortunate to be part of it."

SINGAPORE'S GIFT TO THE WORLD

In Singapore, the Fortitude Kit has been in routine use in 13 public and private hospitals and laboratories since February. To date, the kit has also been deployed in more than 20 countries. While the DxD Hub produced the initial batch of kits, the know-how was transferred non-exclusively to a handful of biotech companies to scale-up and manufacture the diagnostic tests.

From a weekly output of 100,000 tests at the start of the outbreak, the manufacturing transfer exercise has ramped up production to over four times that per week. "Fortitude is now in around 20 countries. It is probably one of the most widely used RT-PCR test kits in the world," shared Yee. Beyond its obvious clinical benefit, Fortitude Kit has also helped spread the branding of Singapore and A*STAR worldwide, she added.

Still, the work continues. "RNA viruses are mutating all the time," explained Maurer-Stroh. Most mutations are tiny changes, but closely monitoring the evolution of the virus allows A*STAR to quickly respond and modify Fortitude if needed.

Amid the fast-evolving coronavirus crisis, one thing is for sure: Singapore will continue to fight with the coronavirus with Fortitude. ★

IDENTIFYING ANTIBODIES THAT WORK

A new test manufactured by A*STAR's Diagnostics Development Hub in partnership with Duke-NUS Medical School could reveal if an individual is likely to be immune to COVID-19.

After months of quiet, countries from Singapore to Spain are emerging from unprecedented lockdowns brought on by COVID-19. Public spaces are buzzing with activity and filled with people once more, providing a much-needed boost to morale—and the economy.

Though it may seem like things are finally going back to the status quo, the pandemic is far from over. Instead of being complacent, we should be more vigilant than ever. To safely guide the influx of people back to the workplace and other venues, some governments have floated the idea of providing 'immunity passports' to those who have recovered from the coronavirus.

Given that approximately 40 to 45 percent of COVID-19 cases are asymptomatic, the true scale of the pandemic has so far remained elusive. Consequently, widespread antibody testing has been suggested as a means to identify individuals who could be given these immunity passports, resulting in the market being flooded with rapid antibody tests. Unfortunately, not only are some of these tests wildly inaccurate, they cannot indicate immunity to reinfection.

Enter the cPass™ kit, a first-of-its-kind rapid test that specifically detects the functional neutralizing antibodies that can clear coronavirus infection. Unlike RT-PCR tests, cPass™ cannot be used to diagnose an ongoing infection. Instead, it indicates if an individual had previously been infected and developed protective antibodies in the process. Invented by Professor Linfa Wang and his team at Duke-NUS Medical School (Duke-NUS), the kit's commercialization was co-facilitated by GenScript Biotech Corporation and A*STAR's Diagnostics Development (DxD) Hub.

"Unlike RT-PCR tests, the main use of cPass™ is not to diagnose an ongoing infection. Instead, it indicates if an individual had previously been infected and developed protective antibodies in the process. This will be a huge boost to current COVID-19 investigations, to determine infection rate, herd immunity, predicted humoral protection, and also vaccine efficacy during clinical trials and after large-scale vaccination," said Wang, Director of the Duke-NUS' Emerging Infectious Diseases program.

ANTIBODIES 101

When a person is infected with SARS-CoV-2, the virus that causes COVID-19, the body mounts an immune response, triggering the production of hundreds to thousands of different antibodies in the blood. While SARS-CoV-2-specific antibodies can recognize and bind to the virus, not all of them can prevent it from invading cells. Some antibodies, for instance, could recognize parts of the coronavirus, but fail to functionally reduce its infection.

These so-called non-neutralizing antibodies may even paradoxically enhance subsequent viral reinfection in a phenomenon known as antibody-dependent enhancement. To guarantee protection against future SARS-CoV-2 infection, we need to look for neutralizing antibodies that mainly bind to the coronavirus' spike protein. By doing so, these antibodies block the spike protein from attaching to the angiotensin-converting enzyme 2 (ACE2) receptor found on the surface of host cells, thereby preventing the virus from infiltrating the cell and replicating within.

Though rapid tests can detect the presence of antibodies against SARS-CoV-2, they are unable to distinguish between the neutralizing and non-neutralizing antibodies. Testing specifically for neutralizing antibodies has proven to be tricky. After all, these tests are based on the blocking of the live virus by neutralizing antibodies present in the cell culture setups. Not only does the process require skilled laboratory personnel and high-level biocontainment facilities, but it also takes several days to complete—making widespread and large scale neutralizing antibody testing a pipe dream. At least, until now.

NEUTRALIZING OR NOT?

With the cPass™ kit, neutralizing antibodies can be detected within an hour in most research and clinical laboratories. In contrast to the other tests, cPass™ does not expose laboratory personnel to potentially dangerous live biological materials. Moreover, the kit can be easily scaled up and fully automated. "It fills a space in the market where no solution exists," remarked Dr Sidney Yee, CEO of A*STAR's DxD Hub.

For SARS-CoV-2 to infect cells, it needs to bind to a protein called ACE2. It does this using the spike proteins that give coronaviruses their name, specifically a part of the spike protein called the receptor-binding domain (RBD). If a person has neutralizing antibodies against SARS-CoV-2, they will prevent the RBD from binding to ACE2, effectively preventing the virus from entering the cell.

The cPass™ kit mimics this virus neutralization process in the lab. The kit contains two key components, namely the RBD protein fragment that has been labeled with an enzyme and the ACE2 protein coated on a plate. In the absence of neutralizing antibodies, the RBD fragments will bind to ACE2, producing a color after exposing to an enzyme substrate.

If neutralizing antibodies are present, however, the RBD fragments are prevented from binding to the ACE2 on the plate and no or less color is formed. By measuring the intensity of color produced by each reaction and comparing it to controls, users can thus determine if their sample contains neutralizing antibodies or not. So far, the cPass™ kit has achieved sensitivity and specificity rates of close to 100 percent—with its performance far outstripping other antibody tests on the market.

ONE KIT, MANY USES

Just like the Fortitude RT-PCR diagnostic kit, the development of cPass™ was an all-out collaborative effort, this time involving Duke-NUS Medical School, A*STAR's DxD Hub and GenScript. According to Yee, the whole process from conception, patent filing, kit development to provisional authorization from Singapore's Health Sciences Authority (HSA) took less than three months, with GenScript assisting in proof-of-concept research, product design and optimization.

