A Two-Pronged Approach

Molly Webster[1] and Vikram Sheel Kumar[2*]

Since their discovery in 1975, monoclonal antibodies have been at the forefront of modern medicine. These monospecific immune proteins target a single antigen. But at the same time that monoclonal antibodies have hogged the spotlight, their distant cousins, bispecific antibodies, have been floating around.

First proposed in 1960, such a protein would be able to target 2 different antigens, or epitopes, on 2 different cells, on the same cell, or even within a single antigen on a single cell. The therapeutic possibilities would be awesome. There was only one catch.

"A discrete, directed pairing of two arms to engage two specific antigens is not achieved in nature," says Christoph Spiess, a scientist in the department of antibody engineering at Genentech Inc, a biotechnology company in San Francisco, CA.

In the more than 50 years after the idea was proposed, bispecific research and breakthroughs have taken off *(1)*. Here we consider a recent report on the first-ever creation of bispecific antibodies from any 2 existing antibodies *(2)*. We also look beyond the therapeutic benefits and consider the clinical applications of these 2-pronged proteins.

What Is the Innovation?

Christoph Spiess

Shaped like a Y, an antibody has a particular molecular structure. It is made up of 4 chains, 2 heavy and 2 light chains. Across antibodies of the same subtype and isotype, the stem of the Y remains constant, explains Spiess. The tips provide the specificity toward an antigen or binding site. This is where the power of bispecific antibodies lies.

To create a tip pairing that will bind to either 2 different antigens or 2 different regions on the same antigen, scientists previously attempted to "zip" together 2 different antibody fragments using linkers, nonnatural sequences. But, "non-natural sequences pose potential immunogenicity," says Spiess. Not only that, prior reports have shown that creating bispecific antibodies from fragments can also lead to a shorter half-life or low yields *(2)*.

Enter Spiess and the Genentech team. They wondered if there was a way to create a bispecific antibody not from fragments, but from 2 complete half-antibodies.

"We were first ones to think about a half antibody," says Spiess, who points out that "it wasn't intuitive that half-antibodies could be expressed—in nature, antibodies are always full."

With their idea in hand, the team of scientists set up an experiment that involved expressing half-antibodies (also known as hemimers), with a distinct set of mutations, in bacterial culture.

How Does It Work?

Ramy Arnaout

The scientists began expressing full-length human antibodies in *Escherichia coli*.

"One of the exciting findings was that half-antibodies were stable structures in the bacterial cell," says Spiess.

The scientists focused on expressing anti–epidermal growth factor receptor (EGFR)[3] and anti-MET proteins, known for fighting off lung cancer. The key was that the antibodies possessed 2 mutations, known as a "knob" and "hole."

"As long as the half-antibodies are kept in two cells during the folding and assembly process," says Spiess, "these alterations to the proteins' genetic sequence al-

[1] Science writer and producer, Brooklyn, NY; [2] Boston Children's Hospital, Boston, MA.
* Address correspondence to this author at: 390 Commonwealth Ave., #605, Boston, MA 02215. E-mail vkumar@dimagi.com.
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[3] Nonstandard abbreviations: EGFR, epidermal growth factor receptor; Fab, antigen-binding fragment.

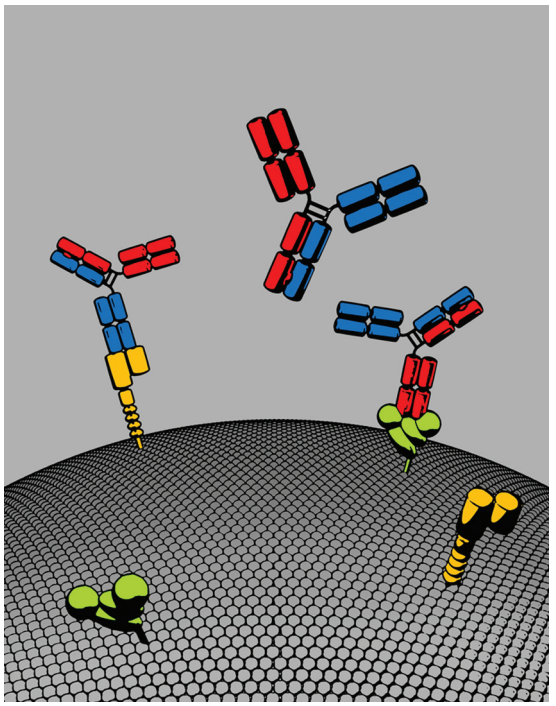

Fig. 1. Bispecific antibody with 2 distinct binding arms (red and blue) that inhibit MET (orange) and EGFR (green). Courtesy of Allison Bruce, Senior Graphics Specialist, Genentech.

low the appropriate heavy and light chains to fold with each other, but do not let the two heavy chains to bond." Thus, the Y stem never forms.

