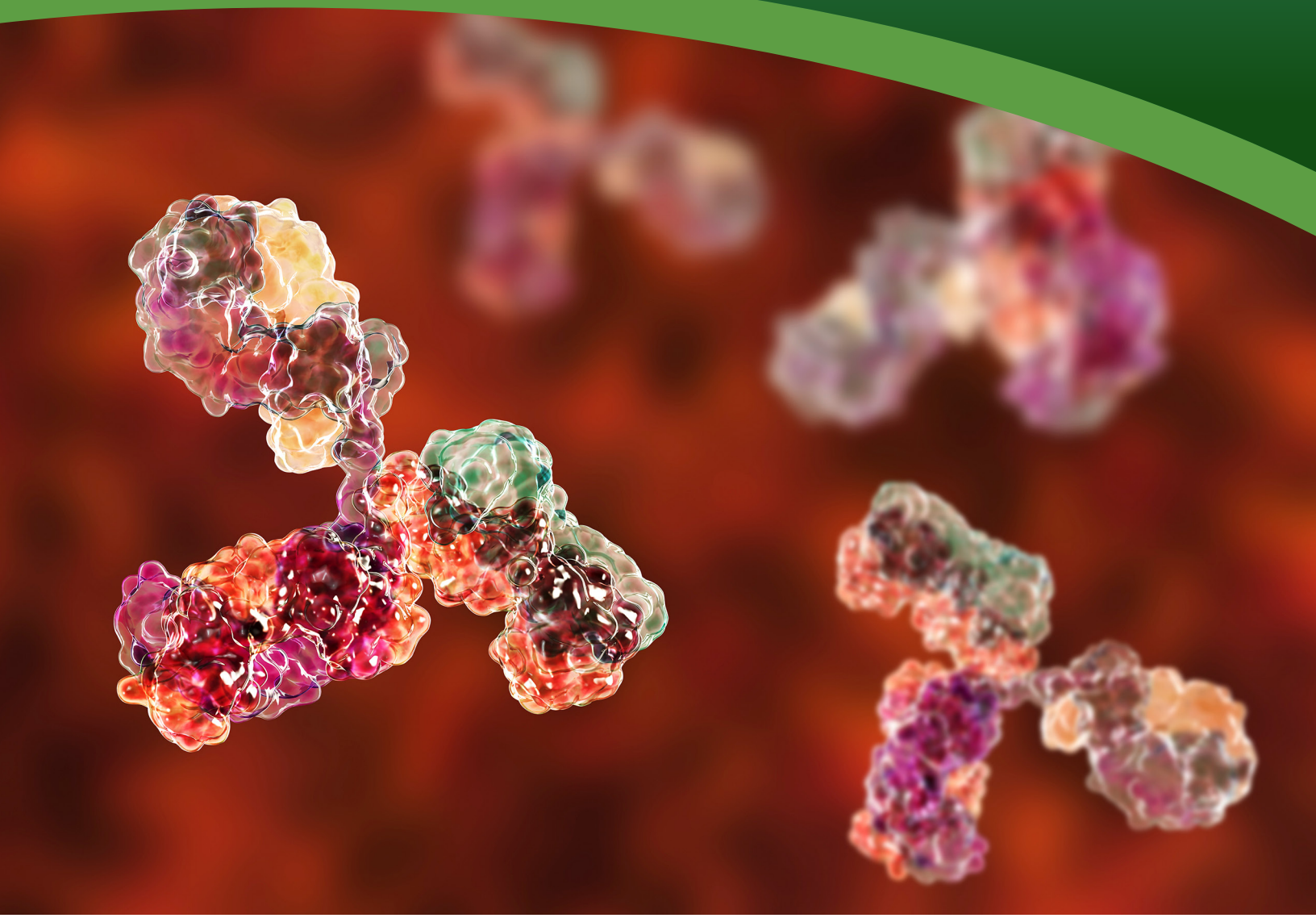
Proper Antibody Design Keeps Specificity in Mind



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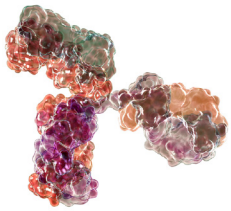
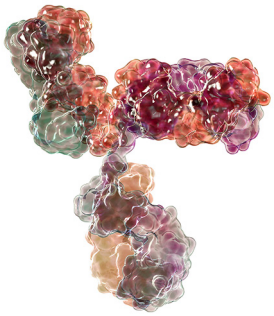
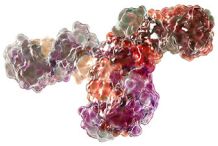
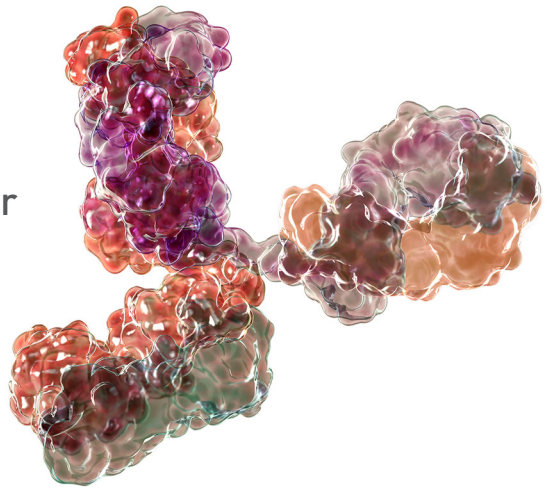


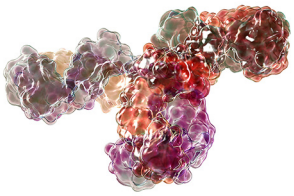






Cover, pp. 3–5: Kateryna Kon / Science Photo Library / Getty Images

INTRODUCTION



Natural antibodies are an amazing design of nature. Our immune system produces proteins of unique structure to bind specifically to incoming foreign agents and protect our body from exogenous invaders. Human ingenuity takes the lesson from nature, turning antibodies into one of the most useful and versatile types of tools for life science research, clinical diagnostics, and even therapeutics. The impact of antibodies has dramatically expanded with recent advancements.

SPECIFICITY! SPECIFICITY! SPECIFICITY!

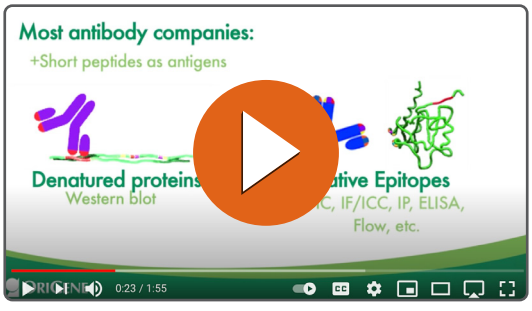
When an antibody is used in a research application or clinical tests, one of the essential features to be critically evaluated is its specificity. Specificity addresses whether the antibody can uniquely bind its intended target while ignoring other similar targets. Promiscuous binding of antibodies can cause misleading results in research and erroneous diagnoses of clinical tests.

So, how then do we make antibodies with high specificity? One of the solutions is to use an extremely unique immunogen. While synthetic peptides are commonly used as immunogens for quick antibody generation, they tend not to recapitulate the 3D structure or post-translational modifications of the native protein. Antibodies generated against such immunogen can fail to recognize native proteins. Additionally, synthetic peptides do not carry post-translational modifications and, therefore, can miss critical epitopes.

On the other hand, full-length proteins made from human cells are the best immunogen to generate highly specific and functional antibodies that recognize native protein targets. This is precisely what OriGene offers—as our TrueMABs are mouse monoclonal antibodies made using full-length human proteins expressed in a human cell as the immunogen. Although this is a slower and more expensive way to make antibodies, we believe this is the method that produces a higher quality, more specific antibody. OriGene has accumulated over 12,000 TrueMABs, and assay developers are discovering new benefits of using TrueMABs all the time.

Why is TrueMAB is the right antibody?