To help secure provisional authorization for the kit and to bring it to the market, the DxD Hub validated the kit using local COVID-19 patient samples from the PROTECT clinical study coordinated by Singapore's National Centre for Infectious Diseases. In addition, the DxD team also developed the manufacturing and quality control protocols, producing the first pilot batch for use in local hospitals. GenScript and local Singapore biotech companies are set to scale up production of the kit.

Aside from Singapore, groups from countries including Malaysia, Thailand, Vietnam, Sri Lanka, China, Australia, New Zealand, USA, Canada, Germany, the UK, the Netherlands and Switzerland are already testing the kit. Approval applications are also being filed in Europe and the US, with GenScript even building up their production lines in China and the US in anticipation of demand—and with good reason. Beyond COVID-19 surveillance, the kit can be used broadly for everything from assessing blood samples for convalescent plasma therapy to tracking coronavirus infections in animals, said Wang.

Besides, cPass™ could be used to calculate just how long the neutralizing antibodies confer protective immunity—a question that has been continuously debated in scientific circles since the beginning of the outbreak. As the race for a vaccine kicks into high gear, pharmaceutical companies will also find the kit useful for determining the efficacy of their candidate with a few vaccine companies already in the process of evaluating cPass™ for this purpose. ★

cPass™ detects the presence of functional neutralizing antibodies against SARS-CoV-2.

PLACING A 'HIT' ON THE CROWN

Could there be a drug that can both treat and protect against COVID-19? The answer may be found in antibody drugs, a promising therapeutic modality against the coronavirus.

Since the emergence of the novel coronavirus in late 2019, multiple approaches for treating COVID-19 have been considered, from the use of repurposed medicines such as the antimalarial drug, hydroxychloroquine, to the Ebola antiviral drug, remdesivir.

Many of these therapeutic strategies have fallen short in terms of their clinical effectiveness, which has propelled scientists to come up with more targeted pharmaceuticals to help COVID-19 patients.

Amidst the frantic race to find ways of stopping this tiny killer, a team of researchers at A*STAR's Singapore Immunology Network (SIgN), led by Senior Principal Investigator Cheng-I Wang, has made a tremendous breakthrough in identifying antibodies that block the SARS-CoV-2 virus from entering human cells, thus thwarting the symptoms of COVID-19.

The team recently established a partnership with Japanese pharmaceutical company Chugai Pharmabody Research and is currently working at full tilt to bring these antibody candidate drugs to the clinic.

TARGETING THE VIRAL CROWN

Over the past six months, a wealth of information around the structure and biology of the coronavirus has been uncovered. Named for its 'crown'—a halo of roughly a hundred sugar-coated protein protrusions on the virus' surface called spike proteins—SARS-CoV-2 uses these spike proteins to bind to receptors on human cells, allowing it to gain entry and initiate infection.

Therapies tailored towards disrupting this binding sequence are thought to be the best way for preventing the virus from harming its host, and antibodies are nature's answer to doing just that. These Y-shaped proteins, produced by immune cells in response to foreign invaders, latch onto specific sites on the viruses, rendering them unable to wreak havoc in the lungs and other organs.

Over the last two decades, the evolution of sophisticated technologies have enabled drug developers to harness antibodies' exquisite target-binding properties, turning them into a safe and reliable therapeutic modality. There are already more than 80 approved antibody drugs on the market, including several blockbusters for treating cancer and inflammatory conditions.

MINING FOR ANTIBODY CANDIDATE DRUGS

Natural immune systems are a rich source of antibody-producing cells that protect us from environmental hazards. This immune landscape is incredibly vast and complex; it has been estimated that humans make around ten billion different antibodies, each with the ability to bind to a specific antigen on a disease-causing agent.

The challenge in COVID-19 drug development is traversing this immense immune universe and finding the handful of antibodies that will strongly latch on to the SARS-CoV-2 spike protein. Even if they do successfully identify these antibodies, only a much smaller subset of these will have the biophysical characteristics required for an effective drug.

To solve the COVID-19 antibody puzzle, Wang and colleagues used a discovery method that involved 'mining' for potential lead candidates from a library of synthetic human antibodies. Instead of searching through naturally-occurring antibody sources, the team panned through a large collection of diverse, pre-constructed antibodies, a sophisticated means of fishing out specific molecules for therapeutic applications rapidly.

"Our antibody discovery team consists of scientists specializing in antibody discovery and engineering. They have deep capabilities in these research areas, having worked on diseases such as chikungunya and dengue fever previously," said Wang, expressing confidence in his team's chances of finding a suitable antibody candidate drug. Wang is currently Head of SIgN's human monoclonal antibody technology platform.

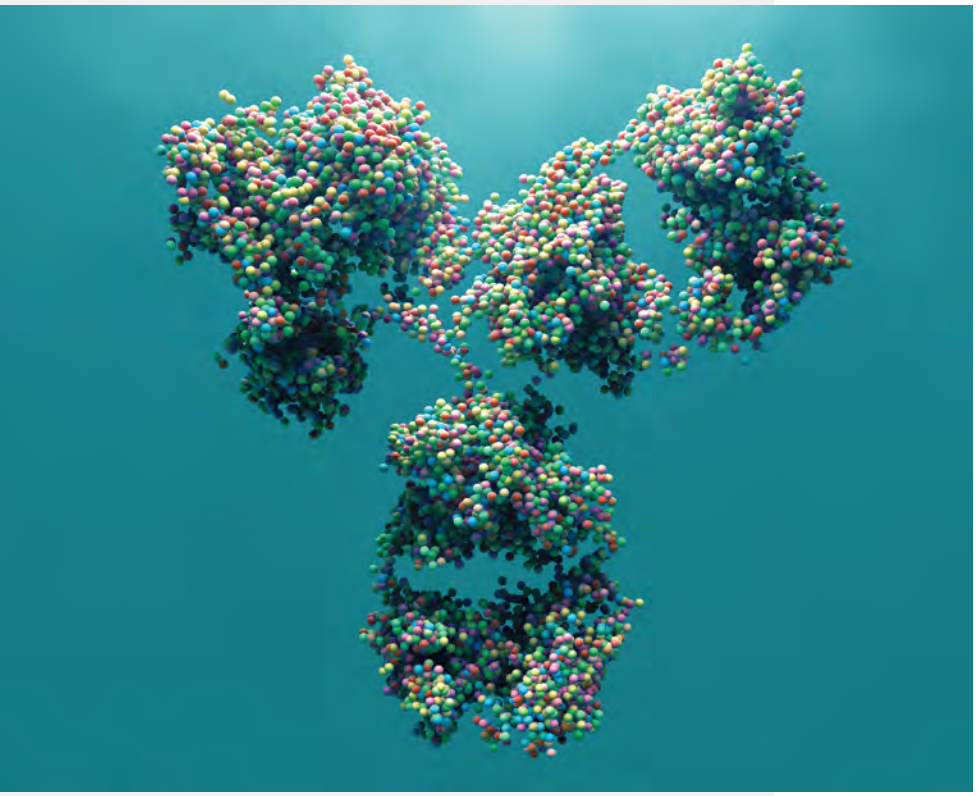


Photo credit: Design_Cells / Shutterstock

We believe that the neutralizing antibody treatment can slow down the replication of the virus and assist clearing virus from the infected patients.

