

Immunomodulating therapies, such as those developed to treat cancer, are being revamped to fight infectious disease, including COVID-19

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In the spring of 2020, just as the COVID-19 pandemic was accelerating, Steven Treon came across a crucial connection. As director of the Bing Center for Waldenstrom’s Macroglobulinemia at the Dana Farber Cancer Institute, Treon was working with cancer immunotherapies. By chance, he found that they might also apply to the symptoms of the SARS-CoV-2 virus.

Immunotherapies are a collection of treatments that modulate the immune system in precise ways, prompting the body’s own defences to more effectively fight disease. A few months into the pandemic, Treon and some of his colleagues reported that an existing cancer immunotherapy, the Bruton’s tyrosine kinase (BTK) inhibitor ibrutinib, could reduce inflammation in the lungs, one of the more lethal effects of a COVID-19 infection.¹

That result came from six patients with Waldenstrom’s macroglobulinemia who were being treated with ibrutinib for cancer, and who happened to become infected with COVID-19. Treon needed to test this idea on a bigger group of patients. Today, an approved cancer drug is in clinical trials as a treatment for COVID-19.

Treon’s idea to trial ibrutinib might have been somewhat of a coincidence, but his impulse to repurpose immunotherapies was not. “There’s never been a time in science when we learned so much and tried to repurpose so much, so rapidly,” says Greta Wegner, vice president of the immunoassay business unit at Bio-Techne.

Bio-Techne is one of a growing number of companies helping to advance immunotherapy research through investment and innovation in GMP antibody and protein production, as well as the development of high-quality reagents, assays, and instruments for assessing immune response and the immune system.

“We’ve been studying immune response for many years, so we clearly understand the immune system’s role in advanced treatment,” Wegner says. “The next step is to explore ways to apply what we know to help broaden the scope and accelerate the pace of success in fighting infectious diseases.”

That flood of research could drive forward new treatments for SARS-CoV-2, but it could also enable researchers to repurpose immunotherapies for other infectious diseases. How easily do immunotherapies fit this need, and how can researchers evaluate their potential?

WHY INFECTIOUS DISEASE LENDS ITSELF TO IMMUNOTHERAPY

In many respects, infectious diseases are well suited to treatment with immunotherapies. “The inability to clear pathogens – virus, bacteria, parasites – from the host is often caused by a deficit in immune response, both innate and adaptive,” explains infectious-disease expert Antonio Bertoletti of Duke-NUS Medical School in Singapore. Immunotherapies can modulate the components of the immune system to overcome such a deficit, allowing it to eliminate threats it might have otherwise ignored.

A number of different classes of immunotherapies exist today, including monoclonal antibodies, cell therapies, vaccines, oncolytic viruses and immunomodulators, such as checkpoint inhibitors and cytokines. While they each have distinct mechanisms, they primarily affect the immune response in two ways – either modifying it directly, as in the case of immunomodulators, or stimulating adaptive immune surveillance carried out by B and T cells.

Vaccines are perhaps the oldest and still most widely used immunotherapies and the knowledge gained in their development led to significant advances in immunology. That science, in turn, fuelled the development of cancer immunotherapies, which today represent the lion's share of approved immunotherapies on the market. More than a dozen checkpoint inhibitors alone are currently approved to treat various cancers.

Like Treon, researchers are now using many of the analytical techniques and assays employed in the development of cancer immunotherapies and turning them back on infectious diseases² That process typically begins by examining the molecular biology of a given illness to determine the mechanisms by which it progresses, and potential strategies to arrest it.

Bertoletti cites recent research into Hepatitis B virus (HBV). Many investigations have shown that HBV can evade innate immunity, but no one knows how.³ "Since the virus does not activate, for example, interferon-alpha production – but is sensitive to the antiviral effect of this and other cytokines – a strategy is to treat patients directly with interferon-alpha or to activate its production in the liver," Bertoletti says. Furthermore, studies of the surface of T cells show that increasing inhibitory receptors deactivate HBV-specific T cells, degrading the adaptive immune response⁴ Scientists are studying ways to restore the functionality of these HBV-specific T cells to control HBV replication.⁵

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HBV is but one example, and an early one at that. There are dozens of preclinical and clinical trials ongoing for repurposed or adapted immunotherapies to treat infectious diseases as diverse as Ebola, Zika, SARS-CoV-2, tuberculosis, and MRSA.⁶ Researchers have found that PD-L1 checkpoint inhibitors, for cancer, have shown promise fighting malaria and leishmaniasis in mice. And in 2018, the U.S. Food and Drug Administration approved the monoclonal antibody ibalizumab to treat HIV-1, the first immunotherapy for that disease.

