ANTIBODIES: WILL THEIR STAR CONTINUE TO RISE?

Antibodies are having a huge impact in basic and translational research, but they are also in crisis. Francesca Lake investigates

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Due to their high specificity and selectivity, antibodies hold great potential for use in the life sciences, in everything from basic research to diagnostics and therapeutics. Since the 1970s when Köhler and Milstein produced hybridoma monoclonal antibodies (mAbs) through the fusion of antibody-producing mouse spleen cells with an immortal myeloma cell line [1], multiple research projects have utilized antibodies and it has been estimated that there are over 300 companies offering 2 million antibodies for research purposes [2].

RISEING STARS IN DIAGNOSTICS & THERAPEUTICS

Antibodies are being used in some hugely important research. They are integral to the future of personalized medicine, being used in various methods to identify biomarkers, such as CITE-seq. What's more, not one of us will be unaware of immunotherapy, with the FDA now having approved immunotherapies for around 20 types of cancer. An antibody-based therapy is also available for preventing rejection of kidney transplants.

Some exciting projects are also underway that are looking at infectious disease. A recent Phase 1b clinical trial has suggested that a combination of 'broadly neutralizing antibodies' is an improvement on current HIV therapies [4], and six Zika antibodies have been produced for utility in testing for and treating the virus [5]. The Pandemic Prevention Program, a co-operation between DARPA and a number of US-based institutions, is seeking to use synthetic biology to turn patients themselves into 'antibody factories'.

One example of this synthetic approach has been shown in preclinical models against Ebola, using DNA-encoded mAbs (DMABs). With Ebola a continuing concern, studies had been looking at mAbs obtained from survivors to try to develop a therapeutic, but this was costly. A team from The Wistar Institute (PA, USA) designed DMABs that, when injected, provide a genetic blueprint for the body to produce its own Ebola-specific antibodies [6].

"Due to intrinsic biochemical properties, some mAbs might be difficult and slow to develop or even impossible to manufacture, falling out of the development process and causing loss of potentially effective molecules," explained David B Weiner, lead researcher of that study. "The DMAB platform allows us to collect protective antibodies from protected persons and engineer and compare them rapidly and then deliver them in vivo to protect against infectious challenge. Such an approach could be important during an outbreak, when we need to design, evaluate and deliver life-saving therapeutics in a time-sensitive manner."

New technological advances are providing us with ever new ways to harness antibodies. One recent study has combined gene sequencing and computing techniques to provide greater insight into how the immune system can be so diverse with so few genes, and to the actual extent of its repertoire. *"Due to recent technological advances, we now have an unprecedented opportunity to harness the power of the human immune system to fundamentally transform human health,"* explained Wayne Koff, CEO of the Human Vaccines Project, which led this effort. *"Decoding the human immune system is central to tackling the global challenges of infectious and non-communicable diseases, from cancer to Alzheimer's to pandemic influenza."* He believes his study marks a key step toward understanding how the human immune system works, *"setting the stage for developing next-generation health products through the convergence of genomics and immune monitoring technologies with machine learning and artificial intelligence"* [9].

The rise of antibody-based therapies is therefore likely to continue, especially considering that new targets are constantly being identified that are likely to be targetable more specifically with antibodies than with traditional approaches, such as small molecules.

EVEN STARS HAVE THEIR FLAWS

Antibodies for use in research – whether that be basic or translational – now come in three flavors – hybridoma monoclonal, recombinant

Figure 1. Hybridoma cells grown in tissue culture. Credit: Gerry Shaw, the EnCor Biotechnology Lab, CC-BY-SA.

monoclonal and polyclonal. Each has its own benefits and drawbacks, making it useful for different kinds of research. Hybridoma mAbs are precise and reproducible, yet come with high requirements in terms of costs, expertise and time. Additionally, they can lose specificity over time, since some hybridomas are prone to genetic drift. Recombinant mAbs (rAbs), produced using nucleic acid sequences in a similar manner to monoclonal proteins, provide researchers better control, but the need for expertise during development is high. Polyclonal antibodies (pAbs) have multi-epitope binding properties; however, there is a finite supply and an inability to rigorously define the clonal composition in a polyclonal mix means performance can vary batch-to-batch. Their ability to bind multiple epitopes also has the potential to be a burden – while advantageous for some applications, this could be a source of troublesome background and cross-reactivity [3].

However, reproducibility concerns are widespread for all antibodies. In just one of the multiple studies on antibody reliability, the Human Protein Atlas examined over 5000 commercial antibodies and demonstrated that over 50% could not be used in the proposed application [2]. This has huge implications for research conducted using these antibodies.

“We now have access to multiple antibody characterization and validation tools such as mass spectrometry and the ability to knock-down or knockout genes to alter protein expression in cells and animals, which allows us to better assess reagent specificity and to be more precise with protein detection than ever before,” noted Alejandra Solache (Abcam, Cambridge, UK). *“However, as these techniques have become more widespread and accessible, we as well as other researchers are finding that some of the reagents that we have relied on for decades might not be as specific as originally*

thought. This has resulted in an important shift in the way that we develop, test and use antibodies.”