A RACE FROM BENCH TO BEDSIDE

Using their *in vitro* antibody discovery platform, Wang's team was successful in singling out antibody molecules that attach strongly to the SARS-CoV-2 spike protein and prevent the virus from interacting with the host cell receptors. In another big win, they found that these antibodies could effectively neutralize live SARS-CoV-2 under experimental conditions, and reduce viral replication in human airway epithelial cells by over 10,000-fold, a functional property that makes these clinical candidates well-poised to perform therapeutically.

"By binding to the crown, the antibody prevents the virus from attaching to human cells, and hence prevents infection," Wang explained. There is a two-fold application potential for this antibody—prevention and treatment. Preventive or prophylactic use of the antibody can provide temporary protection for those at high risk of infection, such as healthcare workers and the families of infected patients, or those for whom COVID-19 vaccines cannot be used, such as in those who are already sick, the immunocompromised, the elderly or young children. The antibody could also be used to treat infected patients, Wang added. "We believe that the neutralizing antibody treatment can slow down the replication of the virus and assist clearing virus from the infected patients, hence allowing the patients a good chance to fight off the disease," he said.

A joint research collaboration between Wang's group and Chugai Pharmaceutical's Singapore-based research center has been established since May 2020, with the goal of promptly bringing this experimental drug to the clinic. To date, A*STAR and Chugai already have a history of fruitful collaborations, including several Global Health Innovative Technology Fund initiatives to create antibody countermeasures against other threats to global health like dengue.

In this partnership, Chugai has committed to leveraging its proprietary antibody engineering technology to further optimize the lead panel selected by Wang's team. This engineering process involves modifying antibody sequences or structures to enhance their clinical functionality such as enhancing their potency, improving their safety profile or extending their circulation half-life in humans, for example.

"The outbreak of novel coronavirus is the most devastating threat that people around the world have faced in decades," said Osamu Okuda, Chugai's President and Chief Operating Officer. "I am thrilled that Chugai can join forces with A*STAR in the global effort to help address this threat, and hope that together, we can open the possibility of clinical use as soon as possible." ★

LEADING THE WAY IN microRNA TECHNOLOGY

After a decade of research and innovation, homegrown biotech company MiRXES can now call themselves a global leader in developing microRNA-based technology to solve real-world issues.

While detecting any disease early can save lives, this is especially true when it comes to cancer. When cancer is caught early enough, patient survival rates and quality of life can be vastly improved. Early diagnosis also opens up more treatment options for patients, which can be key for beating the disease.

A good example of this is gastric cancer, which has a notoriously poor prognosis and is a leading cause of cancer deaths worldwide due to its tendency to produce significant symptoms only at an advanced stage. However, an early gastric cancer detection test is now on the horizon and the key lies in an unexpected source: microRNA, or miRNA for short.

Once thought to be nothing more than 'junk' genetic material, miRNAs are now recognized as central gene regulators and show promise as biomarkers when used as part of early disease diagnostic tools. In response to diseases like cancer, miRNA combinations and their individual amounts can change, leaving a detectable signature. This particular characteristic has led to companies developing ways to harness miRNA signatures as biomarkers for tracking and diagnosing diseases.

One such company is MiRXES, a homegrown A*STAR spin-off led by academics turned self-described "accidental entrepreneurs." Although starting in the then relatively understudied field of miRNA was challenging, MiRXES has since gone on to champion the development of miRNA-based research and technology, including launching the world's first miRNA-based diagnostic tool for gastric cancer.

"It was the culmination of almost eight years of work in collaboration with the Singapore Gastric Cancer Consortium and the Diagnostics Development (DxD) Hub of A*STAR," says Dr Lihan Zhou, Co-founder and CEO of MiRXES. With their expertise in miRNA, MiRXES was perfectly poised to respond to the COVID-19 pandemic, which is caused by an RNA virus, SARS-CoV-2. Building on a test developed at A*STAR and validated by Tan Tock Seng Hospital, MiRXES has scaled up production of the Fortitude Kit 2.0 to 300,000 kits a week, meeting Singapore's testing needs and beyond.

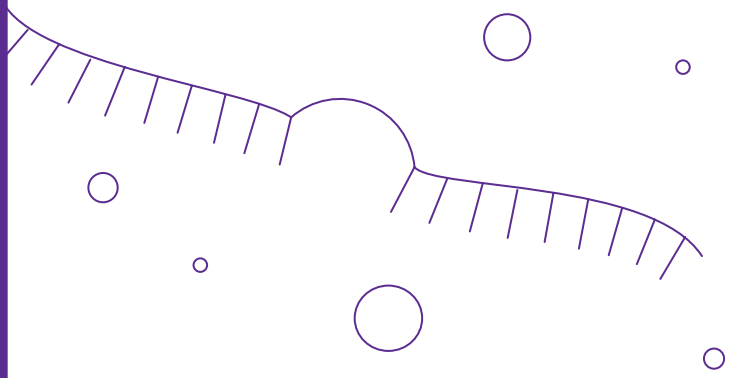
Photo credit: NOBEASTSOFIERCE / Science Photo Library

Here, Zhou shares how the company got its start, the difficulties of pioneering miRNA research, and his hopes of establishing Singapore at the forefront of the global miRNA industry.

Q: WHAT MOTIVATED YOU TO CO-FOUND MiRXES?