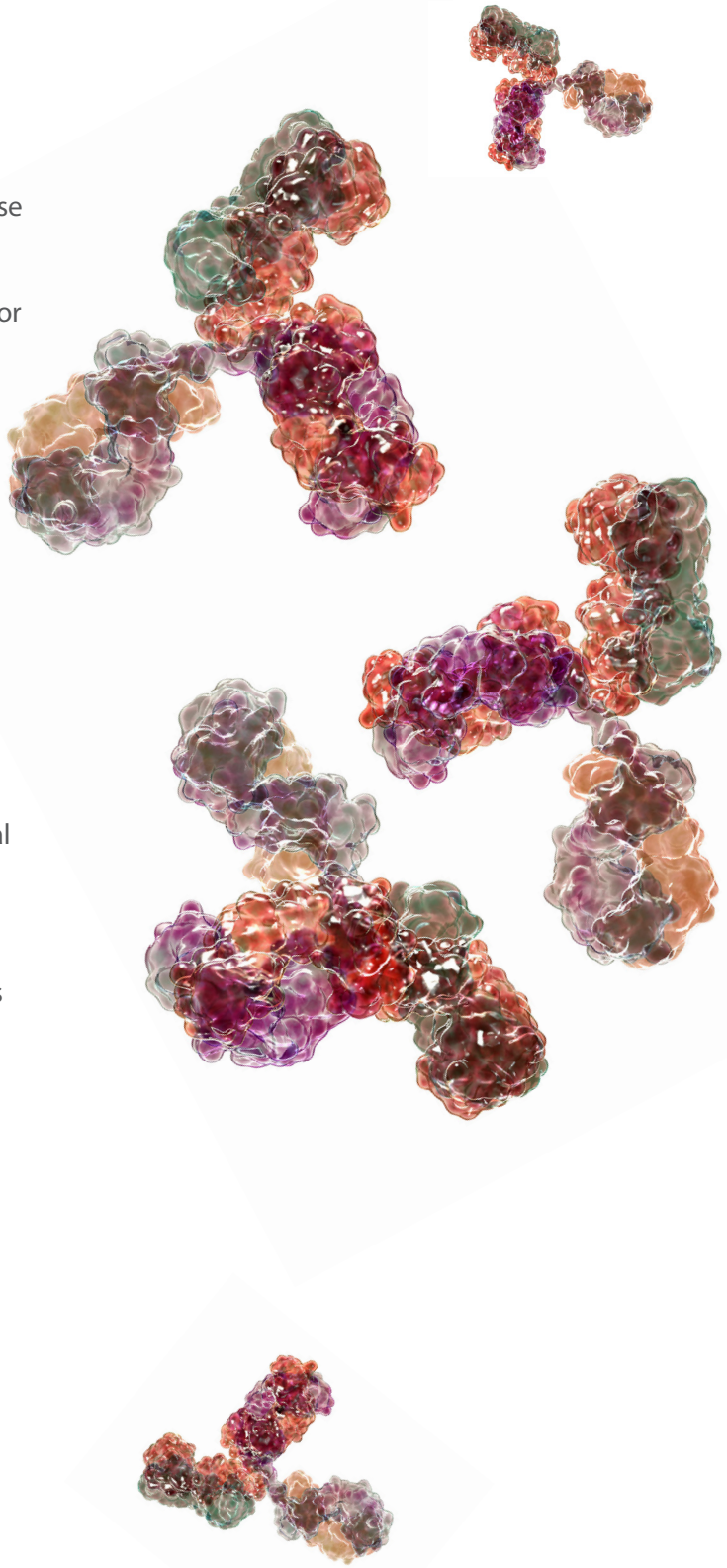
Watch a 2-min video



UltraMAB is a subset of TrueMAB that has gone through rigorous specificity testing: a hybridization test using a microarray chip of 17,000 proteins. Those antibodies that are proven to bind only to their intended target and nothing else can be qualified for UltraMAB.

Thanks to advanced biomedical engineering, scientists can swiftly create designer antibodies with desired features. When the COVID-19 pandemic started, scientists quickly generated human antibodies specific for the virus spike protein—which binds to ACE2 and initiates viral entry into the host cells. Such antibodies can be used to neutralize the virus and serve as a potential treatment option. Moreover, researchers can even create antibody-like proteins that don't exist in nature, such as bi-specific antibodies often used as cancer therapeutics.

In this ebook, you will read about the fascinating ways bioengineering has changed how antibody molecules are applied to research and the clinical world. Despite all the changes, specificity remains the core value determinant of an antibody, albeit naturally or recombinantly made.



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Antibody Characterization Keeps Pace with the Antibody Industry

Biosimilar approvals, production upgrades, and regulatory demands are being matched by advances in monoclonal antibody characterization

By Gareth John Macdonald

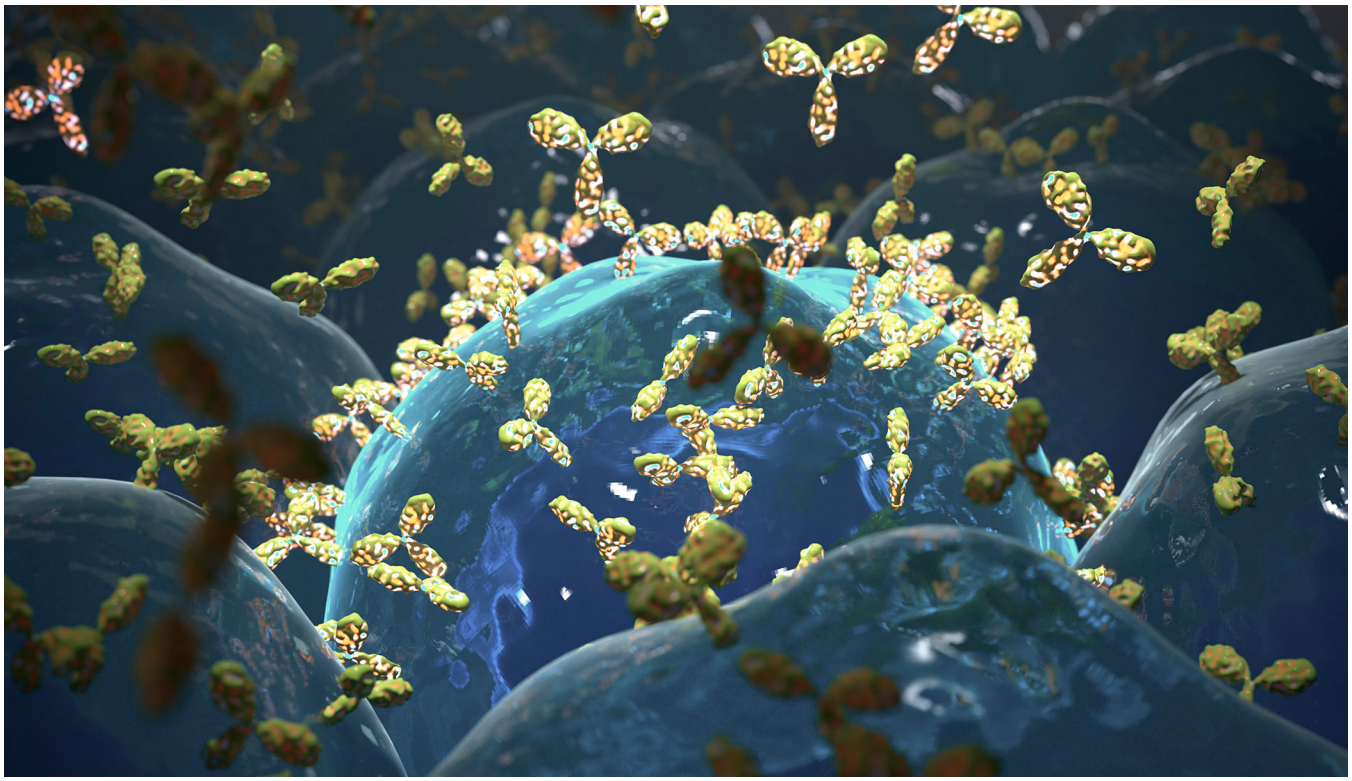
Biologic monoclonal antibody (mAb) drugs are not just more targeted than small-molecule drugs, they are also larger, more complicated, and more variable. Consequently, biologic mAb drugs, which generate sizable revenues, may enjoy some degree of protection from biosimilars, follow-on biologics that are similar but not identical to their corresponding reference biologics. To obtain the maximum degree of protection, biologic mAb drugs need to be intensively and comprehensively characterized. Differences between mAbs and biosimilars, it may be argued, are not merely incidental, but crucial to biological activity and therapeutic action.

Biologic/biosimilar differentiation is just one reason to enhance mAb characterization. Other reasons—potentially more compelling reasons—include the need to collect metrics that can be used to screen mAb drug candidates, optimize production processes, and satisfy increasingly stringent regulations. Also, as mAb processes become more sophisticated, increasingly subtle mAb characteristics become significant.

Back when the U.S. Food and Drug Administration approved the first mAb drug, muromonab-CD3 (Orthoclone OKT3), making mAbs was a complex and cutting-edge undertaking. It involved fusing cells—in the case of Orthoclone OKT3, one from a mouse's spleen and one from a tumor—to create an expression cell line followed by multiple harvesting steps. Yields were low, and production was costly.

Today, mAb production is almost routine. Cell line development and manufacturing techniques have advanced, and more efficient production and harvesting platforms have been developed. Changes such as these mean that the mAb industry faces more nuanced challenges in elucidating mAb molecular structures and characteristics.

Fortunately, industry can meet these challenges by deploying advanced characterization technologies. Such technologies can, for example, help industry find mAb candidates faster, says Anis H. Khimani, PhD, portfolio marketing director, strategy



Nanoclustering / Science Photo Library / Getty Images

leader and applications, for PerkinElmer's Discovery & Analytical Solutions organization. "Monoclonal antibody characterization is important for the biopharmaceutical industry," he says, "because it enables selection of the therapeutic molecule representing greater specificity, efficacy, stability, and functionality."