After expression, the half-antibodies from the 2 different cell cultures were combined in vitro, and through a series of steps of purification, reduction, and oxidation, the interchain disulfides bonded. The result, for the first time, was the creation of bispecific antibodies from complete half-antibodies that maintained the correct architecture of an antibody (Fig. 1).

In their first experiment, the yield was about 0.5–1.0 mg, but the authors have since reported up to 960-mg yields. Pharmacokinetic studies showed that "the bispecific antibody has linear and dose-proportional pharmacokinetics between 5 and 100 mg/kg, resembling that of other antibodies" *(2)*. In a tumor driven by both MET and EGFR, the authors report "potent" activity by the bispecific antibody at both 50 mg/kg and 100 mg/kg (106% and 107% tumor growth inhibition, respectively).

After their tests on the lung cancer antibody team, the group applied their new knowledge, along with a more finely honed method, to a variety of antibody pairings, "creating 27 more that involved the co-culture of both strains, which eliminated the assembly/redox," says Spiess.

"A larger number of pairs than described in any other publication so far," says Spiess.

Where Can This Technology Fit?

The therapeutic implications of bispecifics are obvious, but what could they do to transform diagnostics? In a 2013 review study, a team of scientists wrote that bispecific antibodies have, "real potential to pave the way for improved, next generation diagnostics with applications in low-resource and POC [point-of-care] settings" *(3)*. The authors reported on the use or proposed use of bispecific antibodies in diagnosing diseases such as SARS (severe acute respiratory syndrome), pertussis, *E. coli* infection, and hepatitis B, as well as other infectious diseases, and in assisting with in vivo cancer diagnostic imaging.

We spoke with Ramy Arnaout, associate director of the clinical microbiology laboratories at the Beth Israel Deaconess Medical Center in Boston, who was not involved in the 2013 study, about the applications bispecific antibodies could have in the clinical laboratory.

An area where Arnaout sees promise is in improving the specificity of antibody-based assays.

"It is conceivable that antibodies that can bind two different nearby epitopes on a single analyte (e.g., protein) could provide more specific diagnostics than current monospecific antibodies by allowing for higher affinity," Arnaout wrote in an email to *Clinical Chemistry*. "In fact, assuming the overall affinity of a bispecific antibody to be greater than the sum of the affinities of each Fab [antigen-binding fragment]—that is, assuming synergistic binding of the different Fabs—hybridizing two monospecific antibodies, even ones with so-so affinities, could produce a bispecific antibody with improved affinity and specificity compared with ones that are currently available."

Moreover, Arnaout suggests that of the 3 ends of the bispecific antibodies, 2 variable Fabs and 1 constant Fc, the Fc could be used for signaling.

But there is a catch, says Arnaout.

"In the picture we have imagined, there is signal even if the bispecific antibody has not bound the analyte," he wrote by email. "One can imagine ways to introduce conditionality, but they get somewhat complicated."

Spiess points out one such method, one that would not use an anti-Fc as the detection signal. This method uses a setup that requires engagement with both antigens and is proposed in the 2013 review paper, in which the authors describe the method as follows. "The capture monoclonal antibody is immobilized onto a solid surface

and binds to a specific epitope on its cognate antigen present in the test sample. Upon addition of the corresponding bispecific antibody . . . , one arm binds to a specific epitope on the antigen, while the other arm binds to a reporter molecule . . . and converts the subsequently added substrate to a quantifiable signal or colored product" *(3)*.

All told, "the requirement for conditional signaling is an important obstacle toward introducing bispecific antibodies in the clinical labs in the area where they would seem most naturally to fit, immunoassays," says Arnaout.

For bispecific antibodies to surpass monoclonal antibodies, they would need to be cheaper, more effective, or both, say all parties. Plus, researchers and developers would need to consider "manufacturability, scale, and stability, with issues relating to immunogenicity, pharmacokinetics, and biodistribution being of particular concern for therapeutics" *(3)*.

It's a novel idea, with promise for the clinical setting, according to Spiess. "Anyone can build on this."

References

1. Kufer P. The promise of bispecific antibodies. http://www.discoverymedicine.com/Peter-Kufer/2009/07/12/the-promise-of-bispecific-antibodies/ (Accessed December 2013).
2. Spiess C, Merchant M, Huang A, Zheng Z, Yang N-Y, Peng J, et al. Bispecific antibodies with natural architecture produced by co-culture of bacteria expressing two distinct half-antibodies. Nat Biotechnol 2013;31:753–8.
3. Byrne H, Conroy PJ, Whisstock JC, O'Kennedy RJ. A tale of two specificities: bispecific antibodies for therapeutic and diagnostic applications. Trends Biotechnol 2013;31:621–32.

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