As miRNA began gaining steam in mainstream research, my co-founders and I started to explore the clinical relevance of miRNA in many disease areas. While I was a Research Scientist at A*STAR's Bioprocessing Technology Institute (BTI) in 2012, we showed conclusively that our technology could detect blood-based miRNA biomarkers with unprecedented sensitivity, enabling us to detect subtle signals released by early-stage cancer cells even before clinical symptoms became apparent. The desire to translate this discovery to clinical applications inspired us to found MiRXES in 2014 as a spin-off from A*STAR.

This motivation still drives our biotech start-up today. Though we are very much motivated by scientific curiosity and innovation, we don't just carry out research and develop products for the sake of generating knowledge or proving our capabilities. Instead, we work with the clear goal of developing practical miRNA-based applications that will solve real-world problems, improve healthcare and save lives.



Q: WHAT IS THE CORE TECHNOLOGY OF MiRXES? COULD YOU DESCRIBE HOW IT WORKS?

The development of diseases can be tracked through miRNA signatures, which are specific changes in the quantities of certain combinations of miRNAs. MiRXES' core technology is a quantitative reverse transcription polymerase chain reaction (qRT-PCR) platform that can accurately and robustly detect miRNA signatures from biological fluids like blood, urine and even tears, paving the way for non-invasive liquid biopsy tests for diagnosis and monitoring of diseases.

Because our technology is built on a patented method of designing unique qRT-PCR primers that can detect and amplify miRNAs sensitively, specifically and reproducibly, our method is also more reliable than other commercially available technologies. In addition, our expertise also extends to the formulation of reagents and design of workflows that are optimized for miRNA biomarker and therapeutic candidate discovery.

Q: WHAT WOULD YOU SAY HAS BEEN MiRXES' BIGGEST ACHIEVEMENT SINCE ITS FOUNDING IN 2014?

There are many. But if I had to choose one, it would be receiving approval from the Health Sciences Authority of Singapore in 2019 for GASTROClear, the world's first *in vitro* diagnostic solution for early detection for gastric cancer. With this regulatory approval, MiRXES successfully brought a miRNA-based application from research, product development and manufacturing through to clinical translation and commercialization.

With this first successful application of our core technology, we look forward to working with other partners to develop more game-changing applications that will improve and save lives. In the pipeline are clinical assays for other cancers including lung and breast cancer, as well as other diseases such as pulmonary hypertension.

Beyond that, we started a series of initiatives in 2020 that aims to pave the way for wider clinical validation and adoption of miRNA-based diagnostics at a global level. We hope our work will also continue to help put Singapore at the forefront of the global miRNA industry.

Q: WHAT WERE SOME OF THE KEY CHALLENGES IN CREATING YOUR TECHNOLOGY AND HOW DID YOU OVERCOME THEM?

The challenges we faced in creating the qPCR technology behind our miRNA detection platform were no different from those that all researchers face on a daily basis. We took an idea that nobody knew would work, carried out lots of experiments that failed, and learned from each failure. When we finally succeeded in proving the concept, we repeated the experiment multiple times to convince ourselves that we were not crazy. Even to this day, we continue to work hard in the lab, carrying out thousands of qPCR reactions each day to continually optimize our technology.

Q: HOW DID A*STAR SUPPORT YOUR JOURNEY FROM SCIENTIST TO ENTREPRENEUR?

When we first started working on miRNAs in 2010, few scientists knew what they were, and many of those who did thought they were 'junk' RNAs since they do not code for proteins. At the time, very little was understood about miRNA's role as a master regulator of gene expression. So one of the key hurdles we had to overcome was to convince scientists, clinicians, regulators and investors that miRNAs were, in fact, important biomarkers for detecting diseases and could have other uses.

In addition, few researchers and companies were exploring miRNAs as potential biomarkers for *in vitro* diagnostic applications so there were no ready tools and

processes that we could adopt to develop a commercial miRNA-based test kit. We had to develop a lot of these tools and processes ourselves and even build our own manufacturing capability because there was no way we could have outsourced this and still remain confident in the quality of the product. In short, we had to do a lot of experimentation and build capabilities from scratch over the years. We made lots of mistakes but also learned a lot from our experience. We were fortunate to have the DxD Hub as a partner since 2014 in developing these tools and capabilities.

Finally, because GASTROClear was the first miRNA-based diagnostic test to seek regulatory approval in Singapore, there were no established standards and procedures to follow. We had to work closely with A*STAR's commercialization arm, Exploit Technologies (now A*ccelerate), DxD Hub and the Health Sciences Authority to understand the requirements for regulatory approval.

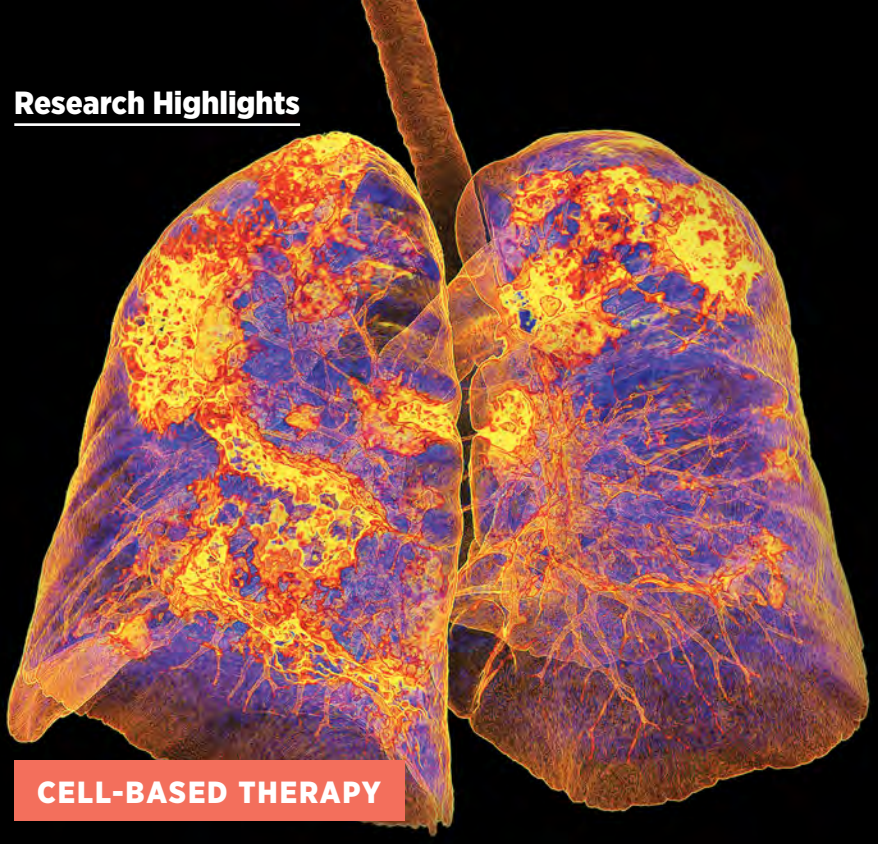
While we still have a lot of work to do to prove the clinical utility and the health economics of GASTROClear, we are working with partners globally to undertake a 20,000-strong lung cancer miRNA study to demonstrate the performance of miRNA biomarkers for the early detection of lung cancer. This is part of our long-term effort in eradicating late diagnoses in cancer and other disease cases.