Characterization through development and production

The rationale for characterizing a mAb differs at each stage of the development and production process. Understanding what you are looking for and why is critical. "It is important," says Gunnar Malmquist, PhD, principal scientist at GE Healthcare, "to distinguish between the mAb characterization that takes place during drug development and the mAb characterization that is performed during process development and routine manufacturing." The former is used to determine that you have the correct mAb and to establish potential critical quality attributes of the antibody. The latter depends, to some extent, on these attributes. "They will identify which product-related impurities you will need to consider during process development and subsequently during manufacturing," Malmquist advises.

The analysis methods used during process development and routine manufacturing fall into three different categories: process control and lot release; stability indicators; and characterization, which is used to confirm that drug properties remain consistent and to confirm acceptable levels of product-related impurities.

Properly executed, analytical methods help manufacturers detect high-molecular-weight

aggregates resulting from the mAbs sticking together during production. Likewise, analytical techniques are used to identify other impurities like host cell proteins or leached Protein A.

To illustrate the importance of such steps, Maryann Shen, PhD, LCMS global marketing program manager, Agilent Technologies, discusses how they relate to a representative characterization activity, glycan profiling. "Many approved biopharmaceuticals are glycoproteins," she says. "Glycans can play an important role in drug efficacy and safety. Regulatory agencies demand detailed analysis of glycosylation. Glycan profiling is a very common step in the characterization of the mAb."

Characterization challenges posed by heterogeneity

Biomanufacturing always involves variability. While production advances have brought greater consistency, the use of living cells in the process makes it impossible for all variables to be controlled.

For mAbs, this variability usually impacts the product's quality attributes more often than the molecule itself, suggests Michael Walker, PhD, technical expert, LC-MS protein analysis, Intertek Pharmaceutical Services. "This route of manufacture," he points out, "leads to heterogenicity in production lots, specifically in relation to differences in post-translational modifications, which are hugely significant as they can impact both the efficacy and safety of the final product.

"Understanding the structural characteristics of the heterogeneous population is consequently not so much concerned with ensuring the correct mAb is formed, although identity testing would always be included, but [with] developing safe, effective therapies through identification of the critical quality attributes. Ideally, these attributes, which need to be controlled through production, should be continuously monitored to ensure the continued efficacy and safety of the product."

This assessment of modifications is an area of active research for industry, says Malmquist. He told us, "The extent of post-translation modifications lead to a severe variability of the mAb structure and therefore to a large number of potential product related impurities that need to be characterized.

"An emerging trend [to achieve this] is to look at so-called multiattribute methods, which are based on a combination of peptide mapping and mass spectroscopy. The goal is to assess multiple quality attributes with one analytical method."

Characterization techniques common to the lab and the factory floor

Another characterization trend has seen drug companies take analytical techniques usually carried out in the laboratory into the production space. According to Malmquist, the approach, which involves firms placing analytical instruments near manufacturing lines, "will increase data frequency, decrease response times, and improve process control."

Eventually, real time release testing may even be possible which, Malmquist says, would significantly

Laboratory techniques to characterize monoclonal antibodies are being developed to satisfy demands for increasingly detailed information. According to Intertek Pharmaceutical Services, which provided this image, better characterization techniques can help biopharmaceutical companies accomplish increasingly ambitious goals with respect to efficiency, safety, and regulatory compliance.

reduce release time by ensuring quality targets are met during the manufacturing process.

Comparing reference biologics and follow-on biosimilars

Biopharmaceuticals, as stated above, are less likely to face competition than small-molecule drugs. Primarily this is due to variability inherent in production, which makes it hard for a company to replicate another's product. That said, off-patent biopharmaceuticals can still face competition from biosimilars. Although biosimilars are not generics, they are conceptually similar.

Biosimilar requirements vary from market to market. In general, securing a biosimilar's approval is a matter of showing that the molecule's active properties are similar to those of a reference product that has already been cleared by regulators. To date, many of the approved biosimilars are versions of mAb-based therapies.

"The biosimilar manufacturer," Malmquist says, "will have to exert a substantial and increased

characterization effort in demonstrating structural similarity to the originator profile to benefit from the simplified regulatory framework available."

The biopharma industry also compares biologics and biosimilars for competitive reasons. It is now common for firms to try to develop as complete and detailed a description of their product's molecular properties as possible to make it harder for biosimilar firms to create matching drugs.

Incorporating advanced analytical technologies

Technology advances support more detailed analyses, which are, Walker points out, becoming increasingly routine as biomanufacturers respond to regulatory demands. "Data-rich techniques like mass spectrometry allow more critical quality attributes to be monitored in a single assay to improve process development," he continues. "The increased throughput and data integrity that technological improvements have allowed open up new parts of the manufacturing pipeline for complex analytical techniques."

The emergence of next-generation mAbs, antibody drug conjugates (ADCs), and fragment-based drugs is also impacting how industry uses characterization technologies.

"Beyond conventional mAbs, related products such as Fc fusion proteins or bispecific antibodies (bsAbs) and antibody-fusion proteins are in development," Walker details. "Each of these brings specific characterization challenges that may require new approaches and technologies.

"For example, Fc fusion proteins are susceptible to proteolytic cleavage. They also have the potential to form higher levels of high-molecular-weight aggregates as compared to conventional mAbs. This drives the need for a definitive suite of approaches to monitor stability and occurrence of aggregation.

"If bispecific antibodies introduce a new product-related impurity, the potential for mismatching of protein subunits needs to be controlled. This is analytically challenging as often there is a high degree of conservation between chains. These types of molecules have led to an increased interest in native mass spectrometry methods hyphenated to methods such as size-exclusion chromatography and capillary electrophoresis."

Consolidating characterization chores

Another dynamic impacting biopharma's approach to mAb characterization in the drive for efficiency. Every company wants to get product to market as quickly as possible. In the mAb space, the focus is on completing the necessary

analysis steps—including characterization—as accurately and efficiently as possible.

"Currently, there is a high desire to combine multiple tasks into one single method, as researchers require both detailed information and faster results," explains Shen. "Monoclonal antibody characterization is complicated, and many tasks are involved in the process. These usually require labor-intensive sample preparations and time-consuming analyses. It is desirable to have instruments that provide reproducibility, robustness, and ease of use."

Walker has also observed this trend: "Monoclonal antibody characterization involves a diverse set of advanced analytical techniques, many of which require specialist equipment and training. As some technologies are maturing, there has been a move toward more automated sample preparation and data analysis to reduce timelines.

"An example of this is with mass spectrometry, where hardware and software are becoming more user-friendly, reducing the time taken by specialist operators. There is still, however, some work to be done in this area so that relatively inexperienced operators can access the technology."

A productive dialectic: characterization needs and technological capabilities

Biopharmaceutical industry demand for more detailed mAb characterization systems will continue to be a major development driver. "The analytical field has witnessed continued development of technologies to enhance characterization, isolation, and purification of mAbs," states Khimani. "The chromatographic and electrophoretic techniques have been combined with mass spectrometry with significant improvements to the sample preparation requirements."

> "Monoclonal antibody characterization is complicated, and many tasks are involved in the process. These usually require labor-intensive sample preparations and time-consuming analyses."
>
> **Maryann Shen, PhD, LCMS**
> global marketing program manager, Agilent Technologies

Khimani cites automated capillary electrophoresis–based separation technologies and advances in assay development as examples of the work being done, explaining that they "have enabled evaluation of mAb in native conformation."

"With continued development of next-generation technologies and tools, characterization of mAb will continue to evolve," he predicts. "It will be a growing space for investigators within the biopharma and biologics segments."

Coronavirus-Neutralizing Human Antibody Discovered

Researchers at Utrecht University, Erasmus Medical Center and Harbour BioMed (HBM) have identified a fully human monoclonal antibody that prevents SARS-CoV-2 from infecting cultured cells. Discovery of the antibody, which also neutralizes the related SARS-CoV coronavirus, represents an initial step towards developing a fully human antibody to treat or prevent COVID-19, and also potentially future diseases caused by viruses from the same coronavirus subgroup.