A prime example of the cost of mistakes in antibody validation can be found in a 2017 study, which suggested that two decades of research attempting to target an estrogen receptor for breast cancer treatment had been wasted owing to insufficiently validated antibodies that were not appropriate for the target in the original paper [7]. Overall, however, the problems facing antibody research have a variety of potential sources.

“Hybridomas are not stable and often not entirely clonal [11] so if you buy something that’s made in a hybridoma today and if you buy something that is made in a hybridoma in a year, the end product that you receive can differ a lot. This can differ in the purity, it can differ in the fine composition, and it can also just be a completely different molecule,” commented Michael Fiebig (Absolute Antibody, Oxford, UK). *“At the same time, it’s also where you get your antibodies from. The antibody market is very diverse and there are a lot of companies that just focus on re-selling antibodies and they might in turn re-sell to other companies. Knowing where your antibody is from and knowing whom to talk to if you are running into any issues is a big challenge, and science is not simple.”*

Longevity and traceability are just some of the potential challenges. Additional complications include not checking properties such as affinity constants and selectivity, the availability of information on reference standards and the original development and production process, and long-term availability [2]. Of course, with researchers continually striving to answer new questions, they will need to use antibodies in new ways that they might not have been validated for. As a result, responsibility falls on both researchers, to ensure their antibodies are useful for their new application, and on manufacturers, to provide robust antibodies that do what they say they will do, and perhaps go a little beyond.

SOLVING THE CRISIS

While the problem is certainly appreciated, the next steps are uncertain. For example, some have suggested the complete phasing out of pAbs is required, yet others disagree [3]. It has also been suggested that recombinant versions of all mAbs should be produced. *“We can use recombinant methods to overcome almost every aspect of the mAb production path,”* explained Fiebig. He believes we will see a drift toward widespread production of rAbs. *“This will be pushed by the end-users who are understanding the advantages with regards to reproducibility, but also the advantages in terms of being able to explore new biology the recombinant production brings. We are going to see a lot of resistance to this from the side of the industry, but in the end, the end-users – the people really doing the science – they are really going to push this through.”*

Carl Ascoli (Rockland Immunochemicals Inc., PA, USA) and Birte Aggeler (Bio-Techne, MN, USA) agree that end-users are key: *“The perspective of researchers will likely evolve, resulting in*

“Using recombinant cloned antibodies is one way to ensure reproducibility, and knock-out cell lines are a good way to confirm specificity. All-in-all, selecting the right antibody comes down to both researchers and suppliers.”

a better understanding of when, and when not, to use an antibody in a 'fit-for-purpose' manner" [3]. They also feel that universal standards for application-specific antibody validation are needed.

Meanwhile, manufacturers are making moves to improve things from their side. "Our most popular products are new versions of traditional, well-established antibodies," noted Fiebig. "A lot of hybridomas are not stable, in fact it's inherent to hybridomas not to be stable as they are B-cell fusions with cancer cells. You can get subtype switching and all of a sudden your blocking antibody starts depleting cells. With the recombinant approach that we're taking, you get a precisely defined product, and you can overcome impurities that might be inherent to the hybridoma, like additional light chains or additional heavy chains. So, it's doing something new but it's doing something that is going to be reliable and reproducible over time."

Using recombinant cloned antibodies is one way to ensure reproducibility, and knock-out cell lines are a good way to confirm specificity. All-in-all, selecting the right antibody comes down to both researchers and suppliers. "Suppliers need to make use of the most appropriate available techniques when producing and validating antibodies, so that researchers can be confident about quality," explained Solache. "And researchers also need to stay as up-to-date as possible on the best type of antibodies to use, and where possible choose an antibody that has been shown to work in the specific applications and/or species relevant to their own experiments." Several open platforms are now available on the internet providing researchers the opportunity to both produce and access reviews of antibodies, such as antibodypedia and pAbmAbs. Checklists have also been produced to help users ensure validation of their antibody [8].

LOOKING TO THE FUTURE OF ANTIBODY RESEARCH

There is a clear need to improve practices in antibody research, and that need appears to be fairly well-known by the research community. With technology constantly improving, there will be ever new ways to harness antibodies to answer novel research questions, while ensuring the antibodies are validated for that use and the resultant research is reproducible. Solache is excited about the future of the field: "With the increased focus on reproducible, high-quality antibodies, we expect to see a continuing shift towards recombinant monoclonals, which in turn will help with reproducibility, and with this we will see even more of the blurring of the distinction between research, diagnostic and therapeutic antibodies that we are already seeing."

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MONEY DOWN THE DRAIN

The use of poorly characterized and ill-defined antibodies wastes materials, researcher time and money

Figure 2. An infographic showing the wastage caused by bad antibodies. Reproduced with permission from [10].

Figure 3. Western blots demonstrating antibody validation using wild-type and knockout cell lines. When an antibody (rabbit monoclonal PKC alpha) that is specific to the target of interest is exposed to its corresponding KO cell line, it does not react and shows up as blank in the KO lane (1), confirming its specificity. By comparison when a nonspecific antibody (here rabbit polyclonal) is used, it reacts with nontarget (non-ILK) proteins in its corresponding KO cell line lysate, resulting in a band in the KO lane (2), demonstrating a lack of specificity. The nonspecific antibody was subsequently removed from the catalogue. Credit: Abcam's Antibody Validation Lab, Cambridge, UK. KO: Knockout; WT: Wild-type.

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