While these research efforts are as diverse as the diseases they're meant to address, one unifying theme is that they all, to a degree, rely on proven analytical techniques at every stage of development.

THE ENDURING APPEAL OF IMMUNOASSAYS

While not a new technology, immunoassays provide valuable, quantitative biomarker data to help researchers rapidly evaluate immunotherapies. Scientists have applied ELISA-based technology in disease studies since the 1970s. One of the biggest advances over the years, Wegner says, came from automating this immunoassay.

In addition to automation, companies developed ELISA-based platforms that can quickly and simultaneously track multiple protein targets. Bio-Techne's Ella platform, for example, can run automated immunoassays for up to eight targets at a time – completing a run in about 90 minutes. Scientists could use such a technology when repurposing drugs for known targets, such as markers of inflammation.

Researchers working to understand disease mechanisms and pathways or develop immunotherapies that attack multiple targets at once – or even test combination therapies – prefer multidimensional methods. For instance, Bio-Techne makes Luminex assays, which can be used to survey the immune response. Scientists can pick from a selection of more than 450 targets to create a custom assay of up to 50 analytes.

"One advantage of multiplexing is that it allows you to examine a wide variety of markers simultaneously," Wegner explains. "Scientists can then use data on multiple biomarkers to refine their drug candidates. Assessing multiple targets in a single assay also allows you to be judicious with precious samples, alleviating the need to prioritize assays based on limited sample volumes."

GROWING POSSIBILITIES IN INFECTIOUS DISEASE RESEARCH

While immunoassays remain the stock-in-trade methods for exploring diseases and evaluating immunotherapy candidates, some researchers are pairing them with other exploratory techniques. Pathologist, Deyu Fang, of Northwestern University, Feinberg School of Medicine in Chicago studies regulatory T cells (Tregs). The cells suppress various components of the immune system, preventing the body from attacking itself. However, they can also aid the progression of certain diseases. For instance, Tregs can enter tumour tissues, which reduces the immune system's ability to fight solid cancers.⁷

Fang, immune-system expert Alexander Marson of the University of California, San Francisco, and their colleagues recently developed a system that would help researchers search for targets on Tregs that could be inhibited by new or existing cancer

immunotherapies. Their focus was to understand the mechanisms that support the expression of a protein named Foxp3, a transcription factor known to control Tregs' suppressive activity. "Foxp3 is the pinnacle for Treg function and proper homeostasis of the immune system," says Elena Montauti, a doctoral student in Fang's lab.

The team used a retrovirus to insert genes for various transcription factors—500 in all—into Treg cells. They then used CRISPR to inactivate those genes, one by one, and antibody-based flow cytometry to measure expression of Foxp3.⁸ If the Treg cells had low Foxp3 expression, the targeted factor stabilized expression; if the Treg cells had high Foxp3 expression, the targeted factor repressed it.

This system, while developed with cancer in mind, could be translated to applications in infectious disease. "Many infectious disease labs are using CRISPR to better understand how infectious pathogens interact with human cells on a molecular level," says Jessica Cortez, who helped develop the CRISPR screen as a doctoral student in Marson's lab and is now a discovery scientist at Sonoma Biotherapeutics. For example, Nevan Krogan and his colleagues at the University of California, San Francisco, used CRISPR to turn off genes in T cells exposed to HIV, and then used antibodies and flow cytometry to find proteins associated with viral infection.⁹ The same methodology could also apply to screenings for T cells, interferons, cytokines or most other targets of new or existing immunotherapies.

While the ongoing battle with COVID-19 has intensified the need to investigate new antiviral therapies, scientists have decades of research to help guide them. They also have available a growing array of tools and techniques for assessing immune response. Bio-Techne recently introduced new techniques for screening and validating clinically relevant immune markers, at various scales, based on the analysis of protein or RNA marker expression.

To Wegner, the potential applications for new immunotherapeutic approaches are as diverse as they have ever been, from fighting emerging infectious diseases to treating cancer. "We're continuing to push the boundaries in the range of techniques and tools available for surveying immune response and understanding its potential for helping to unlock next-generation therapies," she says. "We've only explored the tip of the iceberg for the best treatments based on the characteristics of a person's immune system."

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