Beyond technology and products, MiRXES is also actively shaping the human aspect of our business. I have thoroughly enjoyed the journey of starting and growing with MiRXES. I hope we can continue to forge an open and innovative environment where we can develop ourselves and have fun while growing the business globally. ★

Q: WHAT ARE SOME OF YOUR FUTURE GOALS FOR MiRXES?

We are positioning MiRXES as an RNA-focused platform technology company that develops transformative products for health, disease and industrial applications. We will continue to build and optimize our end-to-end capabilities to enable the discovery of novel biology and the development of quality yet affordable products.

MiRXES' expertise in miRNA technology put them in the position to respond quickly to the national and international need for qRT-PCR kits to detect SARS-CoV-2.



CELL-BASED THERAPY

Call for rigor over COVID-19 exosome therapy

Stem cell experts recognize the potential of exosomes for treating COVID-19 pneumonia, but caution that rigorous clinical testing is needed.

Most people who catch SARS-CoV-2 develop mild to moderate symptoms like shortness of breath, a dry cough and a fever, and recover without hospitalization. However, some are less fortunate—they develop severe pneumonia, a serious illness that can be deadly.

Nicknamed COVID-19 pneumonia, the condition is the result of an immune system spiraling out of control, a 'cytokine storm' where a cascading effect of cellular and molecular processes results in acute respiratory distress syndrome (ARDS).

A rapid uptick in studies on cell-based therapies for COVID-19 pneumonia has focused on mesenchymal stromal cells (MSCs), whose therapeutic activity is thought to be mediated by small extracellular vesicles (EVs). These MSC-EVs—also known as exosomes—are secreted by MSCs and harbor potentially therapeutic components.

"Pre-clinical studies in animal models have suggested that MSC-EVs can reduce inflammation and fibrosis in lung injury and ARDS. These studies suggest that MSC-EVs may also be effective for treating ARDS arising from COVID-19," explained Sai-Kiang Lim, a Research Director at A*STAR's Institute of Molecular & Cell Biology (IMCB), who is also co-chair of the International Society for Cellular and Gene Therapies (ISCT) Exosome Committee and founding member of the International Society for Extracellular Vesicles (ISEV).

However, while recognizing the potential of MSC-EVs, the ISEV and ISCT have published a joint statement recommending the need for rigorous clinical trials before using MSC-EVs as the standard of care to treat patients with severe COVID-19.

"The major issue with the use of MSC-EVs in COVID-19 patients is that the efficacy of MSC-EVs is not known and any use should be conducted in clinical trials under the oversight of the relevant regulatory bodies," said Lim.

In the statement, the expert committee points out that the mechanism by which the exosomes exert their beneficial effects remains incompletely understood. Likewise, the safety and potential for adverse effects of MSC-EV therapies are still unknown.

They highlight the need to consider the source of MSCs, because the tissue and individual from which the MSCs are isolated can affect their function. In the same vein, the potency of EVs, which are thought to be responsible for the therapeutic effects of MSCs, varies depending on their source, preparation, aging and other factors.

"Both ISCT and ISEV recognize there is a strong scientific rationale for the use of MSC-EVs to treat COVID-19-induced pneumonia, but it is premature to offer MSC-EVs as a treatment for COVID-19 pneumonia at this time," said Lim. ★

ABOVE
COVID-19 pneumonia results from a cytokine storm, a severe immune reaction in which the body releases too many cytokines into the blood too quickly.

1. Börger, V., Weiss, D. J., Anderson, J. D., Borràs, F. E., Bussolati, B., *et al*. ISEV and ISCT statement on EVs from MSCs and other cells: considerations for potential therapeutic agents to suppress COVID-19. *Cytotherapy* (2020).

Photo credit: K H FUNG / Science Photo Library

DRUG DISCOVERY

Keeping an AI out for new uses of old drugs

Tipped off by artificial intelligence, a research team is testing whether a rheumatoid arthritis drug could be repurposed as a COVID-19 treatment.

As the worldwide cases of COVID-19 soar past the fifteen-million mark, researchers have devised a clever new way to 'hack' the drug discovery process, by turning to artificial intelligence (AI) to repurpose existing drugs for COVID-19.

Using a custom AI developed in-house, UK bioinformatics startup BenevolentAI mined and analyzed clinical data for approved drugs capable of inhibiting both inflammatory damage and infectivity associated with SARS-CoV-2. Their AI selected baricitinib, a Janus kinase inhibitor, which has been approved for use as an oral, once-daily treatment for adult rheumatoid arthritis.