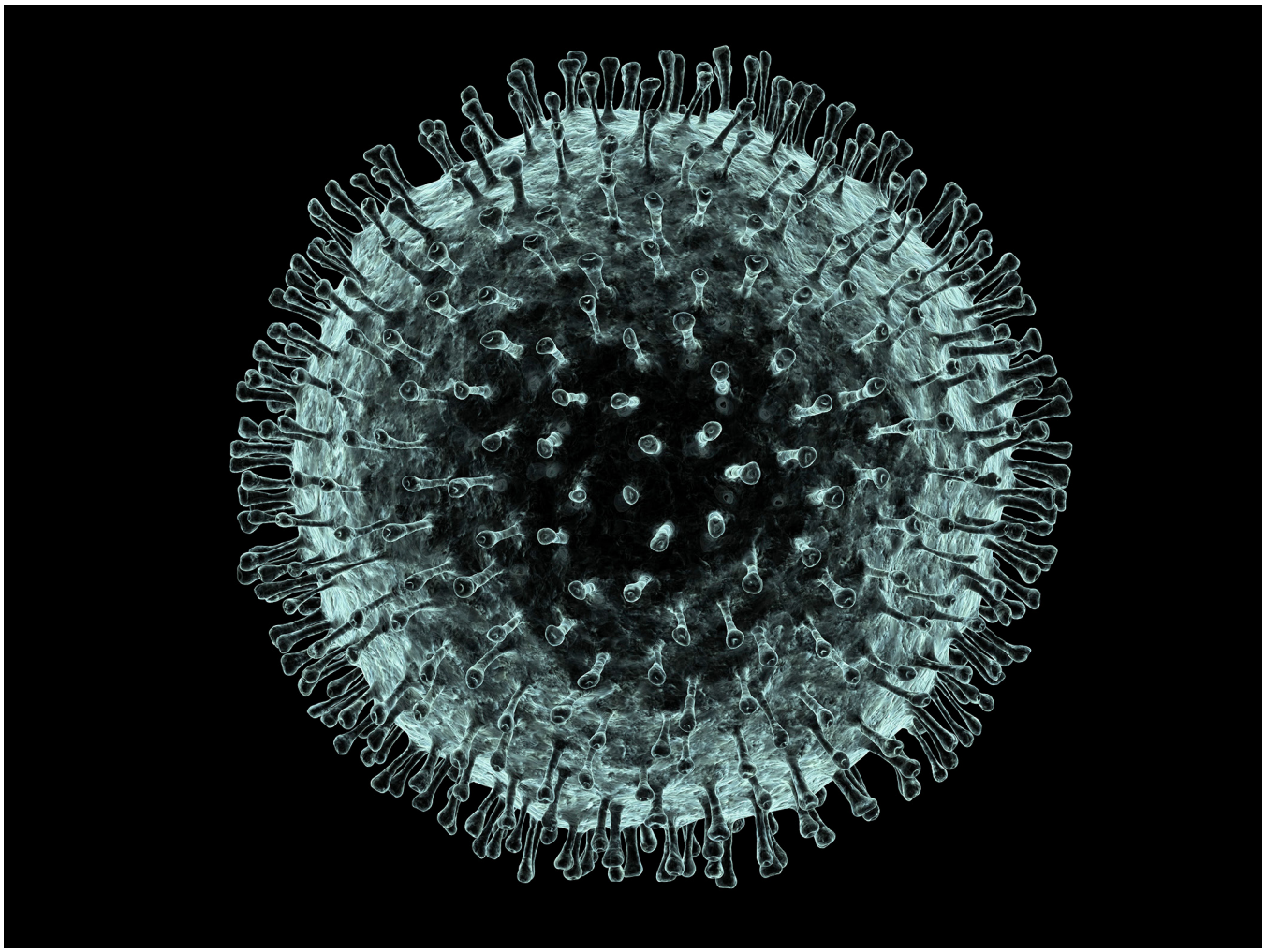
"This discovery provides a strong foundation for additional research to characterize this antibody and begin development as a potential COVID-19 treatment," said Frank Grosveld, PhD. co-lead author on the study, Academy Professor of Cell Biology, Erasmus Medical Center, Rotterdam and Founding CSO at Harbour BioMed. "The antibody used in this work is 'fully human,' allowing development to proceed more rapidly and reducing the potential for immune-related side effects."

Grosveld and colleagues report on the antibody in *Nature Communications*, in a paper titled, "A human monoclonal antibody blocking SARS-CoV-2 infection."

Both SARS-CoV-2 and the SARS-CoV virus that emerged in 2002, belong to the *Sarbecovirus* subgenus of the *Betacoronavirus* family of coronaviruses. The two viruses crossed species barriers from an animal reservoir, and can cause life-threatening respiratory illness in humans. By May 4th 2020 there were more than 3.4 million confirmed cases of SARS-CoV-2 worldwide, and in excess of 230,000 deaths. The SARS-CoV strain caused ~8000 infections, with a lethality of 10%. There are currently no approved targeted therapeutics available for COVID-19, the disease caused by SARS-CoV-2.

Monoclonal antibodies targeting "vulnerable sites" on viral surface proteins are increasingly recognized as a promising class of drugs against infectious diseases, and have shown therapeutic efficacy for a number of viruses, the authors wrote. Coronavirus-neutralizing antibodies primarily target the trimeric spike (S) glycoproteins on the coronavirus surface that mediate entry into host cells. The S protein has two functional subunits. The S1 subunit, which is composed of four core domains, $S1_A$ through to $S1_D$, mediates attachment to the host cell. The S2 domain mediates fusion of the viral and cell membranes.

The spike proteins of SARS-CoV-2 and SARS-CoV share 77.5% identical amino acid sequence, and are structurally very similar, the investigators continued. They commonly bind the human angiotensin converting enzyme 2 (ACE2) protein as the host



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receptor. "Potent neutralizing antibodies often target the receptor interaction site in S1, disabling receptor interactions," the authors continued.

For their reported antibody discovery effort, Grosveld and colleagues built on work that the groups had carried out on antibodies targeting SARS-CoV, explained co-lead author Berend-Jan Bosch, Associate Professor, Research leader at Utrecht University. "Using this collection of SARS-CoV antibodies, we identified an antibody that also neutralizes infection of SARS-CoV-2 in cultured cells." The human antibody identified, 47D11, was generated using Harbour BioMed's H2L2 transgenic mouse technology.

47D11 was shown to bind to cells expressing the full-length spike proteins of both SARS-CoV and SARS-CoV-2, and potently inhibited viral infection of cultured cells. Tests showed that the antibody targeted the $S1_B$ receptor-binding domain (RBD) of the spike proteins of both viruses. The fact that the antibody is cross-reactive indicates that it likely targets the conserved core structure of the $S1_B$ RBD, the investigators suggested. "Such a neutralizing antibody has potential to alter the course of infection in the infected host, support virus clearance or protect an uninfected individual that is exposed to the virus," Bosch stated.

Interestingly, the team's results suggested that

Vichie81 / Getty Images

47D11 neutralizes SARS-CoV and SARS-CoV-2 through "a yet unknown mechanism" that is different from receptor-binding interference. "Alternative mechanisms of coronavirus neutralization by RBD-targeting antibodies have been reported including spike inactivation through antibody-induced destabilization of its prefusion structure, which may also apply for 47D11," the team noted.

"In conclusion, this is the first report of a (human) monoclonal antibody that neutralizes SARS-CoV-2," they concluded. "This antibody will be useful for development of antigen detection tests and serological assays targeting SARS-CoV-2 … this antibody—either alone or in combination—offers the potential to prevent and/or treat COVID-19, and possibly also other future emerging diseases in humans caused by viruses from the *Sarbecovirus* subgenus."

"This is groundbreaking research," said Jingsong Wang, PhD, founder, Chairman & Chief Executive Officer of HBM. "Much more work is needed to assess whether this antibody can protect or reduce the severity of disease in humans. We expect to advance development of the antibody with partners. We believe our technology can contribute to addressing this most urgent public health need and we are pursuing several other research avenues."

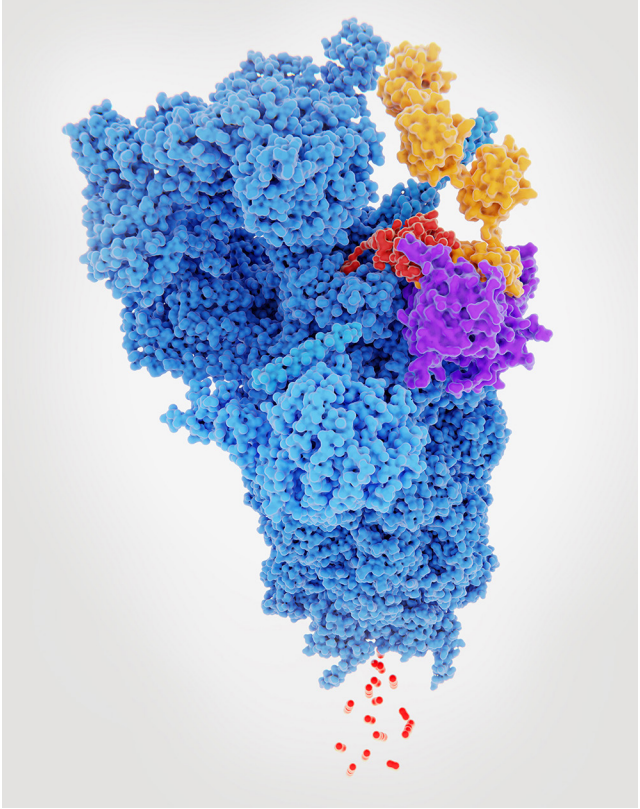
Nanobody Approach Prevents the Culling of Imperfect but Still-Functional Proteins

To ensure the health of the cell's protein herds, the ubiquitination system marks misfolded or damaged proteins for destruction. But the ubiquitination system can be overzealous, targeting proteins that are less than perfect, but still able to carry out their usual, useful functions. Conceivably, if less-than-perfect proteins were to be spared, they could serve therapeutic purposes.