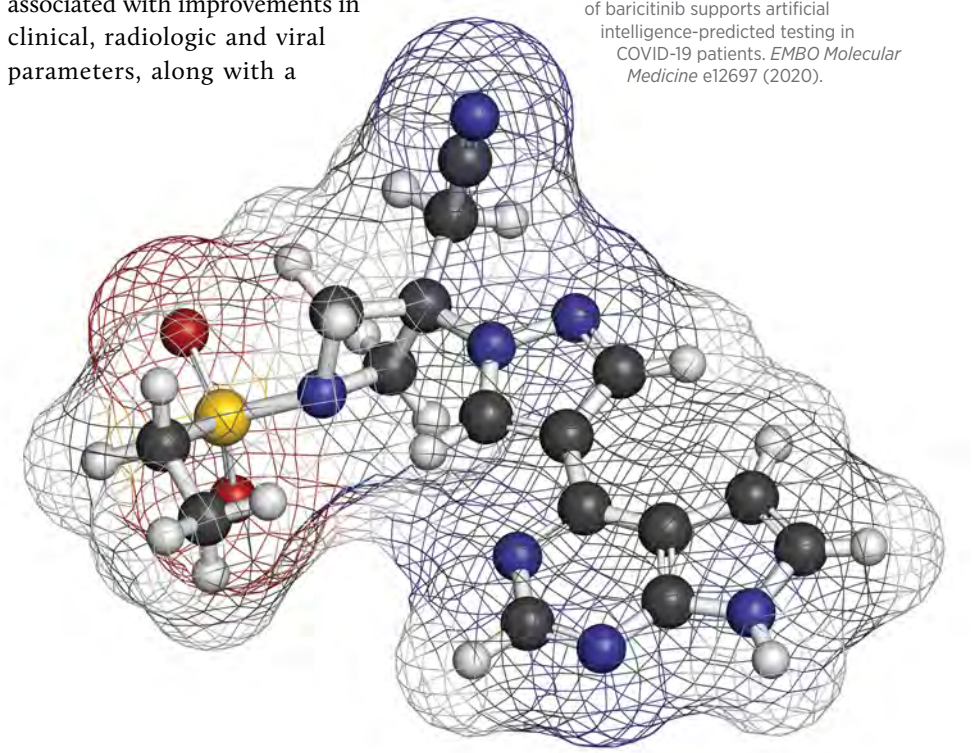
Like any good relay, the researchers next invited collaborators, including Yee-Joo Tan, a Principal Investigator at A*STAR's Institute of Molecular and Cell Biology (IMCB), to validate their AI's predictions about baricitinib. By providing biochemical and cellular evidence from *in vitro* models and clinical studies, the international team showed that baricitinib could indeed stem the cytokine storm and viral propagation seen in hospitalized COVID-19 patients.

Stepwise, the researchers first evaluated the *in vitro* pharmacology of baricitinib to better understand its anti-inflammatory mechanism. When tested on leukocyte subpopulations from a previous randomized trial for rheumatoid arthritis, baricitinib treatment resulted in a statistically significant decline in IL-6 levels—a predictor of mortality in severe COVID-19 infections.

Next, they extended their findings to see if baricitinib could also exhibit antiviral activity. Baricitinib showed nanomolar affinities for human numb-associated kinase (NAK) proteins, which are involved in host viral propagation. Inhibition of NAKs with baricitinib led to a 30 to 40 percent reduction in viral load in coronavirus-infected human primary liver spheroids, validating the AI predictions.

In four patients with bilateral COVID-19 pneumonia, baricitinib treatment was associated with improvements in clinical, radiologic and viral parameters, along with a rapid decline in the levels of inflammatory markers C-reactive protein and IL-6. Patient symptoms improved and all patients regained normal lung function. Importantly, baricitinib promoted a progressive rise in the titer of neutralizing antibodies in follow-up studies.

Given the urgency of the situation, this study provides hope to those working in drug discovery that AI predictions may help them to repurpose existing drugs for COVID-19. "This study represents rapid repurposing from AI to the laboratory to a potential bedside therapeutic and supports the testing of baricitinib in randomized controlled trials in COVID-19 patients," the authors wrote. "Baricitinib is now in clinical trials and if successful, it gives us more options for COVID-19 treatment," Tan added. ★



BOTTOM
Baricitinib is a Janus kinase inhibitor indicated for the treatment of adult rheumatoid arthritis.

1. Stebbing, J., Krishnan, V., de Bono, S., Ottaviani, S., Casalini, G., *et al*. Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients. *EMBO Molecular Medicine* e12697 (2020).

Photo credit: StudioMolekuul / Shutterstock

FINDING THE IDEAL DISINFECTION AGENT

Regular disinfection can help to limit the spread of viral infections such as COVID-19, but only if carried out appropriately, write Xian Jun Loh and colleagues.

Viral outbreaks have posed severe threats to human health and well-being throughout history. The novel coronavirus SARS-CoV-2, which was first reported by China in late 2019, has led to the largest coronavirus outbreak in the past two decades and also caused widespread socioeconomic disruption.

While government interventions can influence the rates and range of viral outbreaks, individuals can play equally—or arguably even more—important roles in limiting its spread. As viral transmissions occur via close human-to-human contact or through contact with contaminated surfaces, the use of sanitizing agents for

personal care and surface disinfection can help to limit viral transmissions by inactivating the viruses before they have a chance to enter the human body.

In a review published in the journal *View*, titled "Sanitizing agents for virus inactivation and disinfection," my colleagues at A*STAR's Institute of Materials Research and Engineering (IMRE) and I evaluate disinfectant agents commercially available on the market for their effectiveness against viruses. We also debunk common myths about viral inactivation and highlight exciting advances in the development of new sanitizing agents.

NO 'ONE-SIZE-FITS-ALL' DISINFECTANT

Depending on the type of surface and ambient conditions, viruses can persist on inanimate surfaces for as short as five minutes to greater than 28 days. A recent study found that SARS-CoV-2 can persist longest on propylene plastic surfaces and stainless steel, with viable viruses found up to 72 h after the initial application, though at a greatly reduced viral titer. Much shorter persistence was observed on copper surfaces, with no viable viruses observed after 4 h.

There are many factors to consider when working with disinfection agents. The key parameters that affect their efficacy include contact time, the concentration of disinfection agent and viral characteristics. Disinfection efficacy can also be influenced by environmental factors such as temperature, humidity and pH; the presence of cell debris, soil and aerosolized droplets can also reduce viral penetration and the corresponding activity of the disinfection agent.

Viruses can be classified into three main types—enveloped viruses, large non-enveloped viruses and small non-enveloped viruses—according to increasing difficulty of chemical disinfectant inactivation. Larger viruses are generally more sensitive to disinfectants, although there are exceptions. Non-enveloped viruses contain a protein coat, and therefore inactivation often requires denaturation of the redundant viral capsid proteins or essential replicative proteins. It is thus challenging to inactivate small non-enveloped noroviruses and several commonly available disinfectants are not able to sufficiently reduce infectivity.

COMMERCIALLY AVAILABLE DISINFECTION AGENTS

Alcohols (isopropyl alcohol and ethanol) are capable of inactivating a wide spectrum of bacterial, fungi and viruses, and are ubiquitous in applications such as skin antisepsis and disinfecting small medical tools. While they are effective in eradicating some types of viruses, other types of disinfectants such as surfactants and oxidizing agents are much better disinfection agents compared to alcohols.

Surfactants are particularly useful against enveloped viruses such as coronaviruses, which include SARS-CoV, MERS and SARS-CoV-2. As the active ingredients found in household disinfectants and detergents, surfactants kill viruses mainly by solvating and disrupting the lipid-based virus envelope. In particular, quaternary ammonium compounds—the most commonly used cationic surfactant—are attractive as they are relatively nontoxic, colorless and odorless.

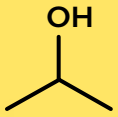
For noroviruses and other small non-enveloped viruses that are difficult to disinfect, strong oxidizing agents are among the most effective disinfectants. The most common oxidizing agent disinfectant in the United States is dilute solutions of sodium hypochlorite, also known as household bleach. Other well-known oxidizing agents are hydrogen peroxide and peracetic acid.

Known for its pungent odor, formaldehyde is often sold as an aqueous solution called formalin and used to inactivate viruses for vaccine production. Other aldehydes such as glutaraldehyde and ortho-phthalaldehyde (OPA) work by the same mechanism, which is to crosslink reactive groups of proteins and nucleic acids. However, not only is formaldehyde a mutagen and suspected carcinogen, it also causes skin and eye irritation.

Common disinfection agents:

Alcohols

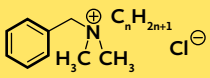
- Thought to work by denaturing proteins
- Able to inactivate a wide spectrum of bacteria, fungi and viruses
- But not as effective as surfactants and oxidizing agents



e.g. isopropanol

Surfactants

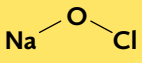
- Work by disrupting the virus envelope
- Quaternary ammonium compounds are attractive as they are relatively nontoxic, colorless and odorless



e.g. benzalkonium chloride

Oxidizing agents

- Particularly effective against non-enveloped viruses that are otherwise difficult to inactivate
- Includes household bleach and hydrogen peroxide



e.g. sodium hypochloride

DEBUNKING MYTHS

While searching for ways to protect ourselves from COVID-19 infection, we must avoid being lulled into a false sense of security by ineffective solutions for which no clear evidence of virucidal properties can be found.

Take for example essential oils, which are topically safe and commonly used in a variety of skincare products to treat dermatological issues such as acne. Despite their popularity, the germicidal abilities of essential oils are mostly bacteria-related; they cannot be assumed to work on viruses.

Another misunderstood antimicrobial compound is antibiotics. It has been estimated that up to 30 percent of antibiotic prescriptions by medical professionals have been inappropriately used to treat viral infections.

A popular dietary supplement, vitamin C, is frequently recommended in the popular literature for treating respiratory infections, while garlic is known to have fairly broad-spectrum antimicrobial effects. Another common belief is that rinsing the nose with saline solution, or gargling with salt water, is effective for treating viral infections. However, there is insufficient evidence to prove that any of these dietary supplements or interventions possess virucidal behavior for disinfection purposes.

In contrast, there has been a long history of applying ultraviolet (UV) light in the elimination of microbial pathogens in laboratory and clinical settings. When combating viruses, however, the efficacy of UV disinfection is highly dependent on the absorption by viral DNA and UV dose.

FROM SMALL MOLECULES TO POLYMERS

To expand the repertoire of virucidal compounds available, considerable research effort has been invested in developing new active materials that have both broad spectrum virucidal activity and low toxicity to humans. Although not yet commercially available, three main types of virucidal agents have received significant research attention—small molecules, metal nanomaterials and virucidal polymers.

Small molecules such as β-cyclodextrins, which are naturally-occurring macrocyclic molecules comprising of seven covalently-joined glucopyranose units, are currently being tested for their virucidal properties. Interestingly, some compounds found in food have been shown to exhibit virucidal activities, such as cinnamaldehyde, an organic compound that is responsible for cinnamon's flavor and odor, which was effective against norovirus surrogates and hepatitis A virus.

Polymers capable of inactivating viruses are a new and exciting area of research.

Metal nanomaterials such as silver and its salts have had a long history of use as antiseptics and disinfectants. Silver nanoparticles between 10 and 100 nm in size are effective biocides in small doses, although their potential toxicities to humans are still under debate. Gold nanoparticles are also promising virucidal agents, but due to the costs involved are unlikely to become commercially viable.

Polymers capable of inactivating viruses are a new and exciting area of research. The vast majority of intrinsically virucidal polymers are charged, and include polyethylenimine (PEI) derivatives, cationic pyridinium-type polyvinylpyrrolidones and cationic quaternary phosphonium polymers. They can be formulated or cast into various forms for customized applications, such as disinfecting coatings, binders in pharmaceutical products, water purification filters and as additives in paper or common household materials.

Airborne transmission of viruses is a major route of human-to-human transmission and can occur in the form of aerosols, which are droplets less than five micrometers in diameter. SARS-CoV-2 virus particles were found in the ventilation systems of the hospital rooms housing COVID-19 patients in China. Photocatalyst (silver ion-doped titanium oxide)-coated air filters and ionizers have recently been demonstrated to be effective in removing viable viruses from the air, although they are not expected to be stand-alone solutions. Chemical alternatives such as chlorine dioxide are also being studied for inactivating airborne viruses, but more research is needed to understand the long-term effects of chlorine dioxide exposure.

BALANCING BIOCOMPATIBILITY AND EFFICACY

In general, disinfectants like aldehydes and oxidizing agents that inactivate viruses by chemically modifying their surface groups are fast-acting and highly potent towards most viruses, but their application remains limited by their higher toxicity and damaging effects to surfaces.

On the other hand, disinfectants like alcohols and surfactants that mostly rely on dissolving lipid envelopes tend to only show potency towards a narrower range of viruses and may require longer exposure durations, but are often more biocompatible.

The ideal disinfectant agent is one that is effective against a broad range of viruses, acts quickly and is highly potent, but still biocompatible and only mildly damaging to surfaces. Thus, a potential direction may be to develop potent disinfectant agents based on natural compounds that may have less toxicity, which allows the product to be child-safe and also suitable for long-term use.

Newer sanitizers with viral inactivation mechanisms that balance broad disinfection efficacy with biocompatibility are thus likely to become the preferred choice for consumers in the future. ★

ABOUT THE AUTHOR:

Xian Jun Loh received his PhD degree in 2009 from the National University of Singapore and joined A*STAR in 2013. A polymer chemist with 20 years of experience working with biomaterials, Loh is currently Executive Director at the Institute of Materials Research and Engineering (IMRE) and Director of Graduate Affairs at the Science and Engineering Research Council (SERC). His research interests lie in the design of supramolecular and stimuli-responsive polymers and hydrogels for biomedical and personal care applications.