This possibility is being explored by scientists based at Columbia University. They are developing engineered deubiquitinases (enDUBs) that can reverse the ubiquitination system's ruthless machinations. Importantly, the enDUBs can discriminate between proteins that should be spared, and those that really do need to be destroyed.

Details of this work recently appeared in *Nature Methods*, in an article titled, "Targeted deubiquitination rescues distinct trafficking-deficient ion channelopathies." This article describes how the Columbia scientists' approach, which relies on a new technology that incorporates synthetic llama antibodies, could be used to treat dozens of diseases, including cystic fibrosis, that arise from the destruction of imperfect but still perfectly functional proteins.

"We developed enDUBs that enable selective ubiquitin chain removal from target proteins to rescue the functional expression of disparate mutant ion channels that underlie long QT syndrome (LQT) and cystic fibrosis (CF)," the article's authors wrote. "In an LQT type 1 (LQT1) cardiomyocyte model, enDUB treatment restored delayed rectifier potassium currents and



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Proteasomes are a large molecular machine that degrade unneeded or damaged proteins that have been tagged with polyubiquitin (yellow). The ubiquitin hydrolase (violet) detaches ubiquitin from the protein that is then unfolded and degraded into small peptides (bottom).

normalized action potential duration. CF-targeted enDUBs synergistically rescued common and pharmacotherapy-resistant CF mutations when combined with [drugs that have already been approved by the FDA]."

> "In the case of one of the cystic fibrosis proteins we tested, we get a remarkable rescue, restoring protein levels in the cell membrane to about 50% of normal. If that happened in a patient, it would be transformative."
>
> **Henry Colecraft, PhD,**
> Columbia University

The enDUBs are modified deubiquitinases. Like ordinary deubiquitinases, the enDUBs remove the ubiquitin tags—small peptides that say "destroy me"—that are applied by the ubiquitination system. The enDUBs, however, incorporate a synthetic nanobody that recognizes a specific protein.

Natural nanobodies are small antibodies produced by llamas, camels, and alpacas. These molecules, which were discovered nearly 30 years ago, bind their targets with exquisite specificity and retain this property inside cells, unlike regular antibodies.

In the current study, nanobodies were produced that recognized and bound only selected targets. One of the targets was a protein mutated in CF. The other was a protein mutated in LQT syndrome, an inherited heart disease that can cause arrhythmia and sudden death.

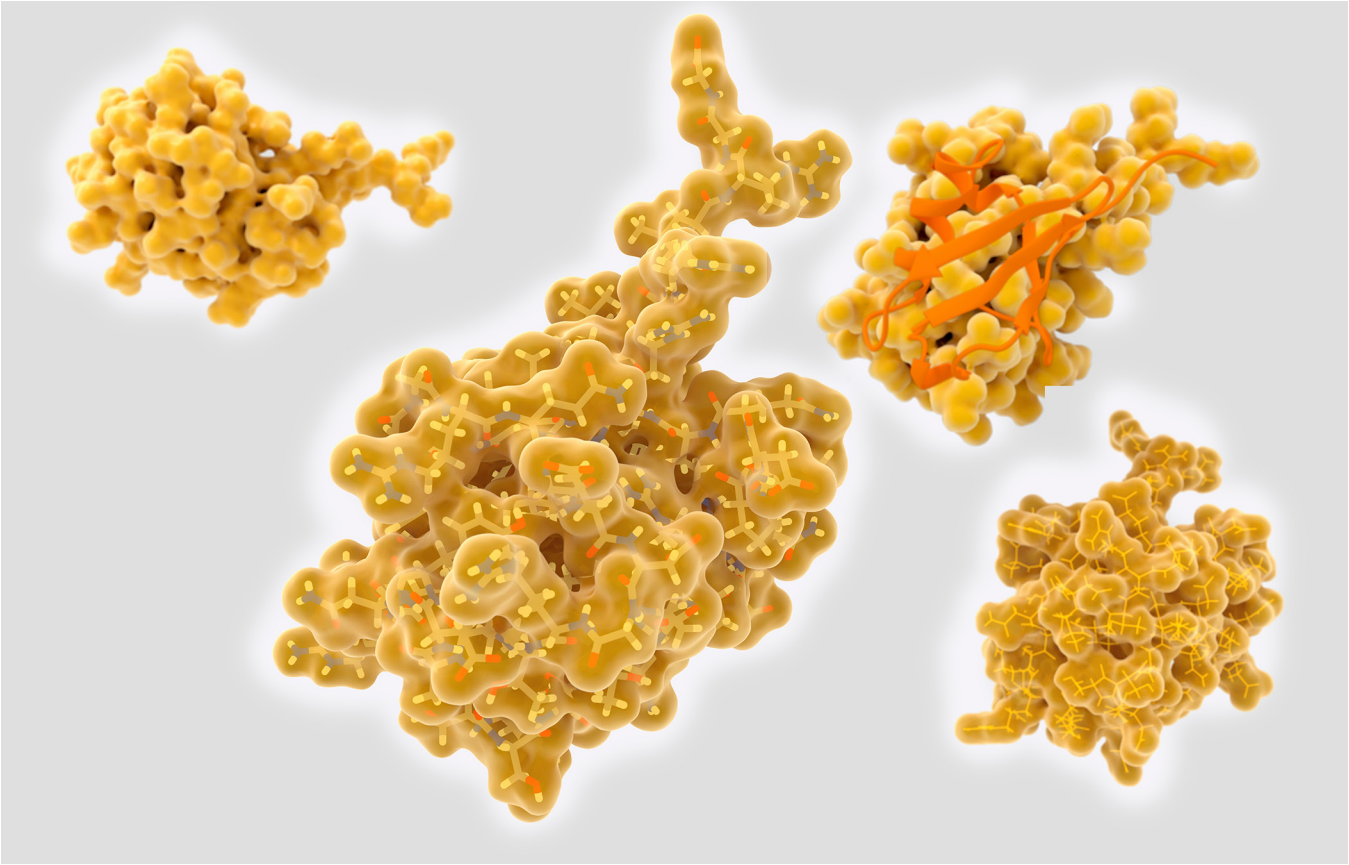
"Altogether," the authors of the *Nature Medicine* article asserted, "targeted deubiquitination via enDUBs provides a powerful protein stabilization method that not only corrects diverse diseases caused by impaired ion channel trafficking, but also introduces a new tool for deconstructing the ubiquitin code in situ."

The Columbia team, which was led by Henry Colecraft, PhD, also noted that deploying enDUBs should be more effective than simply increasing DUB activity and indiscriminately rescuing all proteins in a cell marked for destruction. Suspending all protein destruction in the cell would be harmful.

Colecraft observed that in many genetic diseases, including cystic fibrosis, mutated proteins are capable of performing their jobs but are tagged for destruction by the cell's quality control mechanisms. "The situation is analogous to ugly fruit," he said. "Shoppers reject fruit that doesn't look perfect, even though ugly fruit is just as nutritious. If mutated proteins in cystic fibrosis can escape the cell's quality control mechanisms, they work pretty well."

"A lot of proteins are destroyed by the cell for good reason," Colecraft added, "so a therapy needs to be selective."

To build each enDUB, Colecraft and colleagues, including graduate student, Scott Kanner, first



Juan Gaertner / Science Photo Library / Getty Images

Ubiquitin, molecular model. Ubiquitin is found in all eukaryotic cells. When a protein is damaged or old it will be tagged by several ubiquitin molecules. The protein is then moved to a proteasome, a hollow barrel shaped protein that degrades other proteins into amino acids and small polypeptides.

had to find the right nanobody. Until recently, researchers had to inject their target proteins into llamas, camels, or alpacas and wait for the animal to generate such nanobodies. The Columbia researchers instead fished out binders from a synthetic yeast nanobody display library containing millions of unique nanobodies.

Once created, each enDUB was tested in cells that produced the mutated proteins.

In both cases, enDUBs prevented the destruction of the proteins, and the proteins migrated to their normal locations in the cell membrane where they performed their normal functions.

"In the case of one of the cystic fibrosis proteins we tested, we get a remarkable rescue, restoring protein levels in the cell membrane to about 50% of normal," Colecraft reported. "If that happened in a patient, it would be transformative."

Though both diseases investigated in the study are caused by mutations in ion channel proteins, "the approach can be applied to any protein in the cell, not just membrane proteins or proteins altered by genetic mutations," Colecraft maintained. "It could be applicable to any disease where protein degradation is a factor, including cancer and epilepsy."