1. Lin, Q., Lim, J. Y. C., Xue, K., Yew, P. K. M., Owh, C. *et al.* Sanitizing agents for virus inactivation and disinfection. *VIEW*. e16 (2020).

ENVIRONMENTAL SCIENCE

Minimizing our plastic waste footprint

COVID-19 has catalyzed radical changes in our relationship with single-use plastic, according to a study by an international team of researchers.

COVID-19 continues to devastate communities around the world, with the number of confirmed cases globally crossing the fifteen-million mark. Meanwhile, experts have revealed another unexpected victim of the pandemic: the environment.

As case numbers began to swell, so did the demand for personal protective equipment as a means of limiting the spread of the virus. This, in turn, led to a surge in the volume of medical plastic waste generated, particularly for products used for personal protection and in clinical settings.

In China, for example, the coronavirus outbreak saw a 30 percent dip in solid waste generated by households, but a simultaneous spike in medical waste by a whopping 370 percent in Hubei Province, much of which consists of single-use plastic.

Together with an international team of scientists from the Sustainable Process Integration Laboratory at Brno University of Technology (VUT Brno), Czech Republic, and De La Salle University, the Philippines, Peng Jiang from the Department of Systems Science at A*STAR's Institute of High Performance Computing (IHPC) has now mapped some of the potential negative and positive environmental shifts caused by COVID-19, specifically in the management of plastic waste.

We are witnessing a 'butterfly effect' caused by COVID-19 that has led to significant impacts on the environment.

"Management of the coronavirus requires single-use plastic, even if disposability is largely an environmental liability. We are witnessing a 'butterfly effect' caused by COVID-19 that has led to significant impacts on the environment," the researchers said.

In their study, the authors hypothesize that though ambitious, reducing the environmental burden in the wake of a global health crisis remains possible, given the right combination of plastic use measurement, monitoring and management.

Their study explores six possible strategies that countries can take to respond to the COVID-19 clean-up. Among them, the authors propose a new concept—the plastic waste footprint, or PWFP, which is a tool for measuring and communicating the environmental footprint of a plastic product throughout its life cycle.

By providing a simple and direct means of understanding the environmental burden of a disposable face mask, for example, people can be better informed to make more sustainable choices, the research team noted.

While there are no quick fixes when it comes to protecting Mother Earth from the lingering consequences of plastic waste, the authors believe that metrics such as PWFP can aid policymaking and public engagement around sustainability issues.

"One major challenge is changing the perceptions and behaviors of consumers. We created metrics such as the plastic waste footprint of a product to provide a way of effectively communicating abstract environmental burdens to non-specialists," the researchers said.

The team is now planning to conduct future studies to extend their proposed plastic management framework across countries, taking into account cultural, economic and geographical factors that may influence policy outcomes. This study was funded by the Czech Republic Operational Programme Research and Development, Education (project number: CZ.02.1.01/0.0/0.0/15 003/0000456). ★

IMPACT

COVID-19 has increased plastic usage in some sectors and decreased it in others. The proposed plastic waste footprint helps consumers understand the impact of their choices throughout the entire life cycle of a product.

LEFT
There has been a sharp uptick in the volume of medical plastic waste generated, particularly for personal protection equipment and clinical use.

1. Klemeš, J. J., Fan, Y. V., Tan, R. R., and Jiang, P. Minimising the present and future plastic waste, energy and environmental footprints related to COVID-19. *Renewable and Sustainable Energy Reviews* **127**, 109883 (2020).

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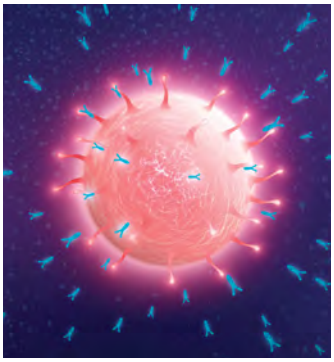
NEXT ISSUE

Here's a sneak peek of the material covered in the next issue of *A*STAR Research*



IMMUNOLOGY

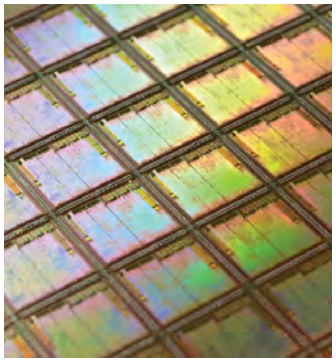
REPURPOSING SARS ANTIBODIES FOR COVID-19

Certain antibodies previously developed against SARS-CoV-1 can be used to detect SARS-CoV-2 as well, A*STAR researchers say.

MATERIALS SCIENCE

FACE-MASK INNOVATIONS, UNCOVERED

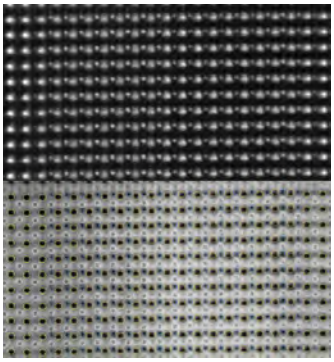
From transparent masks to antimicrobial coatings, newer face masks in development will include advanced design features and materials.



PHOTONICS

INNOVATION AT THE SPEED OF LIGHT

By combining expertise in silicon wafer manufacturing with the emerging field of integrated photonics, Advanced Micro Foundry is helping its customers get the best of both worlds.



MATERIALS SCIENCE

PIEZOELECTRICITY MADE SIMPLE

A*STAR researchers have discovered a way to generate strong piezoelectricity with simpler and greener materials.

Agency for Science, Technology and Research
SINGAPORE

RE-ENVISION THE FUTURE

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- **Work with industry partners in business development and R&D**
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I AM AN EDUCATOR

I enjoy sharing knowledge as well as conducting my own research.

SCIENTIFIC EDUCATOR

- **Be involved in academia and science education**
- **Conduct scientific research at A*STAR**

I AM A THINKER

I have a curious mind and enjoy teamwork and communication.

SCIENTIFIC LEADER

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- **Lead a team of researchers to conduct research and collaborations**

I AM AN INNOVATOR

I have novel ideas to translate research to solutions that would benefit the community.

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- **Develop scientific expertise**

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- A*STAR – University of Warwick (AWP) EngD Partnership
- A*STAR International Fellowship (AIF)

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