Bispecific, Multispecific Antibodies Grapple with Cancer

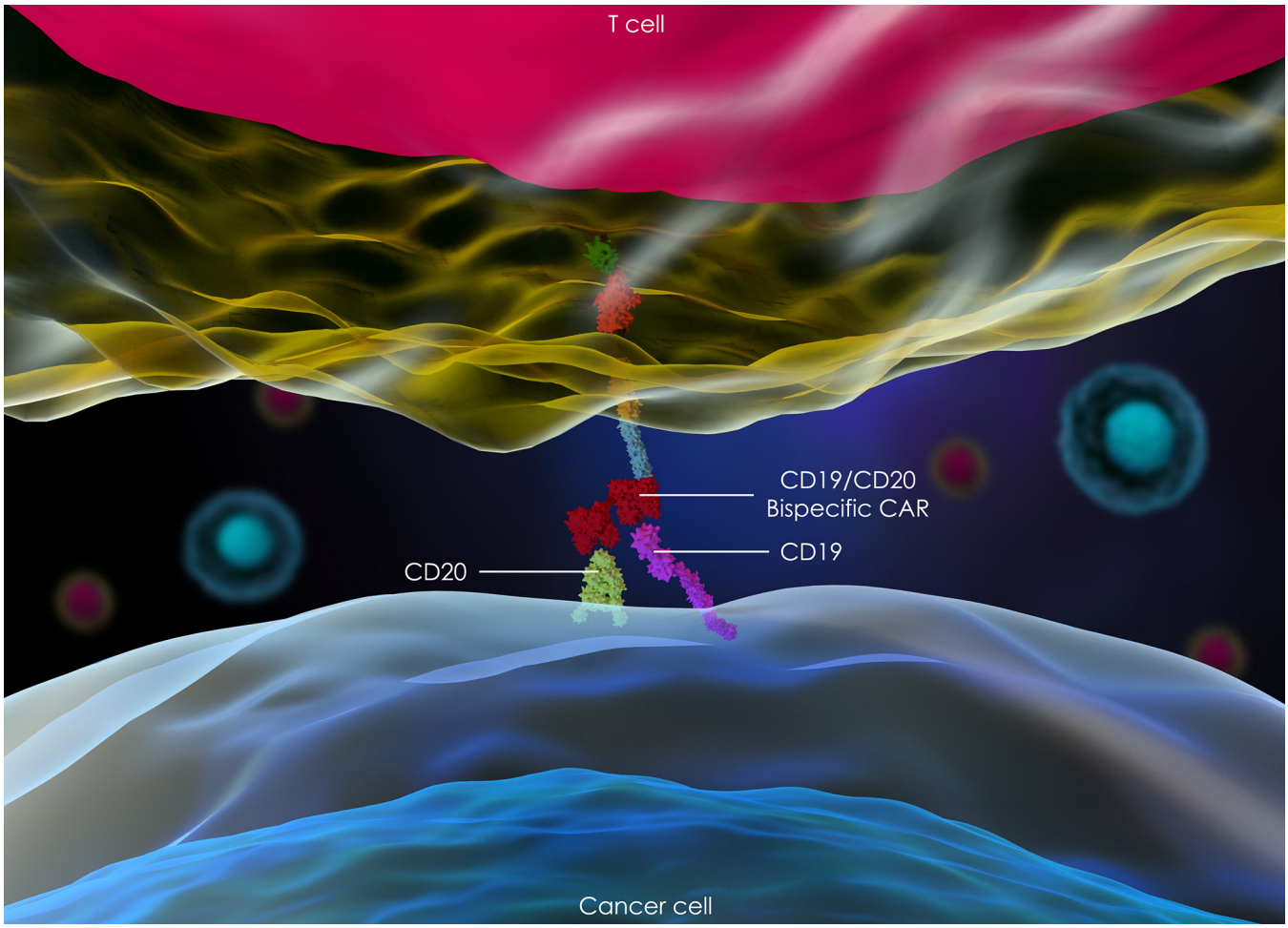
Platforms for novel antibody constructs take hold in cancer immunotherapy development

By Ian C. Clift, PhD, Scientific Communications Consultant, Biomedical Associates and Clinical Assistant Professor, Indiana University

Cancer immunotherapy has been advancing on several fronts, most strikingly in the direction of checkpoint inhibition and chimeric antigen receptor (CAR) T-cell therapy. Another front, however, is about to see its share of action. Here, newly engineered bispecific and multispecific antibodies will be put to the test. Such antibodies may engage two or more antigens at once, serving as force multipliers that can exploit opportunities beyond the reach of monospecific antibodies, whether they are deployed solo or in teams.

Although monospecific antibodies are beginning to show their limitations, they should be recognized as part of a sequence of antibody-based cancer immunotherapy developments, a sequence that reaches back at least as far as the Nobel Prize–winning efforts of James P. Allison, PhD, and Tasuku Honjo, MD, PhD. Allison's work on the CTLA-4 led to the first FDA-approved checkpoint inhibitor drug, ipilimumab (Yervoy, Bristol-Myers Squibb), whereas Honjo's discovery of PD-1 led to the development of anti-PD-1 drugs such as pembrolizumab (Keytruda, Merck). These drugs and other checkpoint inhibitors have profoundly impacted the treatment of cancer.

An alternative cancer immunotherapy approach, namely CAR T-cell therapy, has also demonstrated its potential to combat cancer. In this approach, T cells are engineered to launch sustained attacks on tumors. Although CAR T-cell therapies clearly have fight in them, they may cede some anticancer glory to bispecific antibodies (bsAbs). The first FDA-approved bsAb to directly compete with CAR-T was the CD19/CD3 drug blinatumomab (Blincyto, Amgen). It was introduced in 2014 for indications in B-cell precursor acute lymphoblastic leukemia.



Meletios Verras / Getty Images

A T cell expressing a bispecific CAR interacting with a cancer cell through CD19 and CD20 antigens

Even while monospecific antibody–based checkpoint inhibition therapies and CAR T-cell therapies continue to be improved, bispecific and multispecific antibodies are shaping up as cancer immunotherapy options that may provide significant advantages. At present, companies such as Amunix Operating, Invenra, Glycotope, and Xencor are working independently and in collaboration with larger pharmaceutical companies, such as Novartis, Daiichi Sankyo, and Roche, to bring bispecific and higher-order antibodies into the cancer immunotherapy market. Fundamentally, their engineered expression platforms focus on streamlining novel antibody development, reducing the risk factors to patients, and optimizing tumor destruction.

Increasing Selectivity

bsAbs emerged with the technologies developed by two pioneering companies Amgen and MacroGenics. Amgen introduced the BiTE platform; MacroGenics, the DART platform. Despite the availability of such platforms, it can still be a challenge to produce bsAbs that incorporate an Fc domain, suggests John Desjarlais, PhD, senior vice president of research and CSO at Xencor. "If you don't have an Fc domain," he says, "you have a very short

half-life," necessitating low and frequent injections or continuous infusion in patients.

Xencor's solution was to build a robust and GMP-scalable bispecific platform that includes an engineered Fc domain for the antibody, ensuring that antibodies produced with this platform would have a longer half-life in vivo. Xencor's XmAb Fc platform increases this efficiency of heterodimer Fc formation to 95% out of the gate.

"If I want to make a heterodimeric Fc domain, one that is different on either side," he says of a traditional process, "I'm going to get a mixture of 50% of the heterodimer, and 25% of the different homodimers by comparison."

To improve efficiency yet further, Xencor has engineered an additional feature in the Fc domain. "We perturb the isoelectric point on either side of the Fc heterodimer through substitutions in the Ch3 domains," Desjarlais details. "The idea behind that was, we would have an ability to very easily separate out the small amount of contaminating homodimers just by using ion exchange chromatography."

Xencor is exploring bsAbs that act as dual checkpoint inhibitors, such as anti-PD-1/CTLA-4 and CTLA-4/LAG-3. The field has learned that cancer evolves to suppress the immune system by engaging different pathways meant to protect the body against autoimmunity.

Single checkpoint blockers on the market such as nivolumab (Opdivo; anti-PD1) and ipilimumab (Yervoy; anti-CTLA-4) have been used in combination to improve antitumor activity, but this approach, says Desjarlais, comes at the cost of increased toxicity. Dual-targeting antibodies may promote less toxicity by more selectively targeting the tumor reactive T cells. "The idea is to turn off the brakes," he explains, "and the more brakes you can hit at the same time, the more you can activate those tumor T cells."

In addition to checkpoint inhibitors, Xencor has been successful in establishing two Phase I trials in collaboration with Novartis involving T-cell-engaging bsAbs; one that has an AML indication and binds to CD123 on AML blasts and CD3 on T cells, and a second that binds to CD20 on malignant B cells and CD3 on T cells. The company has a third wholly owned bsAb that binds CD3/SSTR2 (somatostatin receptor 2). Currently in Phase I trials, this bsAb is being explored with dose escalation in neuroendocrine tumors.

"CD3 bispecifics would be considered direct competitors to CAR-T," asserts Desjarlais. CAR T-cell therapies require weeks of preparation including cellular extraction from a patient, engineering in vitro, culturing, speculative dosing, and continued growth in vivo. In contrast, Desjarlais points out, "a bispecific is something in a vial that you have in the pharmacy."

"With a bispecific," he emphasizes, "you know exactly what you're putting in."

T-cell engagers

Volker Schellenberger, PhD, president and CEO of Amunix, affirms that the challenge of the CAR T-cell therapies is that they must be individually created for each patient. "Another challenge," he says, "is that you are injecting live cells into a patient. So, it is very

difficult to control what happens to them. They can even multiply in that person."

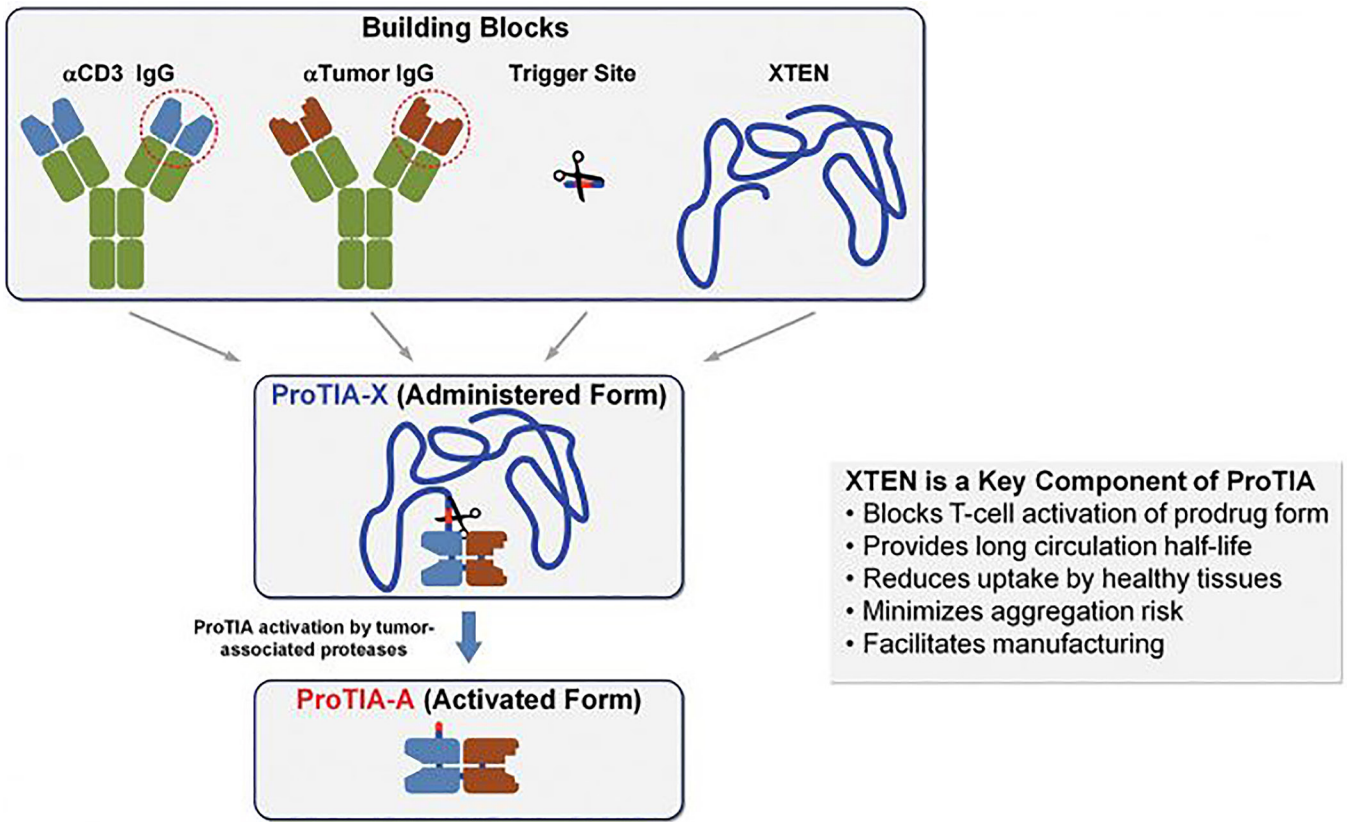
"We need to somehow mitigate the toxicity of these T-cell engagers," insists Schellenberger. "If you have a protein-based drug, then you could give it right away, instead of after the several weeks it takes to develop an individualized CAR T-cell therapy; that would be a big benefit to the patient."

Amunix has developed a new format of bispecific T-cell engagers that can be delivered in a low dose with lower toxicity using XTEN technology, an alternative to PEGylation. "The T-cell engager," Schellenberger explains, "works like an adaptor molecule. It bridges the tumor and the T cell." XTEN is a protein polymer that is engineered to behave like polyethylene glycol (PEG) which is attached to bsAbs to increase their half-life in vivo without the need for an Fc domain.

"XTEN has evolved into kind of a Lego kit for pharmaceuticals," Schellenberger notes. "It allows us to make very complex molecules which by other means we just couldn't produce."

The company's lead XTENylated bsAb, AMX-268, is in preclinical development. It is a T-cell engager

Amunix has used its XTEN platform to develop protease triggered immune activator (ProTIA) molecules. These are bispecific T-cell activators that are designed to outperform other bispecific formats with respect to characteristics such as half-life and safety. An early ProTIA prototype molecule, one that incorporated Amgen's MT110, allowed Amunix to demonstrate the ProTIA format's advantages. Amunix is now replacing the Amgen-specific portions of the prototype to generate additional ProTIA molecules.



that binds to CD3, a T-cell receptor (TCR), and EpCAM, an adhesion molecule overexpressed in 80% of solid tumors.

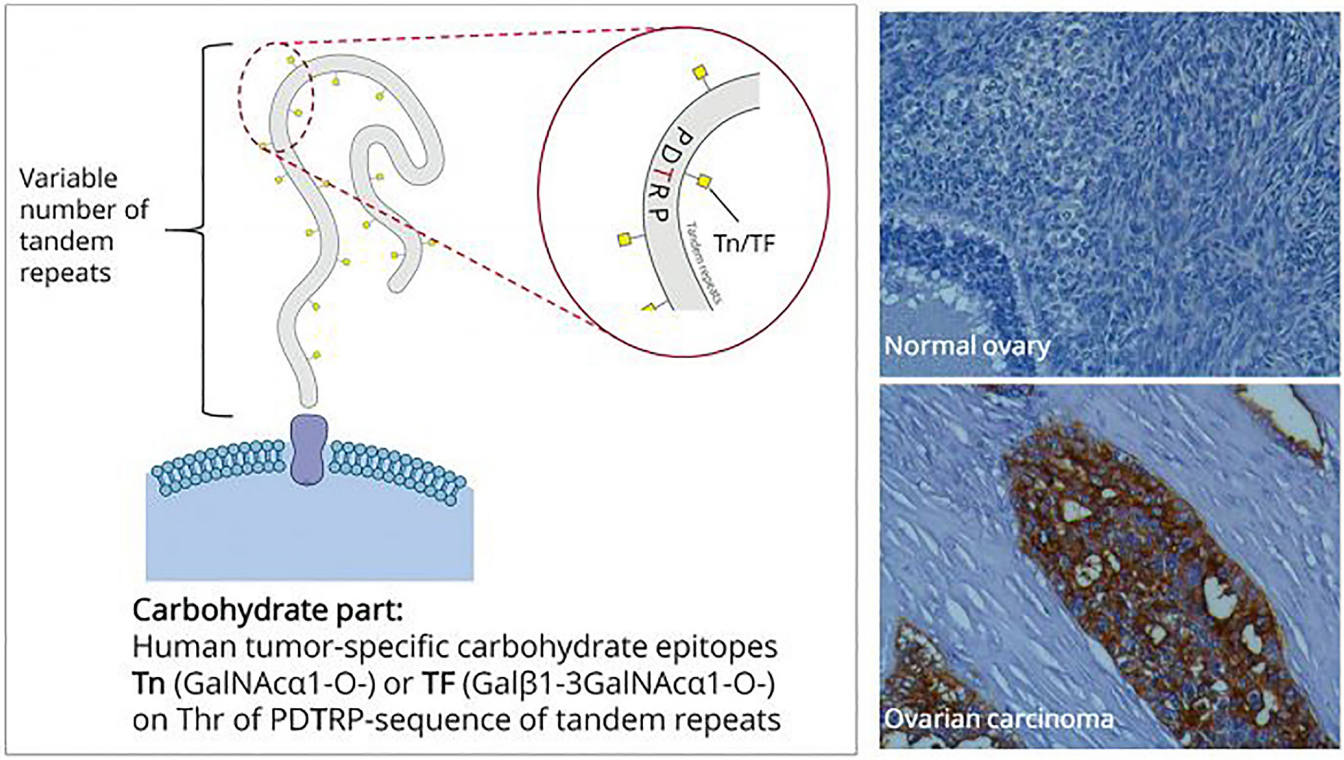
"We give the drug in an inactive form and convert it to the active form only when it is in the tumor environment," Schellenberger says. The company's pro-drug is activated by the inflammatory process found primarily within the tumor microenvironment, reducing off-target toxicity and increasing antitumor selectivity, "so that if our molecule finds that target in a healthy organ, it will still leave it alone."

The active form of the drug is smaller than typical Fc-containing intact antibodies, allowing it to be removed easily and rapidly through the kidney. Schellenberger's data suggests that AMX-268 may have lower immunogenicity and a lower toxicity profile among other potential EpCAM-targeting T-cell engagers such as Removab (Fresenius Biotech) and the investigational MT110 (Amgen).

Moving from mono- to bispecific antibodies

One company that is leveraging its success in developing monospecific antibodies into bi- and trispecific antibodies is Glycotope. According

Aberrant glycosylation patterns specific to cancer cells can be targeted by engineered antibodies, such as those developed by Glycotope using its GlycoExpress (GEX) platform. For example, the company's PankoMab-GEX antibody recognizes a tumor-specific epitope of MUC1 (TA-MUC1). Left: Schematic illustration of MUC1 highlighting the PDTRP motif, which has a conformational epitope induced by the carbohydrate antigens Tn or TF. Upper right: Normal ovarian tissue, which lacks PankoMab-GEX staining. Lower right: Ovarian carcinoma detected with PankoMab-GEX, which can recruit the immune system to destroy tumor cells.



to Anika Jäkel, PhD, the company's director of preclinical pharmacology and cancer immunology, "Glycotope has strong expertise in glycobiology and focuses on the generation of antibodies against tumor-specific glycoepitopes."

The company's first-in-class mAb, Gatipotuzumab, targets the tumor-specific epitope TA-MUC1, a novel combined carbohydrate/peptide conformational epitope on the tumor marker MUC1 (mucin-1). This antibody shows broad therapeutic potential in 80–100% of its main solid tumor indicators (that is, ovarian, lung, and breast cancers).

"Our most advanced pipeline bispecific is a TA-MUC1-targeting T-cell engager (PankoMab-CD3-GEX)," Jäkel points out. "It was designed to combine the high tumor specificity of Gatipotuzumab with activation of polyclonal T cells independent of MHCI engagement upon simultaneous binding of TA-MUC1 and CD3 on T cells."

A second molecule in development at Glycotope is PankoMab-PDL-GEX, which combines binding to TA-MUC1 with immune checkpoint molecule PD-L1 attached to a glycol-optimized functional Fc domain. PankoMab-PDL-GEX is designed to direct checkpoint blockade to the tumor and thereby enhance tumor cell killing.

Glycotope's GlycoExpress (GEX®) technology platform is used for screening and production of biopharmaceuticals, such as those described above, and other glycoproteins for fully human optimized glycosylation. "It consists of a toolbox of proprietary human cell lines generated by glycoengineering," says Jäkel. "It is biotechnologically optimized for product improvement as well as fast, reproducible, and high-yield glycoprotein production."

"We do not use a standard platform approach for our bispecific programs," Jäkel continues, suggesting that by focusing on GlycoTargets, the company has positioned itself to screen several construct formats for each bispecific product idea. "We can produce classical IgGs but also bispecific formats in our GlycoExpress system," she asserts. "We can test different glycosylation variants for identification of a lead candidate with highest antitumor efficacy."

Although Glycotope is not exclusively focusing on the bsAb market, Jäkel suggests that there are many possible advantages to targeting two epitopes over monospecific antibodies, including increased specificity and/or avidity, increased inhibition of tumor growth, enhanced local tumor cell killing, and blockade of immune checkpoint inhibitors.

Beyond bispecifics

In immuno-oncology, a well-trod path is the redirection of tumor T cells. A less-well-traveled path is being explored by Invenra, which seeks to activate functional processes that require a novel mechanism of action through bispecific and higher-order antibody binding.

"A good example is agonist antibodies for the tumor necrosis factor [TNF] receptor superfamily," says Bonnie Hammer, PhD, vice president of biologic development at Invenra. "The ligands for that family are trimeric. To get good activity, you need at least

three receptors coming together, but it is even better if you have even higher-order clustering."

Antibodies that drive this type of receptor clustering are the focus of Invenra's ARCHER (Agonistic Receptor Clustering by High-order Exogenous Rearrangement) technology. One of the receptors in the TNF superfamily, OX-40, is the target of an Invenra bsAb in lead selection.

To engage the higher-order clustering, Invenra used its B-Body multispecific antibody development platform to produce a bispecific with a two by one (2 × 1) format. "The bispecific has three Fab domains," Hammer notes. "But two Fab domains bind to one epitope, and the other Fab domain binds to a different epitope."

"Traditional monoclonal antibodies for OX-40 have suffered in the clinic," Hammer says, pointing out that they are dependent on having Fc engagement to provide the secondary crosslinking needed for activity. In contrast, she continues, Invenra's OX-40 agonist has allowed the company "to achieve activity in the absence of any additional crosslinking by targeting multiple epitopes." Although the OX-40 agonist has yet to see the clinic, Hammer suggests that the agonist "will provide higher activity than has been previously seen with monospecific antibodies."

A bacteriophage library that consists of wholly human Fab fragments and that matches the natural diversity found in the human repertoire can provide the starting point for selecting Fabs of interest used in Invenra's B-Body platform, Hammer says. A domain-substitution strategy with a few orthogonal chain mutations allows for highly specific light chain–heavy chain pairing and enables high-throughput production and purification of bispecific and multispecific antibodies.

"We found that you can predict some things [during antibody design]," she reports, "but a lot of it is through empirical testing. The affinities for the antibodies, the geometry, and the epitopes that you're hitting matter." One other group of multispecific antibodies in Invenra's pipeline consists of discovery candidates that create higher specificity through the targeting of more than one antigen. "These candidates are the bispecific antibodies we call the SNIPERsTM," says Hammer. Currently a regulatory T cell–depleting SNIPER molecule is in lead selection.

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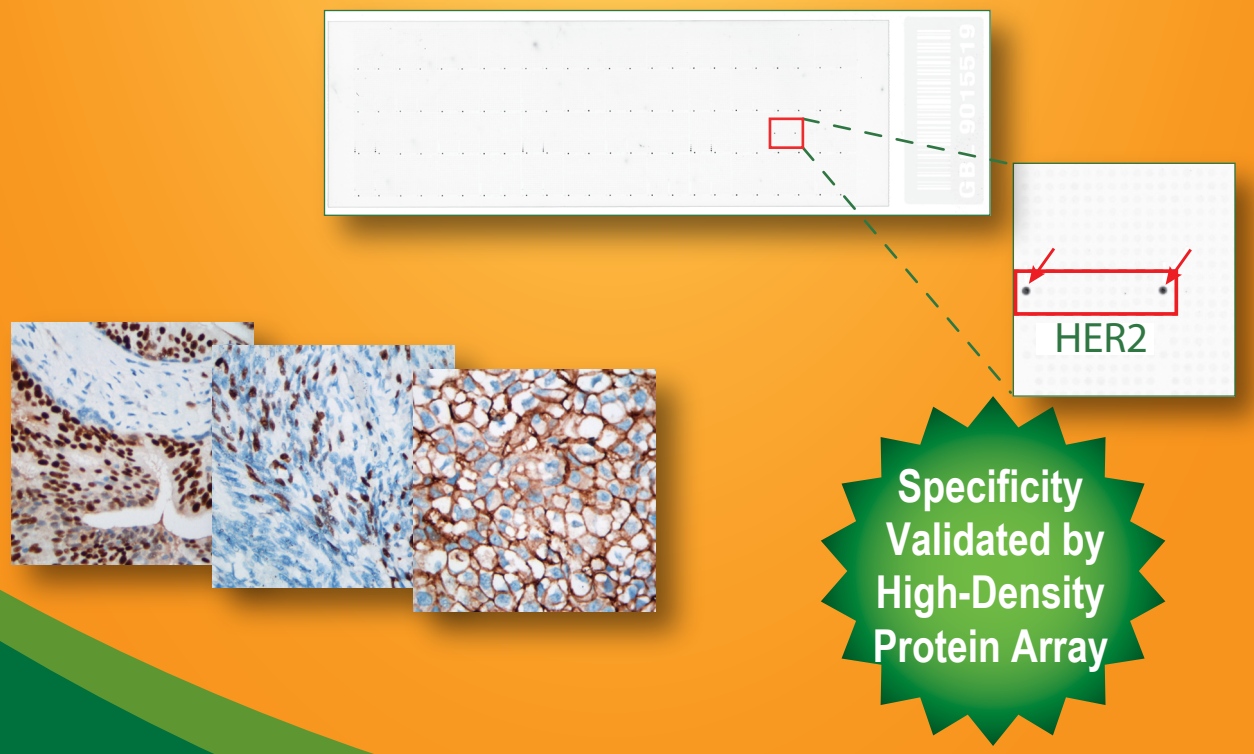

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